

# ACTH Stimulation and Oral Salt Loading Tests Detect Biochemical Outcome Early After Primary Aldosteronism Surgery

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## Abstract

**Background:** In primary aldosteronism (PA), the biochemical outcomes of the Primary Aldosteronism Surgical Outcome study are used to assess aldosterone hypersecretion 6 to 12 months after surgery. However, few studies have investigated whether the outcomes can be predicted in the early postoperative period. In this retrospective study, we evaluated whether the adrenocorticotropin stimulation test (AST) and oral salt loading test (OST) performed immediately after surgery could predict biochemical outcomes 1 year after surgery.

**Methods:** We assessed 268 patients with PA who underwent adrenalectomy at our hospital between 2008 and 2020, underwent AST and OST within 15 days of surgery, and were assessed for biochemical outcomes 1 year after surgery. Patients were divided into 2 groups: biochemical complete success (B-com; n = 219) and incomplete success (B-inc; n = 49). Patients were divided into clinical complete and partial success and absent success groups. The relationships between various AST and OST values and outcomes were analyzed.

**Results:** The B-inc group had significantly higher plasma aldosterone concentration (PAC) and PAC/serum cortisol ratio (PAC/Cort) at baseline and after ACTH loading in AST and 24-hour urine aldosterone in OST than the B-com group. PAC/Cort at 30 minutes after ACTH loading [area under the curve (AUC) = 0.76] and 24-hour urine aldosterone (AUC = 0.77) were relatively superior predictors of the outcome. Parameters after ACTH loading were better predictors of biochemical and clinical outcomes than baseline.

**Conclusion:** AST and OST immediately after surgery can predict biochemical and clinical outcomes 1 year after surgery in patients with PA.

**Key Words:** primary aldosteronism, adrenocorticotropin stimulation test, oral salt loading test, primary aldosteronism surgical outcomes

Primary aldosteronism (PA) accounts for 5% to 10% of hypertension cases and is the most common cause of secondary hypertension (1, 2). Excess aldosterone is associated with the risk of cardiovascular diseases, atrial fibrillation, and end-stage renal failure, independent of the effects of hypertension (3). Thus, specific treatments are required for PA, including adrenalectomy and/or medical treatment with mineralocorticoid receptor antagonists (MRAs). Typically, unilateral PA is treated surgically, and bilateral PA is treated with antihypertensive agents, including MRA (4).

According to the Primary Aldosteronism Surgical Outcome (PASO) study, complete biochemical success after surgery for PA is achieved in 83% to 100% of the cases (5). In patients without complete biochemical success, an unresected adrenal gland may overproduce aldosterone. For these patients, MRA treatment is often initiated after evaluation by PASO. Generally, biochemical outcome evaluation by PASO is performed 6 to 12 months after surgery; thus, MRA may not be administered until such time. In patients without complete biochemical success, MRA treatment should be initiated promptly after surgery to prevent organ damage due to excess

aldosterone. Simultaneously, early prediction of postoperative biochemical outcomes may shorten the postoperative follow-up. Therefore, a method that can predict the biochemical outcomes immediately after surgery is required.

We previously reported that high aldosterone levels in the adrenal veins of the unresected glands in adrenal venous sampling (AVS) are associated with incomplete (partial or absent) biochemical success (6). Therefore, physicians can consider starting MRA immediately after surgery based on the aldosterone levels on the nonoperative side in AVS. However, AVS techniques and interpretation methods vary among centers. Furthermore, it is acceptable to omit AVS and perform surgery in patients presenting with typical aldosterone-producing adenoma (4). Therefore, a simpler method without AVS indices to predict biochemical outcomes is necessary. Plasma aldosterone concentration (PAC) (7) and postoperative ACTH-stimulate aldosterone (8) immediately after surgery can be used to predict biochemical outcomes. However, only a few studies with a limited number of patients exist, which necessitates more research. No study has predicted biochemical outcomes using the results of oral salt loading tests (OST) performed immediately after surgery.

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In the present study, an ACTH stimulation test (AST) and an OST were performed immediately after PA surgery as part of our routine clinical practice. We hypothesized that the results of these loading tests immediately after surgery could predict the biochemical outcome based on the PASO study 1 year after surgery, and their association was analyzed. The relationship between the results of these loading tests and clinical outcomes was also analyzed.

## Materials and Methods

### Transparency and the Openness Promotion Guidelines Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

### Ethics Statement

This study was approved by the Institutional Review Board of Yokohama Rosai Hospital (Yokohama, Japan; approval no. 2024-11). Informed consent was obtained from all participants in the form of opt-outs. This study adhered to the principles of the Declaration of Helsinki.

### Inclusion Criteria

This study assessed 277 patients with PA who met the diagnostic criteria of the Japan Endocrine Society (9). All patients had a PAC to plasma renin activity (PRA) ratio (ARR) of  $\geq 200$ , and at least 1 of the following tests was positive: saline infusion test, OST, captopril challenge test, or furosemide upright test. All patients underwent segmental AVS preoperatively. Patients with unilateral PA were considered for surgery; even patients with bilateral PA were considered if surgical therapy was considered beneficial and they were not excluded from the study. All participants were patients who underwent adrenalectomy in our hospital between 2008 and 2020, underwent AST and OST within 15 days of surgery, and were further assessed for biochemical and clinical outcomes of PASO 1 year after surgery (5). Patients who had serum cortisol levels of  $\geq 5 \mu\text{g/dL}$  after a 1 mg overnight dexamethasone suppression test, a cut-off value considered to ensure the presence of subclinical Cushing's syndrome, were excluded ( $n = 9$ ) (10).

### Biochemical Outcomes 1 Year After Surgery

One year after the surgery, the baseline ARR was measured in all patients to determine the biochemical outcomes based on the PASO criteria (5). If the ARR was  $>200$ , a saline infusion test was used to determine the biochemical outcomes. If a saline infusion test was not performed, an OST was used. A captopril challenge test was used if none were performed. Detailed criteria are described in the supplemental materials (11).

### Biochemical and Clinical Success and Patient Classification According to PASO Criteria

The patients were divided into 2 groups according to biochemical outcomes based on the PASO criteria (5): a biochemically complete success (B-com) group and a biochemically incomplete (ie, partial or absent) success (B-inc) group. Patients were also divided into 2 groups according to the clinical outcomes based on the PASO criteria: the clinically

complete and partial success group (C-suc) and the clinically absent success group (C-abs) (5).

### Aldosterone Secretory Test Immediately After the Operation

AST and OST were performed after the general condition of the patient had improved within 15 days of surgery.

In the ACTH stimulation test, PAC, PRA, and serum cortisol were measured at baseline, 30, and 60 minutes after loading  $250 \mu\text{g}$  of tetracosactide acetate. The test was performed in the morning, and the patient rested in the supine position for 30 minutes prior to blood collection (12).

### Measurement of PAC, PRA, and Serum Cortisol Concentrations

We optimized the prescription of antihypertensive drugs to patients several weeks before blood sampling to measure the PAC and PRA in preoperative aldosterone capacity tests (eg, prescribed only calcium-channel blockers and/or alpha-adrenoreceptor antagonists) according to the Japan Endocrine Society guidelines (9). After the preoperative tests, MRA was prescribed until the day before surgery.

PAC was measured using radioimmunoassay (RIA) (Spac-S Aldosterone Kit; Fujirebio Inc., Tokyo, Japan.) between January 2008 and January 2016, chemiluminescence enzyme immunoassay (CLEIA) calibrated by RIA (Accuraseed Aldosterone; FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan) between February 2016 and August 2021, and CLEIA calibrated by liquid chromatography-mass spectrometry/mass spectrometry (Accuraseed Aldosterone S; FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan) from September 2021 onward. Since the PAC measured by CLEIA was different from that measured by RIA, it was converted to the RIA equivalent value ( $\text{RIA value} = 1.174 \times \text{CLEIA value} + 42.3$ ) in the analysis (13). PRA was measured using RIA (Renin RIABEADS; YAMASA Corporation, Chiba, Japan; the lower limit was  $0.1 \text{ ng/mL}$ ) between January 2008 and December 2015 and enzyme immunoassay (Renin Activity Kit YAMASA; YAMASA Corporation; the lower limit was  $0.15 \text{ ng/mL}$ ) from January 2016 onward. Serum cortisol levels were measured using RIA (Cortisol kit "TFB"; TFB Inc., Tokyo, Japan) between January 2008 and March 2009, CLEIA (Access Cortisol; Beckman Coulter Inc., CA, USA) between April 2009 and January 2016, and CLEIA (Accuraseed Cortisol; Wako Pure Chemical Corporation, Tokyo, Japan) from February 2016 onward.

### Steroidogenic Activity Measured in Segmental AVS

After adrenocorticotrophic hormone stimulation, segmental AVS (sAVS) was performed by collecting blood samples from the central and tributary veins in each adrenal gland. Detailed methods for sAVS have been described previously (14-16). We compared the selectivity index (SI) (17, 18), lateralization index (LI) (17, 18), contralateral ratio (CR) (17), and maximum PAC ( $\text{maxPAC}$ ) in the tributary veins and  $\text{maxPAC}$  to cortisol ratio ( $\text{maxPAC/Cort}$ ) in the same tributary vein of the unresected adrenal gland after ACTH stimulation between the B-com and B-inc groups and the C-suc and C-abs groups.

**Table 1. Preoperative characteristics of biochemically complete and incomplete groups**

	Biochemically complete (n = 219)	Biochemically incomplete (n = 49)	P-value <sup>a</sup>
Age (year)	50 (43–58)	53 (46–58)	.34
Sex (male/female)	99/120	25/24	.53
Body mass index (kg/m <sup>2</sup> )	23.3 (21.5–27.0)	24.5 (21.6–27.2)	.60
Duration of hypertension (year)	7 (2–13)	9 (3–17)	.24
Systolic blood pressure (mmHg)	140 (127–156)	145 (126–153)	.74
Diastolic blood pressure (mmHg)	87 (78–98)	86 (80.5–94.5)	.90
Preoperative DDD of antihypertensive drug	2.0 (1.0–2.8)	1.7 (1.0–3.0)	.43
Serum creatinine (mg/dL)	0.70 (0.56–0.83)	0.68 (0.59–0.88)	.64
eGFR (mL/min/1.73 m <sup>2</sup> )	81.4 (67.3–93.8)	80.4 (66.1–94.5)	.77
Serum potassium (mEq/L)	3.3 (3.0–3.7)	3.6 (3.2–3.8)	.010
Hypokalemia	148 (68%)	23 (47%)	.008
Serum cortisol (μg/dL)	8.2 (5.7–11.0)	8.4 (6.5–10.9)	.49
PRA (ng/mL/hr)	0.3 (0.1–0.5)	0.2 (0.1–0.3)	.011
PAC (pg/mL)	255 (151–412)	189 (134–334)	.049
ARR	863 (403–1880)	860 (545–1920)	.45
24-hour urine aldosterone (μg/day)	18.6 (11.1–33.5)	16.6 (9.6–31.4)	.66
PAC from SIT (pg/mL)	207 (107–377)	160 (92–312)	.16
Largest nodule size (mm)	14 (10–18)	11 (2.5–16)	.024
Right SI	22.6 (12.6–31.0)	21.3 (14.9–36.5)	.57
Left SI	21.1 (14.0–29.5)	20.4 (13.3–28.1)	.55
LI	7.0 (2.3–19.1)	2.9 (1.7–8.8)	.006
CR	0.38 (0.17–0.86)	0.76 (0.43–1.32)	.001
<sub>max</sub> PAC after ACTH loading in the tributary veins of the unresected adrenal gland (pg/mL)	6820 (4380–12 100)	13 400 (7215–24 900)	<.001
<sub>max</sub> PAC/Cort after ACTH loading in the tributary veins of the unresected adrenal gland	9.8 (5.9–17.8)	20.9 (10.4–33.8)	<.001
Positive <i>KCNJ5</i> somatic mutation (n = 97)	32 (36.4%)	2 (22.2%)	.49

Data are presented as the median (interquartile range) or n (%).

Hypokalemia is defined by serum potassium <3.6 mEq/L.

Abbreviations: ARR, plasma aldosterone concentration to plasma renin activity ratio; CR, contralateral ratio; DDD, defined daily dose; eGFR, estimated glomerular filtration rate; LI, lateralization index; <sub>max</sub>PAC, maximum plasma aldosterone concentration; <sub>max</sub>PAC/Cort, maximum plasma aldosterone concentration to serum cortisol ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity; SI, selectivity index; SIT, saline infusion test.

<sup>a</sup>P-value for differences between the biochemically complete and incomplete success groups.

## Detection of the *KCNJ5* Mutation

*KCNJ5* was sequenced using polymerase chain reaction with cDNA reverse-transcribed from the RNA of freshly frozen aldosterone-producing adenoma tissue specimens as described previously (14). Furthermore, the proportions of *KCNJ5* mutants between the B-com and the B-inc groups and the C-suc and the C-abs groups were compared.

## Comparison of Clinical Parameters Between the Clinically Complete/Partial and Absent Groups

Six clinical parameters (duration of hypertension, body mass index, number of antihypertensive drugs, sex, target organ damage, and largest nodule size on imaging) constituted the aldosteronoma resolution score (consisting of 4 parameters, with the exclusion of target organ damage and largest nodule size) (19) and the primary aldosteronism surgical outcome score (20) for the prediction of clinical outcomes after adrenalectomy for unilateral primary aldosteronism (ie, 6 parameters) were compared between the C-suc and the C-abs groups. Target organ damage was defined as the presence of left ventricular hypertrophy (ie, left ventricular mass index >115 g/m<sup>2</sup> in men and >95 g/m<sup>2</sup> in women) (21) and/or microalbuminuria (urinary albumin excretion >30 mg/gCr) (22).

## Blood Pressure Measurements

The measurement and clinical evaluations of blood pressure were based on the guidelines of the Japanese Society of Hypertension (23). Blood pressure values were obtained in the sitting position by using a standard upper arm blood pressure monitor after a 5-minute rest.

## Statistical Analysis

Data are presented as the median (interquartile range) or n (percent), as appropriate. The Mann–Whitney U test was utilized to compare parameters between the B-com and the B-inc groups, as well as the C-suc and the C-abs groups. The relative proportions of categorical variables were assessed using Fisher's exact test. Spearman's rank correlation coefficients were calculated to assess the relationships between the continuous variables. Receiver operating characteristic (ROC) curves were constructed for the ratios of the B-com vs B-inc groups and the C-suc vs C-abs groups, determined by the parameters of the AST and OST immediately after surgery, <sub>max</sub>PAC in the tributary veins, and <sub>max</sub>PAC/Cort in the tributary veins of the unresected adrenal glands. The DeLong test was used for evaluating the difference between the area under the curves (AUCs). We conducted correlation analyses

between the parameters of the postoperative AST and OST and the  $\text{maxPAC/Cort}$  in the tributary veins of the unresected adrenal gland in sAVS. Differences were considered statistically significant at  $P < .05$ . All analyses were conducted using the JMP Pro 12 software (SAS Institute, Inc., Cary, NC, USA) except for the AUC comparisons, which were conducted using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

## Results

### Preoperative Characteristics of B-com and B-inc Groups

A total of 219 and 49 patients were classified into B-com and B-inc groups, respectively. In the B-inc group, 33 patients had biochemical partial success, and 16 patients had biochemical absent success. The B-inc group preoperatively had significantly higher serum potassium, CR,  $\text{maxPAC}$ , and  $\text{maxPAC/Cort}$  after ACTH loading in the tributary veins of the unresected adrenal gland and a lower percentage of hypokalemia, PRA, PAC, largest nodule size, and LI than the B-com group (Table 1).

### Comparison of Parameters of AST and OST Immediately After Surgery Between B-com and B-inc Groups

Immediately after surgery, the B-inc group showed a significantly lower PRA and higher ARR than the B-com group. The B-inc group had significantly higher PAC and PAC/cortisol ratios at baseline and 30 and 60 minutes after ACTH loading than the B-com group. In the OST, the B-inc group had significantly higher 24-hour urine aldosterone levels (Table 2).

### ROC for Biochemically Incomplete Success

Figure 1 shows the ROC curves for biochemically complete success of the remarkable parameters that were significantly different between the B-com and B-inc groups. Particularly good predictors of the biochemical outcomes were PAC/Cort at 30 minutes after ACTH loading and 24-hour urine aldosterone (AUC = 0.76 and 0.77, respectively). These parameters were higher than those obtained from the pre-ACTH loading parameters, such as PAC/Cort at 0 minutes and ARR at 0 minutes (AUC = 0.70 and 0.73, respectively). By loading ACTH, the AUC value significantly increased (PAC/Cort at 0 minutes vs 30 minutes,  $P = .027$ ). PAC/Cort at 30 minutes after ACTH loading and 24-hour urine aldosterone levels were better predictors than the indices from sAVS, including  $\text{maxPAC}$  and  $\text{maxPAC/Cort}$  after ACTH loading, in the tributary veins of the unresected adrenal gland (AUC = 0.69 and 0.71, respectively). No other parameters had superior predictive ability [Supplementary Fig. S1 (11)]. Table 3 shows the cut-off values, sensitivity, and specificity of various parameters of postoperative AST and OST, with the best combination of sensitivity and specificity for complete biochemical success.

### Correlation Analyses Between $\text{maxPAC/Cort}$ After ACTH Loading in Tributary Veins of the Unresected Adrenal Gland and Parameters of AST and OST Immediately After Surgery

As shown earlier, among the immediate postoperative biochemical tests, PAC/Cort at 30 minutes after ACTH loading and 24-hour urine aldosterone were relatively good predictors. Among the indices from sAVS,  $\text{maxPAC/Cort}$  after ACTH loading in the tributary veins of the unresected adrenal gland was a relatively good predictor. Since the aldosterone secretory capacity immediately after surgery may be related to that of the residual adrenal glands after surgery, the

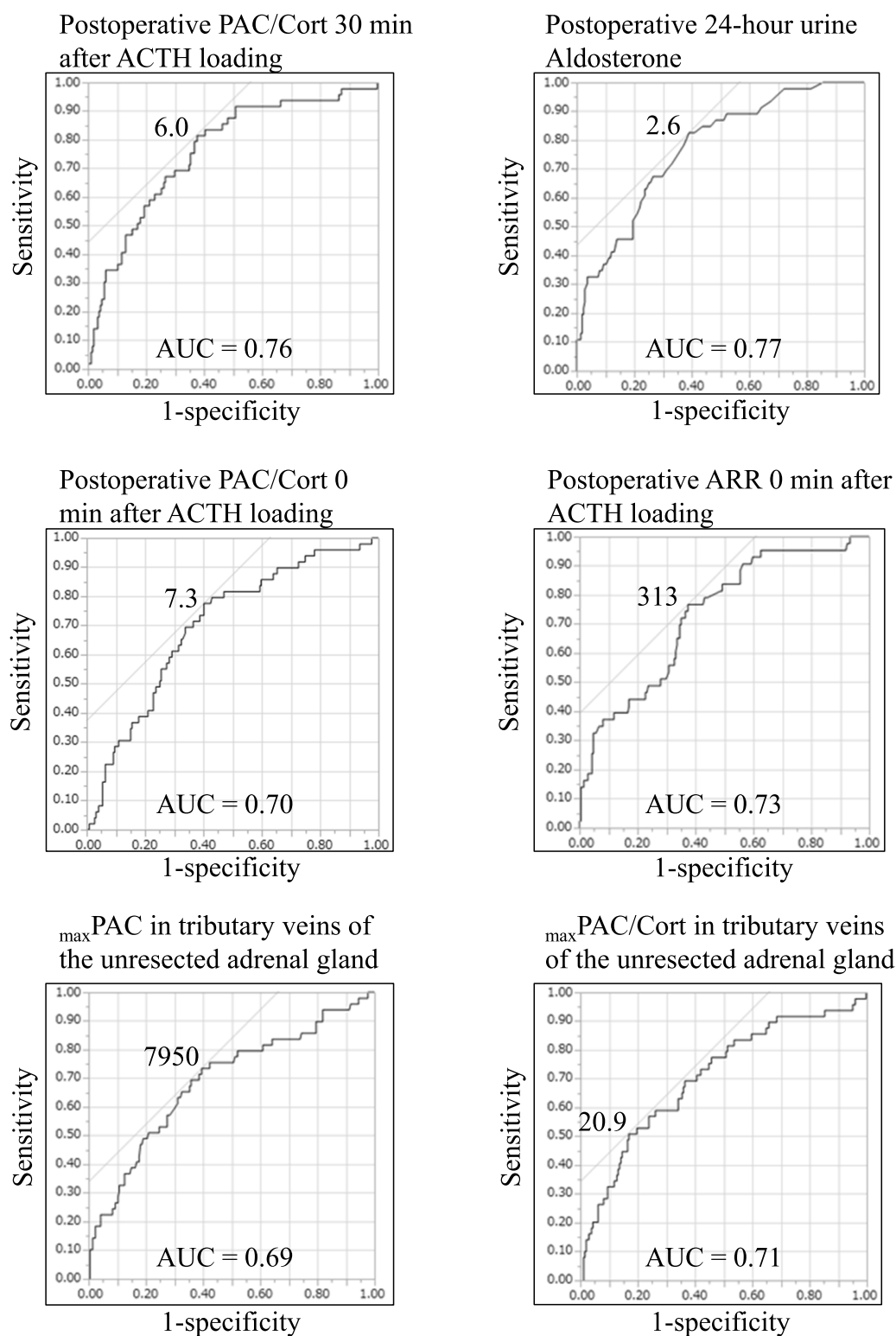
**Table 2. Comparison of parameters of ACTH stimulation and oral salt loading tests immediately after surgery between biochemically complete and incomplete groups**

Postoperative loading test parameter	Biochemically complete (n = 219)	Biochemically incomplete (n = 49)	P-value <sup>a</sup>
ACTH stimulation test			
PRA at 0 minutes (ng/mL/hr)	0.3 (0.1-0.5)	0.2 (0.1-0.3)	.004
PAC at 0 minutes (pg/mL)	68 (51-90)	95 (75-120)	<.001
PAC at 30 minutes (pg/mL)	94 (71-145)	171 (119-230)	<.001
PAC at 60 minutes (pg/mL)	102 (75-148)	167 (119-223)	<.001
$\text{maxPAC}$ after ACTH loading (pg/mL)	105 (80-155)	171 (128-234)	<.001
ARR at 0 minutes	230 (123-540)	475 (313-1170)	<.001
PAC/Cort at 0 minutes	6.6 (4.7-9.2)	9.2 (7.3-12.4)	<.001
PAC/Cort at 30 minutes	5.2 (3.9-7.7)	8.9 (6.3-11.8)	<.001
PAC/Cort at 60 minutes	4.8 (3.5-6.8)	7.8 (5.6-10.8)	<.001
$\text{maxPAC/Cort}$ after ACTH loading	7.0 (5.4-9.6)	10.6 (8.1-13.2)	<.001
24-hour urine aldosterone (μg/day)	2.0 (1.0-3.7)	4.4 (2.8-7.5)	<.001
Preoperative DDD of MRA	1 (1-2)	1 (1-2)	.38
Number of days from the last MRA dose to the test (days)	7 (7-8)	7 (7-8)	.95

Data are presented as median (interquartile range).

Abbreviations: ARR, plasma aldosterone concentration to plasma renin activity ratio; DDD, defined daily dose;  $\text{maxPAC/Cort}$ , maximum plasma aldosterone concentration to serum cortisol ratio; MRA, mineralocorticoid receptor antagonist; PAC, plasma aldosterone concentration; PAC/Cort, plasma aldosterone concentration to serum cortisol ratio; PRA, plasma renin activity.

<sup>a</sup>P-value for differences between the biochemically complete and incomplete success groups.



**Figure 1.** Receiver operating characteristic curves for biochemically complete success of the remarkable parameters.

Abbreviations: ARR, plasma aldosterone concentration to plasma renin activity ratio; AUC, area under the curve;  $\max$  PAC, maximum plasma aldosterone concentration;  $\max$  PAC/Cort, maximum plasma aldosterone concentration to serum cortisol ratio; PAC, plasma aldosterone concentration; PAC/Cort, plasma aldosterone concentration to serum cortisol ratio.

correlation between both indices was analyzed. As a result, PAC/Cort at 30 minutes ( $r = 0.47$ ,  $P < .0001$ ) and 24-hour urine aldosterone ( $r = 0.35$ ,  $P < .0001$ ) had a significant positive correlation with  $\max$  PAC/Cort after ACTH loading in the

tributary veins of the unresected adrenal gland. PAC at 0 minutes ( $r = 0.37$ ,  $P < .0001$ ) and ARR at 0 minutes ( $r = 0.18$ ,  $P = .0041$ ) also had a positive correlation with this index from sAVS (Fig. 2).



Preoperative Characteristics of C-suc and C-abs Groups

A total of 198 and 70 patients were classified into the C-suc and C-abs groups, respectively. In the C-suc group, 88 patients showed complete success, and 110 patients achieved partial success. The C-abs group had significantly lower systolic and diastolic blood pressure, ARR, largest nodule size, and LI and a significantly higher percentage of men, body mass index, serum creatinine level, serum potassium level, PRA, CR, and  $\text{maxPAC}$  and  $\text{maxPAC/Cort}$  after ACTH loading in the tributary veins of the unresected adrenal gland than the C-suc group [Supplementary Table S1 (11)].

Comparison of Parameters of AST and OST Immediately After Surgery Between C-suc and C-abs Groups

Immediately after surgery, the C-abs group showed significantly higher PAC at 30 minutes, 60 minutes,  $\text{maxPAC}$ , and PAC/Cort at 30 minutes after ACTH loading and 24-hour urine aldosterone than the C-suc group [Supplementary Table S2 (11)].

ROC Curves for Clinically Absent Success

Supplementary Fig. S2 (11) shows the ROC curves for clinically absent success of various parameters in postoperative AST and OST. The best predictor among the parameters of the postoperative loading tests was 24-hour urine aldosterone ( $\text{AUC} = 0.69$ ). By loading ACTH, the AUC value significantly increased (PAC/Cort at 0 minutes vs 30 minutes,  $P = .004$ ). Supplementary Fig. S3 (11) shows the ROC curves for clinically absent success in parameters available preoperatively.

Discussion

This study shows that various parameters of AST and OST immediately after surgery for PA were associated with biochemical outcomes based on the PASO criteria 1 year after surgery. PAC/Cort at 30 minutes in AST and 24-hour urine aldosterone in OST were particularly good predictors. The parameters of the postoperative loading tests could predict clinical outcomes. Among them, 24-hour urine aldosterone in OST had a relatively good predictive ability.

In the present study, PAC/Cort at 30 minutes in AST and 24-hour urine aldosterone in OST immediately after surgery were particularly good predictors of biochemical outcomes. In a recent study published by a group from Germany studying 100 patients with PA, the aldosterone levels after ACTH stimulation immediately after surgery were found to predict biochemical outcomes (8). Our study demonstrated that similar results could be achieved in a larger cohort of patients, including Asians who often carry the KCNJ5 mutation (24). Additionally, our research showed that both AST and OST were useful. The cases in our study had a lower LI (median 6.3, interquartile range 2.1 to 16.5) as compared to that previously reported (median 14.22, interquartile range 5.9 to 30.7) (8), including those considered bilateral PA by criteria using LI. Recent reports have suggested the efficacy of surgical treatment for bilateral PA (25), indicating that surgery may become more common in severe cases of bilateral PA. In such cases, the early administration of MRA may be necessary to address aldosterone secretion from residual lesions.

**Table 3. The cut-off values, sensitivity, and specificity of various parameters of postoperative ACTH stimulation and oral salt loading tests with the best combination of sensitivity and specificity for biochemical complete success**

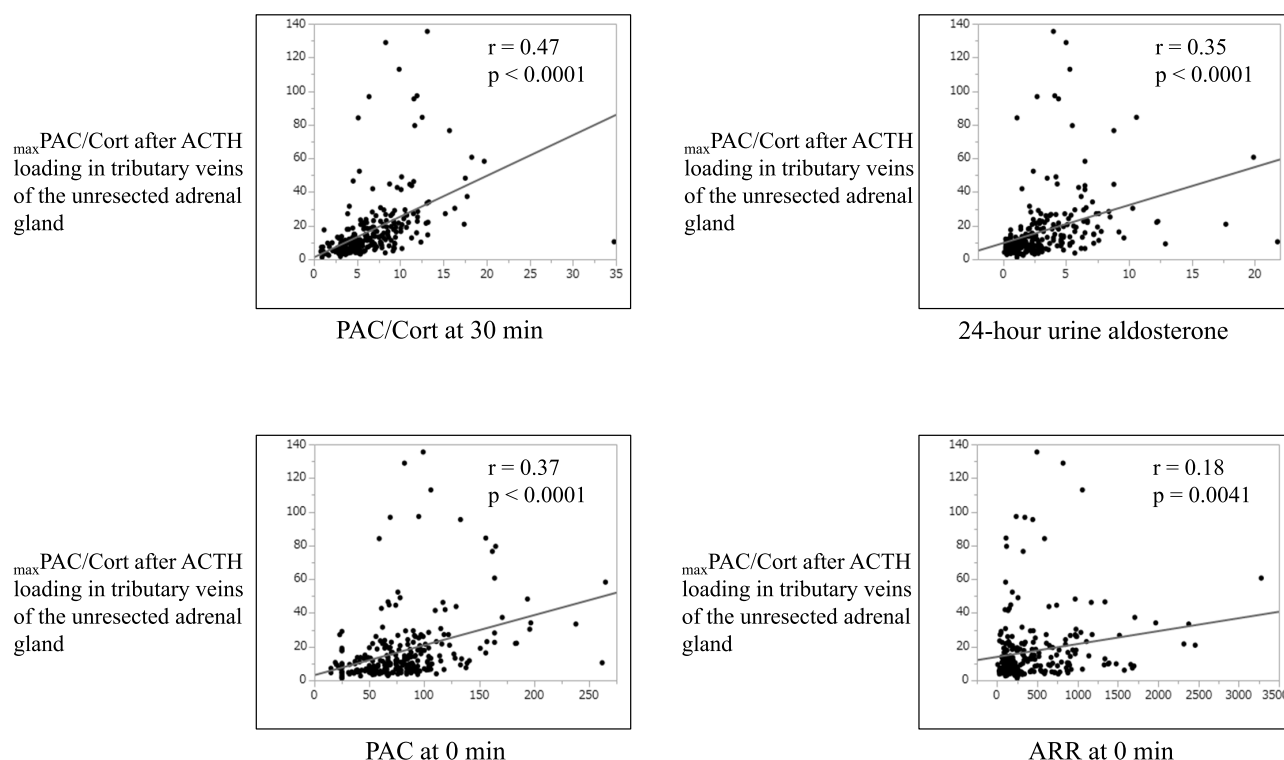
Postoperative loading test parameter	Cut-off values with the best combination of sensitivity and specificity (sensitivity/specificity)
ACTH stimulation test (%)	
PRA at 0 minutes (ng/mL/hr)	0.2 (67/53)
PAC at 0 minutes (pg/mL)	70 (84/54)
PAC at 30 minutes (pg/mL)	95 (90/50)
PAC at 60 minutes (pg/mL)	95 (92/46)
$\text{maxPAC}$ after ACTH loading (pg/mL)	115 (84/55)
ARR at 0 minutes	313 (77/63)
PAC/Cort at 0 minutes	7.3 (78/60)
PAC/Cort at 30 minutes	6.0 (82/63)
PAC/Cort at 60 minutes	5.5 (82/64)
$\text{maxPAC/Cort}$ after ACTH loading	8.1 (78/64)
24-hour urine aldosterone	2.6 (83/61)

Abbreviations: ARR, plasma aldosterone concentration to plasma renin activity ratio;  $\text{maxPAC/Cort}$ , maximum plasma aldosterone concentration to serum cortisol ratio; PAC, plasma aldosterone concentration; PAC/Cort, aldosterone concentration to serum cortisol ratio; PRA, plasma renin activity.

Therefore, the inclusion of a significant number of bilateral cases in the present study underscores its significance.

Furthermore, this study found that the parameters in the postoperative loading tests immediately after surgery had a better predictive ability than the parameters before loading. Autonomous production of aldosterone is usually observed in PA, while endogenous ACTH is known to be 1 of the main regulators of aldosterone-synthesizing enzymes in PA patients with suppressed renin and angiotensin levels because patients with PA always show hyperresponsiveness to PAC in AST and diurnal/daily variations of PAC (26). The lower aldosterone levels in AST in the B-com group than in the B-inc group suggest that PAC hyperresponsiveness to ACTH diminishes early postoperatively. One possible explanation for why the aldosterone response in AST and urinary aldosterone levels in OST have better predictive abilities for biochemical outcomes than preload parameters is that the parameters can be influenced by diurnal/daily variations caused by endogenous ACTH and other factors (27). The results of the present study suggest the importance of considering the influence of ACTH in the evaluation of aldosterone secretory capacity in the immediate postoperative period.

As shown in Fig. 2, the aldosterone secretory capacity in the immediate postoperative loading test was significantly and positively correlated with aldosterone levels in the unresected adrenal glands. This result is reasonable as the aldosterone secretory capacity after surgery depends on that of the residual adrenal gland. Indices from the postoperative loading test were superior to those from AVS when comparing the predictive ability of various factors for biochemical outcomes. The AVS indices indicated local aldosterone levels in the adrenal vein. In contrast, the indices of the immediate postoperative loading tests indicated aldosterone levels in the peripheral blood and urine after systemic distribution and metabolism. Therefore, the latter may be strongly correlated with the



**Figure 2.** Correlation analyses between  $\text{max PAC/Cort}$  after ACTH loading in tributary veins of the unresected adrenal gland and parameters of ACTH stimulation and oral salt loading tests immediately after surgery.  $r$  indicates correlation coefficient.

Abbreviations: ARR, plasma aldosterone concentration to plasma renin activity ratio;  $\text{max PAC}$ , maximum plasma aldosterone concentration;  $\text{max PAC/Cort}$ , maximum plasma aldosterone concentration to serum cortisol ratio; PAC, plasma aldosterone concentration; PAC/Cort, plasma aldosterone concentration to serum cortisol ratio.

biochemical outcome of PASO, which also uses aldosterone levels in the peripheral blood and urine for biochemical evaluation. However, the AVS indices and the indices obtained from the immediate postoperative loading tests have different roles as AVS indices are important indicators of the need for surgery, and the indices obtained from the immediate postoperative loading tests would be indicators of more precise validation of biochemical outcomes early after surgery.

Some parameters of the immediate postoperative AST and OST also predicted clinically absent success 1 year after surgery. There are many reports on predictors of clinical outcome preoperatively, but fewer reports on predictors of clinical outcome of immediate postoperative parameters exist. In PA, blood pressure typically normalizes or shows maximum improvement between 1 and 6 months after surgery but can continue to fall up to 1 year after surgery (4). Based on the results of this study, it may be possible to predict at an early postoperative period whether antihypertensive medication can be reduced.

This study had a few limitations. First, the rate of biochemical complete success in this study was relatively low because bilateral PA was not excluded. A consensus can be achieved in the future for the surgical treatment of bilateral PA based on further investigation (25). Second, MRAs were administered until the day before surgery, and the number of days between the date of surgery and the date the loading test was performed varied, which may have affected the PRA and PAC values. However, there was no difference between the B-com and B-inc groups and C-suc and C-abs groups in the defined daily dose of MRA taken before surgery or the number of days between the date of surgery and the date of the loading tests.

Third, the possibility of bias owing to unknown confounding factors cannot be ruled out. Fourth, this was a single-center, retrospective study. Multicenter prospective studies with uniform protocols and measurement systems may reveal more accurate associations.

In conclusion, aldosterone levels in the ACTH stimulation test and the 24-hour urine aldosterone levels in the OST immediately after surgery were highly accurate predictors of biochemical outcome 1 year after surgery. By performing these tests in the early postoperative period, we can gather valuable information that may help determine the initiation of MRAs.

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## Disclosures

None.

## Data Availability Statement

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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