Parameters of captopril challenge test can predict results of other confirmatory tests for primary aldosteronism and propose the next test to be done

Naohisa Tamura1,2, Kanako Yamada2, Hiroshi Hatakeyama2, Mizuki Torii-Hanakita2, Chika Kyo2, Rieko Kosugi2,3, Takako Yonemoto2,3, Tatsuo Ogawa2, Masato Kotani2, Takeshi Usui1,3 and Tatsuhide Inoue2

1) Research Support Center, Shizuoka General Hospital, Shizuoka 420-8527, Japan
2) Center for Diabetes, Endocrinology and Metabolism, Shizuoka General Hospital, Shizuoka 420-8527, Japan
3) Department of Medical Genetics, Shizuoka General Hospital, Shizuoka 420-8527, Japan

Abstract. In Japan, primary aldosteronism (PA) is diagnosed if any one of the captopril challenge test (CCT), saline infusion test (SIT), furosemide-upright test (FUP), and oral salt-loading test (OST) is positive. The present study aimed to investigate if parameters of CCT, the safest confirmatory test, could predict decisions of other tests and propose the next test to diagnose PA in CCT-negative patients. In a cross-sectional design, 142 patients, who were referred to our hospital for the scrutiny of PA and underwent at least two confirmatory tests, were enrolled. While 123 patients underwent all of the CCT, SIT, and FUP, the OST was successfully done in only six patients and excluded from further analyses. CCT parameters showing correlations of higher degrees with SIT and FUP parameters were selected, and their powers to predict SIT and FUP decisions were investigated by receiver operating characteristic analyses. Proposals of the next test based on the CCT parameters were validated with SIT and FUP decisions in subsets of CCT-negative patients divided by cut-offs of the CCT parameters. The plasma aldosterone concentration and plasma renin activity 60 min after the load of CCT (CCT60-PAC and CCT60-PRA) were selected, and CCT60-PAC ≤59.0 pg/mL and CCT60-PRA ≥1.05 ng/mL/h could predict negativities of SIT and FUP, respectively, with >95% specificities. Based on the validation, the present study suggested the SIT as the next test to be done if the CCT-negative patient belonged to the subset with CCT60-PAC >59.0 pg/mL and CCT60-PRA ≥1.05 ng/mL/h, otherwise the FUP should be selected.

Key words: Primary aldosteronism, Diagnosis, Confirmatory tests, Relation, Captopril challenge test

PRIMARY ALDOSTERONISM (PA) is one of common forms of secondary hypertension, which represents 5–10% of all hypertensive patients [1, 2]. The diagnosis of PA is important, because patients with PA have higher cardiovascular morbidity and mortality than patients with essential hypertension (EHT) where blood pressure, age, and sex are matched, and because specific treatments to ameliorate the consequences of PA are available [3-8]. The consensus statement on the clinical practice of PA in Japan, which was prepared by the Japan Endocrine Society (JES) and the Japan Association of Endocrine Surgeons, and endorsed by the Japanese Society of Hypertension, recommends us to use the captopril challenge test (CCT), salt infusion test (SIT), furosemide-upright test (FUP), and oral salt-loading test (OST) as confirmatory tests to diagnose PA [9]. The SIT is most widely used in the world, but causes severe arrhythmia or exaggeration of heart failure in a subset of patients [10]. The CCT is also widely used in Europe and Japan, which is the safest confirmatory test for PA, unless rare complications such as angioedema occur [10]. The FUP is used only in Japan, and is contraindicated for patients at high risk for cerebrovascular events due to advanced atherosclerosis [10]. The OST also may cause severe arrhythmia or exaggeration of heart failure [10]. It has not yet been established which is the most reliable test to diagnose PA [9]. Relations among results of different confirmatory tests also have not been clarified well. Since PA can be diagnosed if any one of the four confirmatory tests is positive following the consensus statement [9], we cannot completely deny PA until all of the four tests have been confirmed to be negative. This might be
problematic for endocrinologists to diagnose PA for patients referred from general practitioners.

The aldosterone secretion is suppressible by sodium (Na) load in patients with EHT, but not in PA patients due to autonomous aldosterone secretion; this is the rationale of SIT and OST [11]. PRA is increased by the stimulation of furosemide iv load and upright posture in patients with EHT, but it is extensively suppressed by the excess of aldosterone and not increased by the renin stimulation in PA patients; this is the rationale of FUP [12]. The CCT examines both of the autonomous aldosterone secretion and the poor renin response to the loss of negative feedback from hyperaldosteronism in PA patients [13], suggesting the possibility that results of SIT and FUP might be predicted by parameters of CCT. The consensus statement on PA in Japan recommends the CCT as the confirmatory test that would be done first [9]. If we could detect SIT-negative or FUP-negative patients by parameters of CCT with high specificity, we could select the confirmatory test that should be done next to the CCT for CCT-negative patients based on the parameters. We investigated on these issues in the present study.

Materials and Methods

Study design and patients

The present study was a cross-sectional study. The study flow chart was shown in Fig. 1. Patients, who admitted or attended to the Center for Diabetes, Endocrinology and Metabolism, Shizuoka General Hospital from April 1, 2012 to December 31, 2017 and were diagnosed with or suspected as PA, were invited to the study. Inclusion criteria were: 1) patients for whom at least two of the CCT, SIT, FUP, and OST were performed, 2) patients who did not refuse to be included in the study upon information disclosure of the study on the Web site of Shizuoka General Hospital. Exclusion criteria were: 1) patients for whom less than two of the confirmatory tests were performed, 2) patients who refused to be included in the study upon information disclosure of the study. Patients who matched both of inclusion criteria and did not match both of exclusion criteria were included in the study.

In the study period of present study, in our hospital, the CCT, SIT, and FUP were essentially planned to be performed in a series for all PA-suspected patients in daily clinical practice, while the OST was optional. Confirmatory tests that should be avoided as judged by the JES PA guidelines in 2009 [10] or had been performed before the referral to our hospital were not performed. Data of confirmatory tests performed in other institutions...
were excluded. Anti-hypertensive (HTN) medications were not changed during confirmatory testing.

The protocol of this study has been approved by the Clinical Research Ethics Committee of Shizuoka General Hospital (SGHIRB#2017027), and performed in accordance with the Code of Ethics of the World Medical Association (1964 Declaration of Helsinki and its later amendments). This study has been registered in the UMIN Clinical Trials Registry (UMIN-CTR) as UMIN000037304.

**Data collection and measurements**

Data of plasma aldosterone concentration (PAC, pg/mL), plasma renin activity (PRA, ng/mL/h), and aldosterone/renin ratio (ARR, pg/mL over ng/mL/h) were collected before, and 60 and 90 min after the po load of 50-mg captopril in the CCT (CCT0, CCT60, and CCT90), after the drip iv load of 2-L saline over 240 min at the supine position in the SIT (post-SIT), and after 120-min standing or walking following the iv load of 40-mg furosemide in the FUP (post-FUP). Data of urinary aldosterone excretion in the OST were also collected. Data of CCT60-PAC/CCT0-PAC ratio in percent (CCT60-%PAC) were also collected. Basal PAC and PRA values, which were measured in blood samples withdrawn from veins of participants after 30-min bed-rest in the supine position, were also collected. Values of PAC and urinary aldosterone concentration were measured by a radioimmunoassay (RIA) with the SPAC-S Aldosterone RIA kit (Fuji Rebio, Co., Ltd., Tokyo, Japan). Values of PRA were measured by a RIA with the PRA-FR RIA kit (Fuji Rebio, Co., Ltd., Tokyo, Japan). Values of estimated glomerular filtration rate (eGFR) from serum creatinine in Japan were calculated by the revised equations for eGFR from serum creatinine [14].

Diagnostic criteria of confirmatory tests for PA were determined following the JES PA guidelines in 2009 and the consensus statement on the clinical practice of PA in Japan [9, 10]: 1) CCT, CCT60-ARR or CCT90-ARR >200 pg/mL over ng/mL/h; 2) SIT, post-SIT-PAC >60 pg/mL; 3) FUP, post-FUP-PRA <2 ng/mL/h; 4) OST, urinary aldosterone excretion ≥8 μg/day where urinary Na excretion was ≥170 mEq/day. Patients were diagnosed with PA if the result of any one of the four tests fulfilled diagnostic criteria described above. CCT60-PAC or CCT90-PAC >120 pg/mL is included as another diagnostic criterion of CCT in Japan [9, 10]. In an Endocrine Society clinical practice guideline for PA, the decision criterion of CCT is that CCT60-%PAC values or ratios of PAC 120 min after the load of CCT to CCT0-PAC in percent was ≥70% [15]. We did not use these criteria to decide CCT results in the present study, but they were included in the analysis. Where the diagnosis of PA had been confirmed and the patient wished surgical treatments if applicable, the laterality of PA was determined by the adrenal venous sampling (AVS) with cosyntropin loading, following the standard conditions [9]. The AVS was assessed as successfully done if selectivity indexes, which were adrenal vein to inferior vena cava ratios of plasma cortisol concentrations, were greater than 5 for both adrenal veins after cosyntropin loading. PA was determined as unilateral where the lateralizing ratio, which was calculated by dividing the PAC/cortisol ratio of the dominant adrenal vein by that of recessive adrenal vein, was greater than 4 [9].

**Statistical analyses**

Because the D’Agostino and Pearson test revealed that parameters showed neither normal nor log-normal distributions except for 1/post-FUP-PRA and CCT60-%PAC values showing log-normal distributions (Supplementary Fig. 1), correlations among PAC, 1/PRA, ARR, and %PAC values were tested with the Spearman’s rank correlation analysis. The significance of differences between medians was determined by the Mann-Whitney U test. With ARR values, we compare 1/PRA values instead of PAC values, since ARR is calculated by dividing PAC by PRA. Receiver operating characteristic (ROC) analyses were performed to examine if a parameter of one confirmatory test could predict results of other confirmatory tests. Optimal cut-offs were determined as the Youden index or positive likelihood ratio (LR+, the ratio of a positive test result among participants with a particular condition, PA in the present study, to the same result in those without the condition) was maximized [16]. Contingency tables were analyzed with the Fisher’s exact test of independence. Statistical analyses were performed with GraphPad Prism Version 8.1.1 (GraphPad Software, Inc., San Diego, CA), with $p < 0.05$ indicating statistical significance.

**Results**

**Participants**

One hundred forty two patients were invited, and all of them were enrolled. Their characteristics at the first visit to our hospital are shown in Table 1. Blood pressure control was not sufficient with a small number of anti-HTN drugs, which were mainly calcium channel blockers (CCBs) and angiotensin II type 1 receptor antagonists (ARBs). We tried to stop anti-HTN drugs other than CCBs and α blockers before the screening of PA, but 28, ten, nine, and one patients were still taking ARBs, diuretics, β blockers, and central α2 agonists, respectively at the screening. Before the confirmatory testing, number of patients taking ARBs, diuretics, β blockers, and central...
α₂ agonists further decreased to seven, one, four, and one, respectively. While the number of patients prescribed with potassium (K) supplements was only three at the first visit to our hospital, it increased to eight at the screening and twelve before the confirmatory testing to adjust plasma K levels. The majority of patients did not show impaired eGFR. In significant number of patients, although basal ARR values were >200 pg/mL over ng/ml/h at general practitioners’ offices, those values were ≤200 pg/mL over ng/ml/h at our hospital. One hundred twenty eight (90.1%) of the 142 patients were clinically diagnosed with PA by the positivity of any of the confirmatory tests performed. However, all of the four confirmatory tests were performed in only four patients (Fig. 1), and the OST was not performed in patients negative for all of the CCT, SIT, and FUP. It was possible that some patients clinically diagnosed with non-PA in the present study were PA patients. So, we presented the characteristics without dividing into PA and non-PA. The AVS was performed in 54 PA patients, and eleven patients were diagnosed with unilateral PA.

**Implementation statuses and decisions of confirmatory tests**

The CCT and SIT were performed in 137 and 134 patients without adverse events, and their positive rates were 59.1% and 45.5%, respectively (Supplementary Table 1A). The FUP was tried in 139 patients, but was interrupted by blood pressure drop or bad feeling in four patients; the FUP-positive rate in the remaining 135 patients was 82.2% (Supplementary Table 1A). Decision concordance rates were 70.0% between CCT and FUP, 53.4% between CCT and SIT, and 50.8% between SIT and FUP (Supplementary Table 1B). In 123 patients, all of the CCT, SIT, and FUP were successfully performed. The positive rate of each test was almost not affected by including only these 123 patients in the analysis (Fig. 2). Eleven (8.9%) of the 123 patients were negative for all of the three tests (Fig. 2). In the 123 patients, while patients positive only for the CCT or SIT were rare, 14.6% of the patients were positive only for the FUP (Fig. 2). By contrast, the OST was carried out in eleven patients without adverse events, but only six patients fulfilled the condition of urinary Na excretion that ensured sufficient Na load [10]. The OST was excluded from following analyses, because the number of patients, for whom the test could be successfully done, was too small.

**Table 1** Characteristics of participants at the first visit to our hospital

| Participants | 142 |
|-------------|-----|
| Sex (F/M)   | 84/58 |
| Age (yr)    | 52.6 (11.7) |
| Height (cm) | 160.0 [155.0, 169.2] |
| Weight (kg) | 61.0 [52.7, 75.5] |
| Body mass index (kg/m²) | 23.9 [21.2, 27.0] |
| Systolic blood pressure (mmHg) | 148 [133, 161] |
| Diastolic blood pressure (mmHg) | 90 [78, 101] |
| Number of anti-HTN drugs* | 1 [0, 2] |
| Participants prescribed with CCBs or α blockers | 95 (66.9%) |
| Participants prescribed with ARBs | 39 (27.5%) |
| Participants prescribed with BBs or Central α₂ agonists | 12 (8.5%) |
| Diuretics | 12 (8.5%) |
| Participants prescribed with K supplements | 3 (2.1%) |
| eGFR (mL/min/1.73 m²) | 87.4 (23.8) |
| Plasma K concentration (mEq/L) | 3.66 (0.51) |
| Basal PAC (pg/mL) | 135.0 [103.0, 186.0] |
| Basal PRA (ng/mL/h) | 0.50 [0.30, 0.70] |
| Basal ARR (pg/mL over ng/mL/h) | 305.0 [188.8, 510.0] |

For parameters that normally distribute, data are shown in the form of “mean (SD)”. For parameters that do not normally distribute, data are shown in the form of “median [25, 75 percentiles]”. Numbers of participants prescribed with anti-HTN drugs or K supplements are shown with percentages to all participants in parentheses. Basal values of PAC, PRA, and ARR are those at the screening of PA in our hospital. *The number of anti-HTN drug was calculated by dividing the dose, at which the drug was used, by its normal dose. Abbreviations: CCB, calcium channel blocker; ARB, angiotensin II type 1 receptor antagonist; BB, β blocker; eGFR, estimated glomerular filtration rate.

**Fig. 2** Classification of patients by decisions of the CCT, SIT, and FUP. Classification of 123 patients, for whom all of the CCT, SIT, and FUP were performed, by decisions of the three tests is shown in a Venn diagram. The number of patients in each subset is shown with percent of total in a parenthesis. One hundred twelve patients were positive for at least one test, and 85 patients were positive for at least two tests.
Relations of parameters among the CCT, SIT, and FUP

CCT90-ARR, CCT90-PAC, and 1/CCT90-PRA values showed positive correlations of high degrees with CCT60-ARR ($\rho = 0.903$), CCT60-PAC ($\rho = 0.924$) and 1/CCT60-PRA values ($\rho = 0.922$), respectively (Supplementary Fig. 2). We, therefore, used the latter parameter set in following analyses. With post-SIT-PAC values, CCT60-ARR values showed a positive correlation of medium degree (Fig. 3A), but CCT60-PAC values showed a positive correlation of high degree (Fig. 3B). With 1/post-FUP-PRA values, both of CCT60-ARR and 1/CCT60-PRA values showed positive correlations of medium degrees (Fig. 3C and D); the coefficient of correlation in the latter combination was a little bit higher than that in the former combination. CCT60-%PAC values showed a positive correlation of low degree with post-SIT-PAC values ($\rho = 0.200$), but no significant correlation with 1/post-FUP-PRA values (not shown).

Therefore, we selected CCT60-PAC and CCT60-PRA as CCT parameters to predict decisions of SIT and FUP, respectively. No significant correlations were observed between post-SIT-PAC and 1/post-FUP-PRA values (Fig. 3E). Patients with higher CCT60-ARR values tended to show higher CCT60-PAC values (Fig. 3F).

Predicting SIT decisions by CCT parameters

CCT60-PAC values in SIT-positive patients were significantly greater than those in SIT-negative patients (Fig. 4A). The ROC analysis revealed that CCT60-PAC values could differentiate SIT-positive patients from SIT-negative patients (Fig. 4B). Cut-offs of CCT60-PAC maximizing the Youden index and LR+ were >82.4 pg/mL (91.2% sensitivity, 70.8% specificity) and >141.0 pg/mL (38.6% sensitivity, 98.6% specificity), respectively (Fig. 4A and B, Supplementary Table 2A). In addition to the ARR-based criterion, the JES PA guidelines in 2009 and the consensus statement on PA in Japan have PAC-based diagnostic criterion of CCT: CCT60-PAC or CCT90-PAC >120 pg/mL [9, 10]. CCT60-PAC >120 pg/mL could detect SIT-positive patients with 50.9% sensitivity and 91.7% specificity (Fig. 4A and B, Supplementary Table 2A).

Among 56 ARR-based CCT-negative (CCT60-ARR and CCT90-ARR ≥200 pg/mL over ng/mL/h) patients, seven patients were PAC-based CCT-positive (Supplementary Table 3). Among them, one patient showed a CCT60-PAC value >141.0 pg/mL (Fig. 3F). In this patient, a basal PRA value was 1.0 ng/mL/h, but CCT0-PRA and CCT60-PRA values were 3.5 and 33 ng/mL/h, respectively. The results of CCT fulfilled the screening criteria of renovascular hypertension (RVH): CCT60-PRA and the absolute increase from CCT0-PRA to CCT60-PRA were greater than 12 and 10 ng/mL/h, respectively [17]. Although renal artery stenosis was not indicated by renal artery ultrasound, this patient appeared to be a patient with secondary aldosteronism. However, the CCT was performed in ten of the eleven AVS-confirmed unilateral PA patients, and eight of the ten patients belonged to the subset, in which the CCT was positive by both of ARR-based and PAC-based criteria (Fig. 3F).

On the other hand, the smallest cut-off of CCT60-PAC that gives >95% sensitivity (actually 96.5%) to detect SIT-positive patients was >59.0 pg/mL (Fig. 3A and B, Supplementary Table 2A), suggesting that CCT60-PAC ≤59.0 pg/mL could detect SIT-negative patients with 96.5% specificity.

Predicting FUP decisions by CCT parameters

CCT60-PRA values in FUP-positive patients were significantly smaller than those in FUP-negative patients (Fig. 4C). The ROC analysis revealed that CCT60-PRA values could differentiate FUP-positive patients from FUP-negative patients (Fig. 4D). Cut-offs of CCT60-PRA maximizing the Youden index and LR+ were <0.65 ng/mL/h (84.0% sensitivity, 69.6% specificity) and <0.55 ng/mL/h (77.4% sensitivity, 73.9% specificity), respectively (Fig. 3C and D, Supplementary Table 2B).

On the other hand, the largest cut-off of CCT60-PRA that gives >95% sensitivity (actually 95.3%) to detect FUP-positive patients was <1.05 ng/mL/h (Fig. 4D, Supplementary Table 2B), suggesting that CCT60-PRA ≥1.05 ng/mL/h could detect FUP-negative patients with 95.3% specificity.

SIT and FUP decisions in subsets of ARR-based CCT-negative patients

Among the 123 patients, for whom all of the CCT, SIT, and FUP were performed, 47 patients were ARR-based CCT-negative. As shown in Table 2, they were divided into four subsets: A) CCT60-PAC ≤59.0 pg/mL and CCT60-PRA ≥1.05 ng/mL/h, B) CCT60-PAC ≤59.0 pg/mL and CCT60-PRA <1.05 ng/mL/h, C) CCT60-PAC >59.0 pg/mL and CCT60-PRA ≥1.05 ng/mL/h, and D) CCT60-PAC >59.0 pg/mL and CCT60-PRA <1.05 ng/mL/h. For each subset, we investigated positive and negative rates of SIT and FUP to validate the power of CCT parameters to select the next test to be performed. Decision concordance rates of SIT and FUP were not so high in all subsets, and the rate was the lowest in subset C. In the subset B, for which only the SIT was predicted to be negative, 90.0% of patients were actually SIT-negative, and 60.0% of them were FUP-positive (Table 2). In subset C, for which only the FUP was predicted to be negative, 66.7% of patients were...
Fig. 3  Relations between parameters of different confirmatory tests. Correlations of CCT60-ARR (A) and CCT60-PAC (B) values with post-SIT-PAC values, and those of CCT60-ARR (C) and 1/CCT60-PRA (D) values with 1/post-FUP-PRA values are shown. The correlation between post-SIT-PAC and 1/post-FUP-PRA values (E) and the relation between CCT60-PAC and CCT60-ARR values (F) are also shown. Post-SIT-PAC of 60 pg/mL, CCT60-ARR of 200 pg/mL over ng/mL/h, CCT60-PAC of 120 pg/mL, and post-FUP-PRA of 2 ng/mL/h (1/post-FUP-PRA of 0.5) are indicated by dotted lines. In panels A-E, numbers of patients analyzed and Spearman’s rank correlation coefficients (\( \rho \)) with 95% confidence intervals in parentheses are shown. In panel F, data of patients with AVS-confirmed unilateral PA, those with AVS-confirmed bilateral PA, and those, for whom the AVS was not performed, are shown in red triangles, blue squares, and black closed circles, respectively. CCT60-PAC of 141.0 pg/mL is indicated with a dashed line. An arrow indicates one ARR-based CCT-negative patient with CCT60-PAC >141.0 pg/mL. Because one patient, for whom all of the CCT, SIT, and FUP were performed, lacked CCT60-PRA and CCT60-ARR values, numbers of patients in panels A, C, D, and F were one less than those shown in Supplementary Table 1.
actually FUP-negative, and 77.8% of them were SIT-positive (Table 2). In subset A, for which both of the SIT and FUP were predicted to be negative, all patients were actually SIT-negative, but half of them were FUP-positive. In subset D, for which neither the SIT nor FUP was predicted to be negative, 61.5% of patients were SIT-negative, and 76.9% of them were FUP-positive; SIT-negative and FUP-positive rates were similar to those in all ARR-based CCT-negative patients (Table 2).

**Discussion**

**Predicting SIT decisions by CCT parameters**

In the present study, the degree of correlation between
CCT and SIT was low (Supplementary Table 1B). How‐ever, CCT60-PAC value >141.0 pg/mL appeared to be a

ever, CCT60-PAC values showed a positive correlation

might be able to enrich unilateral PA patients. The cut‐off

was poor.

Where the post-CCT-PAC cut-off was set to >120 pg/mL, an alternative diagnostic criterion of CCT in

Japan [9, 10], about half of ARR-based CCT-positive

patients could be detected with moderately high speci‐cifi‐city (Supplementary Table 3). This cut-off could detect

also half of SIT-positive patients with >90% specificity (Fig. 4A and B, Supplementary Table 2A). Taken together,

CCT60-PAC >120 pg/mL would be suitable as an alterna‐tive diagnostic criterion of CCT. In the present study,

the majority of unilateral PA patients were CCT-positive

by both of ARR-based and PAC-based criteria (Fig. 3F).

Using these two diagnostic criteria in combination

might be able to enrich unilateral PA patients. The cut‐off

maximizing the LR+ (>141.0 pg/mL) could predict

SIT-positivity with almost 100% specificity (Supplemen‐tary Table 2A). However, we should be careful of using solely post-CCT PAC as a decision criterion of CCT, because one ARR-based CCT-negative patient with a

CCT60-PAC value >141.0 pg/mL appeared to be a patient with secondary aldosteronism in the present study (Fig. 3F). RVH should be ruled out where either basal or CCT0-PRA values are >3 ng/ml/h [18].

Predicting FUP decisions by CCT parameters

The positivity of FUP could be predicted by CCT60-

PRA values, and the decision concordance between the

CCT and FUP was moderately high. However, the power

of CCT60-PRA values to detect FUP-positivity was not

so good (Fig. 4D). A significant portion of the patients

were positive only for the FUP, while patients positive

only for the CCT or SIT were rare (Fig. 2). These observa‐
tions suggested that clinical characters of FUP-positive

patients might be unique compared with those of patients

positive for other confirmatory tests.

Proposal of the order of remaining confirmatory
tests by CCT parameters

The present study indicated that CCT60-PAC ≤59.0 pg/mL and CCT60-PRA ≥1.05 ng/mL/h could detect SIT-negative and FUP-negative patients, respectively, with >95% specificities (Fig. 4B and D, Supplementary Table 2A and B). We might be able to decide which of SIT and FUP should be done by the CCT parameters in ARR-based CCT-negative patients, because SIT and FUP decisions were discordant in significant portion of patients (Table 2). To validate this hypothesis, we investigated SIT and FUP decisions in four subsets divided by the CCT60-PAC and CCT60-PRA cut-offs (Table 2). In subset B (CCT60-PAC ≤59.0 pg/mL and CCT60-PRA <1.05 ng/mL/h), selecting the FUP as the next test would lead to a higher probability of PA diagnosis than selecting the SIT, which was predicted to be negative (Table 2); the FUP should be selected as the next test (Fig. 5). In subset A (CCT60-PAC ≤59.0 pg/mL and CCT60-PRA ≥1.05 ng/mL/h), while all patients were SIT-negative as predicted, we still had a chance of FUP-positivity (Table 2); the FUP should be selected as the next test (Fig. 5). In subset D (CCT60-PAC >59.0 pg/mL and CCT60-PRA <1.05 ng/mL/h), because the FUP-positive rate was two-fold higher than the SIT-positive rate (Table 2), we should select the FUP as the next test. In subset C (CCT60-PAC >59.0 pg/mL and CCT60-PRA ≥1.05 ng/mL/h), because the SIT-positive rate was significantly higher and the FUP-positive rate tended to be lower as predicted than in other subsets, selecting the SIT would lead to a higher probability of PA diagnosis than selecting the FUP (Table 2). We, therefore, should

| Subsets | Numbers of patients (percentage to subset total) | SIT decisions | FUP decisions |
|---|---|---|---|
|    |          | negative | positive | negative | positive | concordant |
| ≤59.0 pg/mL | ≥1.05 ng/mL/h | 2 (100.0%) | 0 (0.0%) | 1 (50.0%) | 1 (50.0%) | 1 (50.0%) |
| <1.05 ng/mL/h | 10 | 9 (90.0%) | 1 (10.0%) | 4 (40.0%) | 6 (60.0%) | 5 (50.0%) |
| >59.0 pg/mL | ≥1.05 ng/mL/h | 9 (22.2%)* | 7 (77.8%)* | 6 (66.7%) | 3 (33.3%) | 3 (33.3%) |
| <1.05 ng/mL/h | 26 | 16 (61.5%) | 10 (38.5%) | 6 (23.1%) | 20 (76.9%) | 14 (53.8%) |
| Total |         | 47 | 29 (61.7%) | 18 (38.3%) | 17 (36.2%) | 30 (63.8%) | 23 (48.9%) |

Data of ARR-based CCT-negative patients, for whom all of the CCT, SIT, and FUP were performed, were analyzed. *p < 0.05 vs. other subsets.

Table 2 Decisions of SIT and FUP in subsets of CCT-negative patients divided by CCT60-PAC and CCT60-PRA values
select the SIT as the next test in subset C (Fig. 5). If the SIT or FUP should be avoided, we should select the other test.

CCT60-ARR values positively correlated with both of post-SIT-PAC and 1/post-FUP-PRA values (Fig. 3A and C), and CCT60-ARR ≤49.5 pg/mL over ng/mL/h predicted both of SIT and FUP negativities with >95% specificities (not shown). For CCT-negative patients with this condition, neither the SIT nor FUP could be proposed as the next test. Using CCT60-PAC and CCT60-PRA values in combination could avoid this problem.

**Limitations**

There are several limitations in the present study. The first one is the retrospective design of the study. Participants were patients referred to seek scrutiny on PA. This made the prevalence of PA in participants more than 90%; the large bias in the diagnosis would make the accuracy of investigation on PA-discriminating power poor. Moreover, the OST was successfully performed in only six patients (Fig. 1), which made definitive diagnosis with non-PA impossible, where strictly adhering to the consensus statement on PA in Japan [9]. Therefore, we gave up to directly investigate PA-diagnosing power of CCT60-PAC and CCT60-PRA values. However, since the consensus statement on PA in Japan recommends us to perform confirmatory tests only on screening-positive patients [9], the present study would be performed in a real-world setting. The CCT and SIT were performed in more than 100 patients, and their positive rates were 40–60%, which supported the accuracy of investigation on relations between their results. The high FUP-positive rate could interfere with the analyses related to the FUP.

The second one is related to the standardization of aldosterone measurements. The serum certified reference material of aldosterone (NMIJ CRM 6402) was established in 2016 [19], and the JES issued the operational guidelines that recommended manufacturers to make measurement results of each aldosterone assay kit metrologically traceable to SI unit using this primary reference material in 2018. For the SPAC-S Aldosterone RIA kit, the traceability to the primary reference material has not been established, and PAC values measured by this kit were reportedly different from those measured by the liquid chromatography tandem mass spectrometry [20]. However, the SPAC-S Aldosterone RIA kit was the standard kit to measure PAC values in the JES PA guidelines in 2009 [10], and the majority of evidence, on which Japanese guidelines relied [9, 10, 21], was established on PAC values measured by this kit. Results of the present study should not be applied to PAC values measured by kits other than the SPAC-S Aldosterone RIA kit without appropriate conversion.

Thirdly, the present study was a single center study, and regional factors might influence the results. Results might be different, if the screening of PA would be performed for all patients belonging to the high-PA prevalence group [21]. Multicenter studies on issues of the present study are awaited.

**Conclusion**

With >95% specificities, CCT60-PAC ≤59.0 pg/mL could predict the SIT-negativity, and CCT60-PRA ≥1.05 ng/mL/h could predict the FUP-negativity. The present study proposed the SIT as the next test to be done to diagnose PA for CCT-negative patients if they belonged to the subset with CCT60-PAC >59.0 pg/mL and CCT60-PRA ≥1.05 ng/mL/h, otherwise the FUP should be selected.

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**Disclosure**

None of the authors have any potential conflicts of interest associated with this research.
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