Saline Infusion Test highly associated with the incidence of cardio- and cerebrovascular events in primary aldosteronism

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Abstract. Primary aldosteronism (PA) is caused by excess secretion of aldosterone and is an independent risk factor for cardio-cerebro-vascular (CCV) events. The goal of treatment of PA should include prevention of CCV events. A definitive diagnosis of PA is established by confirmatory tests [saline infusion test (SIT), furosemide upright test (FUT) and captopril challenge test (CCT)]. However, there is no information on whether the hormone levels measured by these confirmatory tests are associated with CCV events. The aim of this retrospective study was to elucidate the relationship between the results of the above confirmatory tests and prevalence of CCV disease in patients with PA. The study subjects were 292 PA patients who were assessed for past history of CCV events at the time of diagnosis of PA. CCV events were significantly higher in patients with positive than negative SIT (12.8% vs. 3.3%, p=0.04). There were no differences in the incidences of CCV events between patients with positive and negative CCT and FUT (CCT: 11.0% vs. 3.9%, p=0.13, FUT: 6.1% vs. 5.7%, p=0.93). Our results demonstrated a higher incidence of CCV disease in PA SIT-positive patients compared to those with negative test. SIT is a potentially useful test not only for the diagnosis of PA but also assessment of the risk of CCV events.

Key words: Primary aldosteronism, Cardio- and cerebrovascular (CCV) events, Saline infusion test, Captopril challenge test, Furosemide upright test

Recently, The Japan Endocrine Society and The Japan Hypertension Society developed guidelines for the diagnosis of PA. The recommended screening tests for PA are plasma aldosterone concentration (ng/dL) (PAC) and plasma renin activity (ng/mL/h) (PRA), as well as the PAC/PRA ratio (ARR) [15]. Patients with ARR of more than 20 should undergo confirmatory tests, including saline infusion test (SIT), furosemide upright test (FUT) and captopril challenge test (CCT), to establish the diagnosis of PA [15]. PAC and PRA levels are known to fluctuate with the general condition of the patient, such as body posture and dietary salt intake. Therefore, it is difficult to determine the capacity of aldosterone secretion by the ARR value [16]. On the other hand, SIT evaluates the suppression of aldosterone production in response to volume expansion with isotonic saline. PA patients do not show suppression of aldosterone production in this test [17, 18]. FUT induces volume depletion and stimulates renin release, and PA patients do not show renin release [19]. In the CCT, captopril is used to inhibit the activity of angiotensin-converting enzyme (ACE),
block renin effect and reduce aldosterone production. ARR is not decreased in PA patients [20]. Since these tests can estimate hormonal levels under standardized conditions, their results are used for the diagnosis of PA, and also estimate the severity or clinical status of the patients. Weigel et al. [21] reported recently that PA patients with high post-SIT aldosterone levels had shorter duration of hypertension, higher systolic blood pressure and lower serum potassium, compared to those with low post-SIT aldosterone levels [21]. That is, post-SIT aldosterone levels possibly reflect the clinical severity of PA. It is possible that the confirmatory tests, including SIT, could also reflect the capability for autonomous aldosterone production in patients with PA, and might correlate with CCV risk. To our knowledge, however, there is little or no information on whether hormone levels after these confirmatory tests correlate with CCV disease.

The aim of this retrospective study was to elucidate the relationship between the three confirmatory tests (SIT, FUT, CCT) and the prevalence of CCV disease in patients with PA.

### Subjects and Methods

#### Subjects

In this study, we recruited 292 PA patients who had been diagnosed at our department between January 2004 and March 2015. The median age of these patients was 58.0 years (range: 47.0-67.0), median body mass index (BMI) was 23.7 kg/m² (range: 21.4-26.9) and the median duration of hypertension at the time of PA diagnosis was 6.0 years (range: 2.0-15.0) (Table 1).

The study protocol was approved by the Human Ethics Committee of Osaka University (no. 15078) and conformed to the Declaration of Helsinki.

#### Diagnosis of PA

PA was diagnosed according to the guidelines of Japan Endocrine Society [15]. To exclude the effects of antihypertensive agents on PRA and PAC levels, all patients were asked to stop treatment with angiotensin II receptor blockers (ARB), angiotensin-converting enzyme (ACE) inhibitors, β-blockers, and diuretics 14 days before the diagnostic tests. We measured PAC (ng/dL) and PRA (ng/mL/hr), and calculated the ARR. When the ARR was positive (more than 20), we performed at least two of the three confirmatory tests (SIT, FUT, CCT) to confirm the diagnosis of PA. The tests were considered positive using the following cutoff levels: post-loading PAC of >6 ng/dL (SIT), post-loading ARR ≥20 (CCT), post-loading PRA <2.0 ng/mL/hr (FUT). The diagnosis of PA was established when one of these tests was positive. We excluded patients who were under 18 years of age, had end-stage kidney disease, or were clinically diagnosed with PA without confirmatory tests.

#### Cardio-cerebro-vascular events

We defined CCV events as cardiovascular disease (CVD) (nonfatal myocardial infarction or angina diagnosed by coronary angiography), AF, HF requiring hospitalization, symptomatic cerebral hemorrhage (CH) and cerebral infarction (CI). All patients were assessed for past history of CCV events at the time of PA diagnosis.

#### Laboratory tests

Blood samples were collected in the early morning after fasting and a period of bed rest. PAC was measured by the Aldosterone radioimmunoassay (RIA) Kit II (YAMASA Corporation, Tokyo) before 31 January, 2007 (reference range: 2-13 ng/dL, intra- and inter-assay CV: ≤15%, the measurement unit was converted using the formula shown blow) or the Spac S Aldosterone Kit (TFB Corporation, Tokyo) after 1 February, 2007 (reference range: 2.99-15.88 ng/dL, intra- and inter-assay CV of ≤20%). To correct the difference in value between the two assays, the PAC value

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**Table 1 Clinical characteristics of the 292 patients with primary aldosteronism**

| n   | Gender Males/Females (%) | Age, years | Body mass index, kg/m² | Duration of hypertension, years | Systolic blood pressure, mmHg | Diastolic blood pressure, mmHg | Number of antihypertensive classes | Ever smoker | Diabetes mellitus | Hyperlipidemia | Hypokalemia at diagnosis | eGFR, mL/min/1.73 m² | Urinary albumin excretion, mg/g Cr | Estimated salt intake, g | Plasma aldosterone, ng/dL | Plasma renin activity, ng/mL/h |
|-----|--------------------------|------------|------------------------|-------------------------------|-------------------------------|-------------------------------|----------------------------------|------------|-------------------|----------------|----------------------|----------------|----------------------------|----------------|----------------|-----------------------------|
| 292 | 118/174 (40/60)          | 58.0 (47.0-67.0) | 23.7 (21.4-26.9)       | 6.0 (2.0-15.0)               | 137 (124-150)                | 82 (75-92)                    | 1.0 (1.0-2.0)                    | 38.0%      | 23.1%             | 52.3%          | 34.9%                 | 75.3 (64.5-88.1) | 9.3 (2.8-30.1)                        | 8.7 (7.6-9.9) | 16.9 (11.7-29.6)   | 0.2 (0.1-0.4)                         |

Data are median (first and third quartiles) values.
measured by Aldosterone RIA Kit II was converted using the following equation: PAC (ng/dL) = 0.767 × PAC (Aldosterone RIA Kit II) (ng/dL) − 0.67.

PRA was measured by radioimmunoassay (YAMASA renin rear beads, YAMASA Corporation, Tokyo), with a reference range of 0.2-2.7 ng/mL/hr, and intra- and inter-assay CV of <15%.

The estimated salt intake was calculated using urinary sodium excretion, as described in detail by Tanaka et al. [22].

**Statistical analysis**

Variables were expressed as median values (first and third quartiles). The unpaired t-test was used to compare patients’ characteristics of two groups. The relation between the results of the PA confirmatory tests and the incidence of CCV events was evaluated by Pearson correlation coefficient. The Cochran-Armitage trend test was used to evaluate the relation between the quartile results of the PA confirmatory tests and the incidence of CCV events. Two-sided p<0.05 was considered to denote the presence of a statistically significant difference. Statistical analyses were performed using the JMP Pro software for Window (ver. 11, SAS Institute, Cary, NC).

### Results

**Comparison of clinical characteristics according to PA confirmatory tests**

Among the 292 patients with PA, 233 (80%), 251 (86%) and 199 (68%) patients underwent SIT, CCT, FUT, respectively (Table 2). Patients with positive SIT were mostly young males with higher BMI and higher diastolic blood pressure compared to those with negative SIT (Table 2). Patients with positive SIT and CCT were treated with a larger number of antihypertensive drugs and more likely to have hypokalemia compared to those with negative tests. There was no significant difference in the estimated salt intake between the positive and negative test groups. Patients with positive SIT and CCT were more likely to have albuminuria than the negative tests groups (Table 2). There were no significant differences in clinical characteristics between patients with positive and negative FUT.

**Comparison of incidence of CCV events according to PA confirmatory tests**

The median PAC after SIT was 9.2 ng/dL (5.8-16.7 ng/dL), ARR after CCT was 39.8 (22.8-102.0), and PRA after FUT was 0.9 ng/mL/hr (1.6-0.3 ng/mL/hr).

### Table 2 Comparison of clinical characteristics in each PA confirmatory test

| Test  | Negative group | Positive group | Negative group | Positive group | Negative group | Positive group |
|-------|----------------|----------------|----------------|----------------|----------------|----------------|
| SIT   | 61             | 172            | 51             | 200            | 35             | 164            |
| CCT   | 58.8%          | 60.5%          | 58.8%          | 60.5%          | 57.3%          |                |
| FUT   | 56 (43-66)     | 58 (47-67)     | 56 (43-65)     | 57 (45-66)     |                |                |
| Age, years | 66 (58-73) | 54 (44-64) | 56 (43-66) | 58 (47-67) | 56 (43-65) | 57 (45-66) |
| Body mass index, kg/m² | 23.0 (20.0-25.3) | 23.9 (21.7-27.0) | 24.0 (21.7-27.3) | 23.7 (21.5-26.8) | 23.2 (21.3-27.3) | 23.8 (21.4-26.9) |
| Duration of hypertension, years | 5 (1-13) | 6 (2-16) | 5 (2-12) | 6 (2-16) | 4 (1-9) | 7 (2-15) |
| Systolic blood pressure, mmHg | 134 (121-143) | 140 (122-147) | 132 (120-145) | 137 (123-148) | 132 (120-145) | 137 (123-148) |
| Diastolic blood pressure, mmHg | 78 (72-87) | 83 (76-94) | 85 (77-94) | 82 (74-92) | 80 (70-93) | 82 (76-92) |
| Number of anti-hypertensive classes | 1 (1-2) | 1 (1-3) | 1 (1-2) | 1 (1-3) | 1 (1-3) | 1 (1-3) |
| Ever smoker | 39.3% | 43.2% | 35.3% | 37.2% | 44.1% | 36.4% |
| Diabetes mellitus | 22.6% | 18.8% | 15.0% | 24.6% | 13.3% | 18.6% |
| Hypertension at diagnosis | 14.6% | 40.1% | 13.7% | 39.0% | 22.9% | 29.9% |
| eGFR, mL/min/1.73 m² | 77.2 (67.1-84.6) | 75.8 (65.7-91.7) | 75.8 (66.7-89.9) | 76.4 (64.8-90.0) | 73.0 (59.9-83.5) | 76.1 (66.4-87.7) |
| Albuminuria | 11.1% | 24.2% | 13.3% | 22.4% | 22.2% | 16.4% |
| Estimated salt intake, g | 8.9 (7.6-9.7) | 8.5 (7.4-9.8) | 8.9 (7.6-10.2) | 8.6 (7.8-9.7) | 8.5 (7.4-9.8) | 8.9 (8.0-9.9) |
| Plasma aldosterone, ng/dL | 10.5 (8.1-13.8) | 13.8 (11.3-24.2) | 13.8 (11.3-24.2) | 17.9 (12.3-31.5) | 16.0 (11.6-24.9) | 16.9 (12.3-30.0) |
| Plasma renin activity, ng/mL/h | 0.2 (0.1-0.4) | 0.3 (0.1-0.5) | 0.5 (0.2-0.8) | 0.2 (0.1-0.3) | 0.5 (0.3-0.9) | 0.2 (0.1-0.4) |
| Aldosterone-producing adenoma | 62.1% | 58.9% | 57.1% | 64.1% | 55.6% | 61.7% |

Data are median (first and third quartiles) values. * p<0.05, † p<0.01 vs. the negative group of the same test (by the unpaired t-test). Albuminuria was defined as urinary albumin excretion greater than 30 mg/g Cr. SIT, Saline infusion test; CCT, Captopril challenge test; FUT, Furosemide upright test.
PA patients were divided into four groups according to the post-SIT PAC levels, four groups according to the post-CCT ARR levels and four groups according to the post-FUT PRA levels (Fig. 1). The incidence of CCV events increased significantly in proportion with high post-SIT PAC levels [Post-SIT PAC level: first quartile: 3.3% (2/60), second quartile: 10.3% (6/58), third quartile: 12.3% (7/57), fourth quartile: 15.5% (9/58) (p=0.03) (Fig. 1)]. Similar trends were noted in the other two tests. Thus, the incidence of CCV events increased significantly with increases in post-CCT ARR levels (p=0.02, Fig. 1) and also with increases in post-FUT PRA levels (p=0.09, Fig. 1).

Analysis according to the results of each test showed significantly higher incidence of CCV events in the SIT-positive group compared with the negative group [12.8% (22/172) vs. 3.3% (2/61), respectively, p=0.04, Fig. 2]. However, there was no significant difference in the incidence of CCV events between CCT positive and negative groups [11.0% (22/200) vs. 3.9% (2/51), respectively, p=0.13] and FUT [6.1% (10/164) vs. 5.7% (2/35), respectively, p=0.93] (Fig. 2).

**Effects of sex and age on CCV events in the SIT group**

PAC levels after SIT as well as before SIT were significantly lower in female PA patients than male PA patients (before SIT: p<0.01, after SIT: p<0.01, Table 3). However, there was no difference in estimated salt intake between male and female patients (8.9 g (7.6-10.1 g) vs. 8.5 g (7.6-9.5 g), respectively, p=0.36). There was also no difference between the SIT-positive and -negative groups with respect to the percentages of male patients who developed CCV events (p=0.43, Fig. 3A). On the other hand, SIT-positive female patients were likely to suffer CCV events compared with their SIT-negative counterparts (p=0.11, Fig. 3A).

With regard to the effect of age, PAC levels before and after SIT were significantly lower in patients aged more than 65-years than those less than 65-years (before SIT: p<0.01, after SIT: p<0.01, Table 3). There was no difference in the estimated salt intake between the elderly and younger groups [8.9 g (7.9-9.9 g) vs. 8.4 g (7.4-9.5 g), p=0.06]. The incidence of CCV events tended to be higher in SIT-positive patients aged less than 65 years than those with negative test (p=0.09, Fig. 3B). On the other hand, a significantly higher incidence of CCV events was noted in elderly patients with positive and negative SIT compared to their younger counterparts (Fig. 3B).
PRA, and CCT measures PRA and PAC to calculate ARR. In other words, SIT can directly assess aldosterone production while FUT and CCT evaluate aldosterone production and renin secretion, secondary to diminishing extracellular fluid by furosemide, upright position and inhibition of angiotensin I conversion to angiotensin II. Considering the different mechanisms of these confirmatory tests, the reason that SIT, but not FUT and CCT, related to CCV events could be because it directly assesses aldosterone production per se.

The results showed that SIT- and CCT-positive patients were more likely to use antihypertensive drugs and a large proportion of these patients had hypokalemia compared with the SIT-negative patients. These findings suggest that SIT- and CCT-positive PA patients have aldosterone excess. Salt intake plays an important role in aldosterone-induced organ damage [23-27]. In this study, estimated salt intake was 8.7 (7.6-9.9) g/day. This was lower than the Japanese mean salt intake (men for 10.9 g/day and women for 9.2 g/day), but was higher than the ideal salt intake (<6 g/day). Although there was not any difference in salt intake between the positive and negative groups in each confirmatory test, the salt intake in both groups may have been enough to induce organ damage.

Furthermore, the SIT-positive group included significantly higher proportion of young males than the negative group. PAC levels before and after SIT were significantly lower in female PA patients than male PA patients. Previous study reported that estrogen decreased while testosterone increased PAC levels [28]. Our results suggest that PAC is less common in female PA patients than male patients. With regard to the effect of age on SIT, Nakama et al. [29] reported that aging could influence SIT results, and that patients aged over 65 years had lower PAC compared to those aged less than 65 years. Our results showed significantly lower pre- and post-SIT PAC levels in patients aged more than 65-years than those aged less than 65-years. These results indicate that PAC levels at rest and after SIT are affected by sex and age. However, the incidence of CCV events was significantly higher in patients positive for SIT than those with negative tests, independent of sex and age (Fig. 3).

We often see PA patients who do not have typical clinical manifestations like young age, refractory hypertension or hypokalemia. These PA patients are suspected to have mild PA, with relatively lower aldosterone production than typical PA. It is important to

### Table 3 PAC levels according to sex and age, before and after SIT

|                | Pre-SIT, PAC (ng/dL) | Post-SIT, PAC (ng/dL) |
|----------------|----------------------|-----------------------|
| Males          | 20.7 (14.4-34.7)     | 12.8 (7.9-20.3)       |
| Females        | 14.7 (9.8-21.4)      | 7.2 (5.0-10.9)        |
| <65 years      | 17.9 (12.9-28.7)     | 10.1 (6.9-19.5)       |
| ≥65 years      | 14.2 (9.6-21.1)      | 6.4 (4.5-10.5)        |

Data are median (first and third quartiles) values. † p<0.01, compared with data of male patients (by the unpaired t-test). ‡ p<0.01, compared with data of the younger group (by the unpaired t-test).

### Fig. 3 Effects of sex (A) and age (B) on CCV events in patients with negative and positive saline infusion test (SIT)

Data on each bar represent the percentage of patients who developed CCV events. * p<0.05 (by Pearson test).

### Discussion

The goal of management of PA patients is not only to control blood pressure but also to prevent CCV disease associated with excessive aldosterone production. In this study, we showed that the incidence of CCV events was significantly higher in SIT-positive patients compared with SIT-negative patients, but not between the CCT- and FUT-positive and -negative groups. These results suggest that SIT is potentially useful not only for the diagnosis but also for assessment of the severity or risk of CCV disease.

SIT evaluates the effect of aldosterone production by volume expansion with isotonic saline [17, 18]. On the other hand, FUT evaluates whether reduction of extracellular fluid by furosemide and upright position affect renin secretion [19], while CCT evaluates whether inhibition of conversion of angiotensin I to angiotensin II by captopril affects renin secretion and aldosterone production [20]. SIT measures PAC, FUT measures
clarify the type of patients that are at high risk of CCV disease. Our results showed that SIT can be used not only for the diagnosis of PA but also potentially to predict CCV risk in PA patients. In other words, the results suggest the clinical superiority of SIT.

In this study, there was no difference in the performing rate of CCT or SIT between the patients with past history of CCV events and without past history of CCV events [CCT (76.7% vs 86.9%, p=0.13), SIT (77.4% vs 80.1%, p=0.73)]. On the other hand, the performing rate of FUT was significantly lower in the patients with past history of CCV events than without past history of CCV events (35.0% vs 70.1%, p<0.01). When the PA confirmatory tests are performed, the characteristics of each patient have to be considered. FUT tends to be avoided in patients with past history of CCV events because using diuretics has the risk factor of dehydration. This had caused selection bias in this study. For this reasons, we could not accurately elucidate the relationship between FUT and the prevalence of CCV disease in this study. Since our study was retrospective in design, our results need to be verified in a prospective study to clarify the true relationship between the confirmatory tests and CCV disease.

In conclusion, a higher incidence of CCV disease was associated with positive SIT in PA patients, compared with patients with negative test. SIT could be a potentially useful test not only for the diagnosis of PA but also assessment of the risk of CCV events.

**Disclosure**

The authors declare no personal financial or institutional interest in this article.

**References**

1. Conn JW (1966-1967) The evolution of primary aldosteronism: 1954-1967. *Harvey Lect* 62: 257-291.
2. Williams JS, Williams GH, Raji A, Jeunemaitre X, Brown NJ, et al. (2006) Prevalence of primary hyperaldosteronism in mild to moderate hypertension without hypokalaemia. *J Hum Hypertens* 20: 129-136.
3. Nishikawa T, Omura M (2000) Clinical characteristics of primary aldosteronism: its prevalence and comparative studies on various causes of primary aldosteronism in Yokohama Rosai Hospital. *Biomed Pharmacother* 54 Suppl 1: 83s-85s.
4. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, et al. (2008) Case detection, diagnosis, and treatment of patients with primary aldosteronism: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 93: 3266-3281.
5. Catena C, Colussi G, Marzano L, Sechi LA (2012) Aldosterone and the heart: from basic research to clinical evidence. *Horm Metab Res* 44: 181-187.
6. Catena C, Colussi G, Brosolo G, Iogna-Prat L, Sechi LA (2012) Aldosterone and aldosterone antagonists in cardiac disease: what is known, what is new. *Am J Cardiovasc Dis* 2: 50-57.
7. Mulatero P, Monticone S, Bertello C, Viola A, Tizzani D, et al. (2013) Long-Term Cardio- and Cerebrovascular Events in Patients With Primary Aldosteronism. *J Clin Endocrinol Metab* 98: 4826-4833.
8. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, et al. (2005) Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol* 45: 1243-1248.
9. Takeda R, Matsubara T, Miyamori I, Hatakeyama H, Morise T (1995) Vascular complications in patients with aldosterone producing adenoma in Japan: comparative study with essential hypertension. The Research Committee of Disorders of Adrenal Hormones in Japan. *J Endocrinol Invest* 18: 370-373.
10. Catena C, Colussi G, Nadalini E, Chiuch A, Baroselli S, et al. (2008) Cardiovascular outcomes in patients with primary aldosteronism after treatment. *Arch Intern Med* 168: 80-85.
11. Savard S, Amar L, Plouin PF, Steichen O (2013) Cardiovascular complications associated with primary aldosteronism: a controlled cross-sectional study. *Hypertension* 62: 331-336.
12. Rossi GP, Bernini G, Desideri G, Fabris B, Ferri C, et al. (2006) PAPY Study Participants. Renal damage in primary aldosteronism: results of the PAPY Study. *Hypertension* 48: 232-238.
13. Sechi LA, Novello M, Lapenna R, Baroselli S, Nadalini E, et al. (2006) Long-term renal outcomes in patients with primary aldosteronism. *JAMA* 295: 2638-2645.
14. Tanase-Nakao K, Naruse M, Nanba K, Tsuiki M, Tagami T, et al. (2014) Chronic kidney disease score for predicting postoperative masked renal insufficiency in patients with primary aldosteronism. *Clin Endocrinol (Oxf)* 81: 665-670.
15. Nishikawa T, Omura M, Sato H, Shibata H, Takahashi K, et al. (2011) Guidelines for the diagnosis and treatment of primary aldosteronism—the Japan Endocrine Society 2009. *Endocr J* 58: 711-721.
16. Stowasser M, Ahmed AH, Pimenta E, Taylor PJ, Gordon...
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17. Holland OB, Brown H, Kuhnert L, Fairchild C, Risk M, et al. (1984) Further evaluation of saline infusion for the diagnosis of primary aldosteronism. *Hypertension* 6: 717-723.

18. Rossi GP, Belfiore A, Bernini G, Desideri G, Fabris B, et al. (2007) PAPY Study Investigators; Prospective evaluation of the saline infusion test for excluding primary aldosteronism due to aldosterone-producing adenoma. *J Hypertens* 25: 1433-1442.

19. Hirohara D, Nomura K, Okamoto T, Ujihara M, Takano K (2001) Performance of the basal aldosterone to renin ratio and of the renin stimulation test by furosemide and upright posture in screening for aldosterone-producing adenoma in low renin hypertensives. *J Clin Endocrinol Metab* 86: 4292-4298.

20. Castro OL, Yu X, Kem DC (2002) Diagnostic value of the post-captopril test in primary aldosteronism. *Hypertension* 39: 935-938.

21. Weigel M, Riester A, Hanslik G, Lang K, Willenberg HS, et al. (2015) Post-saline infusion test aldosterone levels indicate severity and outcome in primary aldosteronism. *Eur J Endocrinol* 172: 443-450.

22. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, et al. (2002) A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. *J Hum Hypertens* 16: 97-103.

23. Rocha R, Rudolph AE, Friedich GE, Nachowiak DA, Kekek BK, et al. (2002) Aldosterone induces a vascular inflammatory phenotype in the rat heart. *Am J Physiol Heart Circ Physiol* 283: H1802-1810.

24. Martinez DV, Rocha R, Matsumura M, Oestreich E, Ochoa-Mayo M, et al. (2002) Cardiac damage prevention by eplerenone: Comparison with low sodium diet or potassium loading. *Hypertension* 39: 614-618.

25. Rocha R, Chander PN, Khanna K, Zuckerman A, Stier CT Jr (1998) Mineralocorticoid blockade reduces vascular injury in stroke-prone hypertensive rats. *Hypertension* 31: 451-458.

26. Blasi ER, Rocha R, Rudolph AE, Blomme EA, Polly ML, et al. (2003) Aldosterone/salt induces renal inflammation and fibrosis in hypertensive rats. *Kidney Int* 63: 1791-1800.

27. Pimenta E, Gordon RD, Ahmed AH, Cowley D, Leano R, et al. (2011) Cardiac Dimensions Are Largely Determined by Dietary Salt in Patients with Primary Aldosteronism: Results of a Case-Control Study. *J Clin Endocrinol Metab* 96: 2813-2820.

28. Komukai K, Mochizuki S, Yoshimura M (2010) Gender and the renin-angiotensin-aldosterone system. *Fundam Clin Pharmacol* 24: 687-698.

29. Nakama C, Kamide K, Kawai T, Hongyo K, Ito N, et al. (2014) The influence of aging on the diagnosis of primary aldosteronism. *Hypertens Res* 37: 1062-1067.