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Impact of the transition from radioimmunoassay (RIA) to chemiluminescent enzyme immunoassay (CLEIA) for the measurement of plasma aldosterone concentration (PAC) on the diagnosis of primary aldosteronism (PA) *via* retrospective analyses in Okinawa, Japan

Ken-ichiro Honma, Yoshiro Nakayama, Atsuko Tamaki, Moriyuki Uehara, Taiki Teruya, Takamitsu Yabiku, Yohei Ishiki, Ken Yonaha, Rei Chinen, Tsugumi Uema, Shiki Okamoto and Hiroaki Masuzaki

Division of Endocrinology, Diabetes and Metabolism, Hematology and Rheumatology, Second Department of Internal Medicine, Graduate School of Medicine, University of the Ryukyus, Okinawa 903-0215, Japan

Abstract. In Japan, the traditional method for measuring plasma aldosterone concentration (PAC) was radioimmunoassay (RIA), which had several challenges, including poor traceability of certified reference materials and reduced detection sensitivity at low concentrations. To overcome these issues, a chemiluminescent enzyme immunoassay (CLEIA) for PAC measurement was introduced in April 2021 and the Japan Endocrine Society published new guidelines for primary aldosteronism (PA). This study aimed to evaluate the impact of the transition from RIA to CLEIA for PAC measurement on PA diagnosis. Data from 190 patients admitted to the Second Department of Internal Medicine, University of the Ryukyus Hospital, between April 2012 and March 2021 were analyzed. Patients who were diagnosed with PA underwent adrenal venous sampling. The PAC measured by RIA (PAC(RIA)) was converted to the estimated PAC measured by CLEIA (ePAC(CLEIA)) using a conversion formula. The present study evaluated the discordance rates in diagnoses based on screening (SC), captopril challenge test (CCT), saline infusion test (SIT), and diagnosis of PA between results judged by PAC(RIA) according to the previous guidelines and those judged by ePAC(CLEIA) according to the new guidelines. The results revealed discordant diagnosis rates of 6.4% for SC and 10.1% for CCT, with no discordance for SIT. The discordant diagnosis rate for PA was 3.7%. Our study reveals the challenges in establishing appropriate diagnostic criteria for PA using PAC(CLEIA) and highlights the demand for further research on provisionally positive categories.

Key words: Hypertension, Primary aldosteronism, Plasma aldosterone concentration, Radioimmunoassay, Chemiluminescent enzyme immunoassay

Introduction

Primary aldosteronism (PA) is a major cause of secondary hypertension, accounting for 5–20% of hypertension cases [1-3]. Accurate diagnosis of PA is extremely important because it is associated with a higher prevalence of cerebral, cardiovascular, and renal complications than essential hypertension, including stroke, left ventricular hypertrophy, atrial fibrillation, coronary artery disease, heart failure, and proteinuria [4-6].

Historically, the establishment of plasma aldosterone concentration (PAC) assays and adoption of the aldosterone-renin ratio (ARR) for screening (SC) have led to an increase in the number of patients diagnosed with PA [7]. PA is diagnosed using specific confirmatory tests including captopril challenge test (CCT) and saline infusion test (SIT).

In Japan, PAC measurement by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [8] is not widely used because it is not covered by the National Health Insurance system. Therefore, PAC measurement was traditionally performed using radioimmunoassay (RIA). However, RIA presents several challenges, including the use and disposal of radioactive isotopes,

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Correspondence to: Hiroaki Masuzaki, MD, PhD, Division of Endocrinology, Diabetes and Metabolism, Hematology and Rheumatology, Second Department of Internal Medicine, Graduate School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan.
E-mail: hiroaki@med.u-ryukyu.ac.jp

poor traceability of certified reference materials (CRM), and reduced detection sensitivity at low concentration ranges [9, 10]. To address these issues, a two-site sandwich chemiluminescent enzyme immunoassay (CLEIA) for PAC measurements was introduced in Japan in April 2021. PAC measurement by CLEIA (PAC(CLEIA)) demonstrated good CRM traceability and aligned well with the LC-MS/MS values [11].

Notably, PAC(CLEIA) tends to be lower than that measured by RIA (PAC(RIA)) [12], meaning that the direct application of PAC(CLEIA) to conventional diagnostic criteria may lead, at least in some cases, to the misdiagnosis of PA as essential hypertension. Given this point, the latest clinical guidelines published by the Japan Endocrine Society (JES) in 2021 (GL2021) [13] recommend that, for SC, PAC(CLEIA) ≥60 pg/mL and ARR ≥200 should be considered positive, and PAC(CLEIA) ≥60 pg/mL and ARR 100–200 should be considered provisionally positive. While the previous guidelines [14] had a PAC(RIA) cutoff value of 120 pg/mL for SC, GL2021 sets this value at half, based on a regression analysis of PAC(RIA) and PAC(CLEIA) against PAC measured by LC-MS/MS [9, 11, 15, 16]. Similarly, for CCT and SIT, provisional positive criteria were established in addition to the positive criteria. However, these provisionally positive criteria were initially established pending the widespread availability of PAC(CLEIA) and determination of optimal cutoffs, and their validity has not been thoroughly evaluated.

Therefore, the present study aimed to evaluate the impact of changes in the diagnostic criteria for SC, CCT, and SIT, resulting from the transition from RIA to CLEIA, on PAC measurement in PA diagnosis. Furthermore, it sought to identify the clinical characteristics that distinguish positive cases of SC, CCT, and SIT from those that are considered provisionally positive.

Materials and Methods

Setting and participants

This study retrospectively reviewed the data of 190 patients admitted to the Second Department of Internal Medicine at the University of the Ryukyus Hospital between April 2012 and March 2021. Patients were diagnosed with PA and underwent adrenal venous sampling (AVS). Data were extracted from electronic medical records, including age, sex, body mass index (BMI), systolic and diastolic blood pressure, heart rate, random blood glucose level, HbA1c, serum creatinine, serum sodium, serum potassium, serum chloride, semi-qualitative test for urinary protein, presence of adrenal nodules (defined as a tumor diameter ≥10 mm) on abdominal CT, and results of SC, CCT and SIT. PAC was determined using RIA (SPAC-S Aldosterone kits; Fuji Rebio Co., Ltd., Tokyo, Japan). All participants who underwent AVS also underwent CCT and/or SIT; therefore, none were excluded. Hypokalemia was defined as a serum potassium level of <3.5 mEq/L. The study protocol was approved by the Ethics Review Committee for Life Sciences and Medical Research Involving Human Subjects at the University of the Ryukyus (approval number: 23-2138-01-00-00). Informed consent was obtained through an opt-out option on the hospital website.

Diagnosis of PA using PAC (RIA)

PA diagnosis was based on a consensus statement on the clinical practice of Primary Aldosteronism in Japan (CS) [14], a guideline previously developed by the JES. For SC, a PAC(RIA) >120 pg/mL and ARR >200 were considered positive; for CCT, an ARR >200 at 60 or 90 min after 50 mg captopril intake was considered positive; and for SIT, a PAC(RIA) >60 pg/mL after 240 min of a 2 L saline infusion was considered positive. CCT and SIT were performed not only in patients with a positive SC result but also in those with a negative SC result if PA was suspected based on the physician's judgment. Prior to testing, antihypertensive treatment with calcium channel blockers (CCBs) and/or alpha-blockers was attempted. Mineralocorticoid receptor antagonists (MRAs) and/or angiotensin receptor blockers (ARBs) were also used in some patients with inadequate blood pressure control.

PA subtype differentiation (adrenal venous sampling)

Plasma samples were collected from both the right and left adrenal veins and the inferior vena cava 30 minutes after a 250 μg bolus injection of cosyntropin. The PAC-to-plasma-cortisol concentration ratio (A/C ratio) was calculated for each sample. Successful catheterization of the adrenal vein was determined by the selectivity index (SI), defined as the ratio of plasma cortisol concentration in the adrenal vein to that in the inferior vena cava, with a value ≥5 indicating successful catheterization [13, 17-19]. The lateralized ratio (LR) was used for subtype differentiation and was calculated by dividing the A/C ratio on the dominant side by that on the non-dominant side, with an LR ≥4 indicating unilateral PA (uPA) [13, 14, 20, 21]. If the LR was between 2 and 4, it was considered borderline, and subtype differentiation was determined concerning an adrenal vein PAC(RIA) ≥14,000 pg/mL or the contralateral ratio (CR) [13, 14]. The CR was calculated by dividing the A/C ratio on the non-dominant side by that of the inferior vena cava, with a CR ≤1 indicating uPA. Patients diagnosed with uPA underwent laparoscopic adrenalectomy, whereas those not diagnosed with uPA were diagnosed with bilateral

PA (bPA) and treated with MRAs. Histopathological diagnosis was performed by a well-trained pathologist at our university hospital following laparoscopic adrenalectomy. In the present study, patients diagnosed with bPA and those with uPA but found to have adrenal hyperplasia rather than aldosterone-producing adenoma (APA) on histopathological examination were defined as having idiopathic hyperaldosteronism (IHA).

Calculating estimated PAC(CLEIA) (ePAC(CLEIA))

PAC(RIA) was converted to the estimated PAC(CLEIA) (ePAC(CLEIA)) using the following conversion formula:

ePAC(CLEIA) = 0.852 × PAC(RIA) – 36.0 [9]

This formula was derived from a regression analysis comparing PAC measured by RIA and PAC measured by CLEIA using PAC measured by LC-MS/MS as a reference [9].

Simulating determinations in GL2021 using ePAC(CLEIA)

Initially, the SC, CCT, and SIT determinations in GL2021 were simulated using ePAC(CLEIA), and the outcomes were compared with those obtained from the CS. The cutoff values of PAC(RIA) in CS, the corresponding cutoff values of ePAC(CLEIA) in CS, and the cutoff values of PAC(CLEIA) in GL2021 for SC, CCT, and SIT are shown in Supplementary Table S1. We calculated the percentage of discrepant results for each of the SC, CCT, and SIT tests between the GL2021 and CS. Subsequently, the process was extended to simulate PA diagnosis in GL2021 using ePAC(CLEIA). In the present study, a PA diagnosis was defined as either SC positive or provisionally positive, and positive in at least one of the CCT or SIT. SC positive or provisionally positive and provisionally positive on at least one of the CCT or SIT was defined as a provisional diagnosis of PA. Discrepancies in diagnosis were then assessed, and patients diagnosed with PA or provisionally diagnosed with PA in GL2021, who also received a PA diagnosis in CS, were classified as having concordant diagnoses. In contrast, cases diagnosed or provisionally diagnosed with PA in GL2021, but not in CS, were considered discordant. Similarly, patients with a negative PA diagnosis in GL2021 who were not diagnosed with PA by CS were labeled as concordant; however, cases with a negative PA diagnosis in GL2021 but diagnosed as PA by CS were identified as discordant. Finally, the clinical characteristics of the patients with discordant diagnoses were reviewed.

Additional analysis

We conducted an in-depth analysis of the clinical characteristics of patients with confirmed, provisional, and negative PA diagnoses using the GL2021. This included a comparison of uPA and bPA in terms of clinical parameters. We further subdivided the patients diagnosed with PA based on the presence or absence of hypokalemia or adrenal nodules to compare the prevalence of APA among these subgroups.

Statistical methods

All statistical analyses were conducted using EZR (version 1.64; Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria). It is a modified version of R Commander, enhanced with additional statistical functions frequently used in biostatistics [22]. Continuous variables are presented as mean ± standard deviation (*SD*) or median (interquartile range (*IQR*)), depending on their distribution, and were analyzed using Mann-Whitney *U* test for comparisons between two groups. One-way analysis of variance (ANOVA) was used to assess statistical differences among the three groups. For *post-hoc* comparisons, the Bonferroni correction method was used to adjust for multiple comparisons. Fisher's exact test was used to compare the proportions of categorical variables among the three groups, and the Bonferroni correction method was used for *post-hoc* comparisons to adjust for multiple comparisons. The threshold for statistical significance was set at $p < 0.05$.

Results

Clinical features

This study included 27 patients with APA and 163 patients with IHA who underwent AVS at our institution between April 2012 and March 2021. The clinical characteristics of all 190 patients are shown in Table 1.

Comparison of screening results using PAC(RIA) according to CS and ePAC(CLEIA) according to GL2021

In a retrospective analysis, SC using PAC (RIA) according to CS identified 148 positive and 42 negative cases. However, by applying ePAC(CLEIA) and aligning it with GL2021, we identified 117 positive, 43 provisionally positive, and 30 negative cases. Of the PAC(RIA) SC positive cases, ePAC(CLEIA) reclassified 38 cases as provisionally positive and retained 110 cases as positive, with no negatives. Conversely, among the SC negative cases identified by PAC(RIA), ePAC(CLEIA) classified seven cases as positive, five as provisionally positive, and 30 as negative. This reclassification resulted in diagnostic discordance in 12 cases (6.4%), as illustrated in

Table 1 Clinical characteristics of all patients

Number	190
Male (%)	70 (36.8)
Age (year)	51 ± 12
Body mass index (kg/m^2)	26.5 ± 4.6
Systolic blood pressure (mmHg)	134 (124–144)
Diastolic blood pressure (mmHg)	80 (70–88)
Heart rate (/min)	75 (68–82)
Random blood glucose (mg/dL)	94 (88–102)
HbA1c (%)	5.6 (5.3–6.0)
Cre (mg/dL)	0.65 (0.55–0.78)
Na (mEq/L)	140 (139–141)
Hypokalemia (%)	30 (15.8)
K (mEq/L)	3.8 (3.5–4.0)
Cl (mEq/L)	104 (103–106)
Semi-qualitative test for urinary protein (%)	
–	157 (84.9)
±	18 (9.7)
1+	9 (4.9)
2+	1 (0.5)
Adrenal nodules (%)	95 (50)
Number or antihypertensive drugs (%)	
0	42 (22.1)
1	127 (66.8)
2	19 (10.0)
3	2 (1.1)
Classification of antihypertensive drugs (%)	
Calcium channel blockers	145 (76.3)
Alpha-blockers	12 (6.3)
Angiotensin receptor blockers	8 (4.2)
Mineralocorticoid receptor antagonists	3 (1.6)
Beta-blockers	2 (1.1)
Diuretics	1 (0.5)
Potassium supplements (%)	27 (14.2)
Aldosterone-producing adenoma (%)	27 (14.2)

Data are expressed as mean ± *SD*, median (*IQR*), and *n* (%). HbA1c, glycated hemoglobin; Cre, serum creatinine; Na, serum sodium; K, serum potassium; Cl, serum chloride.

Fig. 1A, all of which were identified as IHA. Of these discordant cases, only four cases showed provisional or positive results on either CCT or SIT, leading to three cases with a provisional diagnosis and one case with a confirmed diagnosis of PA, as detailed in Supplementary Table S2.

Comparison of CCT results using PAC(RIA) according to CS and ePAC(CLEIA) according to GL2021

CCT using PAC(RIA) according to CS identified 129 positive and 59 negative cases. Using ePAC(CLEIA) according to the GL2021 criteria, 69 results were positive, 49 were provisionally positive, and 70 were negative. Of the PAC(RIA) CCT positive cases, 69 were positive, 45 were provisionally positive, and 15 were negative with ePAC(CLEIA). Among the PAC(RIA) CCT negative cases analyzed with ePAC(CLEIA), none were reclassified as positive, four were identified as provisionally positive, and 55 cases remained negative, resulting in diagnostic discordance in 19 cases (10.1%) between PAC(RIA) and ePAC(CLEIA) (Fig. 1B). Among the 19 patients with discordant CCT results between PAC(RIA) and ePAC(CLEIA), one was diagnosed with APA, while the remaining 18 were diagnosed with IHA. Of the 15 cases that were CCT positive by PAC(RIA) and negative by ePAC(CLEIA), in SC, seven were positive, one was provisionally positive, and the remaining seven were negative. In SIT, of the 11 cases, one case was positive, three were provisionally positive, and the remaining seven were negative (SIT was not performed in four cases). None of these cases were ultimately diagnosed or provisionally diagnosed with PA because of negative SC results, despite four cases having at least one positive or provisionally positive CCT or SIT result. Among the four cases that were CCT negative by PAC(RIA) and provisionally positive by ePAC(CLEIA) in SC, two of the four cases were positive, and two were provisionally positive. In SIT, three of the four cases were positive or provisionally positive, with all four cases having at least one positive or provisionally positive CCT or SIT. This resulted in three cases being provisionally diagnosed with PA and one case being diagnosed with PA (Supplementary Table S3).

Comparison of SIT results using PAC(RIA) according to CS and ePAC(CLEIA) according to GL2021

The SIT using PAC(RIA), in accordance with the CS, identified 66 positive and 30 negative cases. Using ePAC(CLEIA) according to the GL2021 criteria, 21 results were positive, 45 were provisionally positive, and 30 were negative. Of the 66 SIT positive cases identified by PAC(RIA), 21 were positive by ePAC(CLEIA), 45 were provisionally positive, and none were negative. Among the 30 cases that were SIT negative by PAC(RIA), none were identified as positive or provisionally positive by ePAC(CLEIA), and all were confirmed as negative by SIT. There were no discordant results between the PAC(RIA) and ePAC(CLEIA) groups (Fig. 1C).

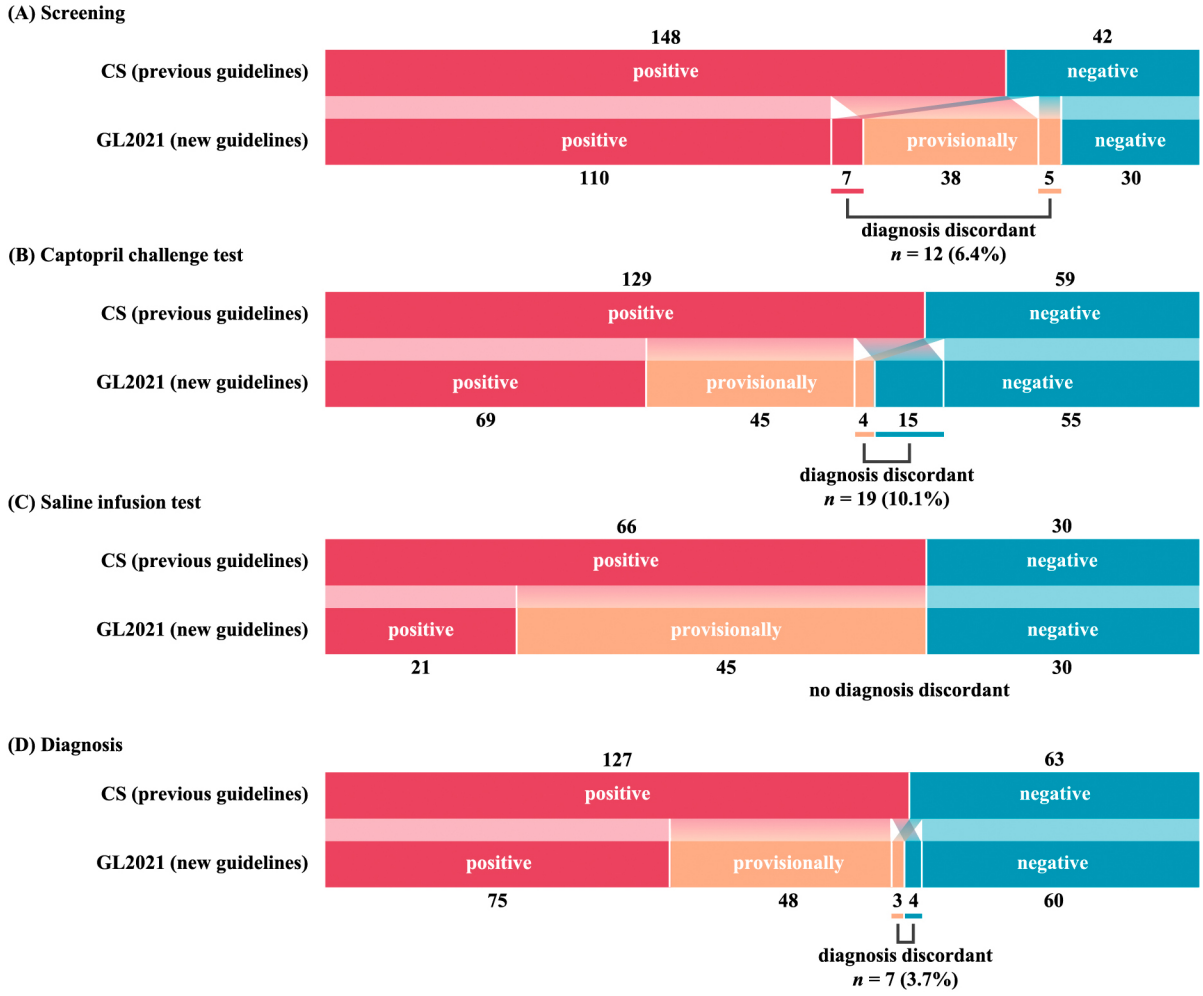

Fig. 1 Comparison of the consensus statement on the clinical practice of primary aldosteronism in Japan and the Japan Endocrine Society Clinical Practice Guideline for the Diagnosis and Management of Primary Aldosteronism 2021.

(A–D) Comparison of (A) screening, (B) captopril challenge test, (C) saline infusion test and (D) overall diagnosis of primary aldosteronism results. The upper and lower bars of each figure show the number of positive (red), negative (blue) and provisionally positive (orange) cases according to CS and GL 2021, respectively. Gradient bands indicate shifts in classification between the two criteria, with red-to-blue or blue-to-red or orange gradients representing diagnostic discordance. CS: Consensus Statement on the Clinical Practice of Primary Aldosteronism in Japan [14]; GL2021: Japan Endocrine Society clinical practice guideline for the diagnosis and management of primary aldosteronism 2021 [13].

Comparison of PA diagnosis using PAC(RIA) according to CS and ePAC(CLEIA) according to GL2021

Using PAC(RIA) and following the CS guidelines, 127 patients were diagnosed with PA and 63 were not. Among the 63 cases diagnosed as PA negative according to CS, 42 showed a negative SC result when strictly applying the cutoff criteria of CS (Fig. 1A). However, owing to the strong suspicion of PA by the attending physicians, these patients underwent CCT or SIT. As the results of the confirmatory tests were positive, the patients were diagnosed with PA and underwent AVS. The remaining 21 cases showed a positive SC result but were negative for CCT and SIT when strictly applying the cutoff criteria for CS. However, these patients showed CCT or SIT results very close to the cutoff values and were diagnosed with PA by attending physicians based on findings including serum potassium level, blood pressure level, and the presence of adrenal nodules. Consequently, the patients also underwent AVS.

Using ePAC(CLEIA) and adhering to the GL2021 guidelines, 75 patients were diagnosed with PA, 51 were provisionally diagnosed, and 64 were not diagnosed. Among the 127 patients diagnosed with PA using PAC(RIA), 75 were diagnosed with PA using ePAC(CLEIA), 48 were provisionally diagnosed, and four were not diagnosed. Of the 63 patients not diagnosed with PA using PAC(RIA), none were diagnosed

with PA using ePAC(CLEIA), three were provisionally diagnosed, and 60 were not diagnosed. Thus, there were seven cases in which the diagnosis differed between PAC(RIA) and ePAC(CLEIA), all of which were IHA. The diagnostic discordance rate was 3.7% (7/190) (Fig. 1D). Among the four patients diagnosed with PA by PAC(RIA) but not by PAC(CLEIA), three were SC positive, and one was provisionally positive. All four patients were CCT negative, and three of the four cases were SIT negative (one case did not undergo SIT). Among the three patients not diagnosed with PA by PAC(RIA) but provisionally diagnosed by PAC(CLEIA), one was SC positive, and two were provisionally positive. In CCT, two of the three cases were provisionally positive, and one case was negative. In SIT, two of the three cases were provisionally positive, and one case was negative. All three patients were provisionally diagnosed with PA because at least one of either CCT or SIT was provisionally positive (Table 2).

Clinical characteristics of APA and IHA

The clinical characteristics of the patients with APA and IHA are shown in Table 3. The APA group had significantly more male cases (55.6% *vs.* 33.7%, $p = 0.034$), higher serum sodium levels (141 mEq/L *vs.* 140 mEq/L, $p < 0.001$), and lower serum potassium levels (3.3 mEq/L *vs.* 3.8 mEq/L, $p < 0.001$) than those of the IHA group. The IHA group had significantly higher BMI (26.7 kg/m^2 *vs.* 23.5 kg/m^2, $p = 0.02$), diastolic blood pressure (80 mmHg *vs.* 74 mmHg, $p = 0.043$), and HbA1c (5.7% *vs.* 5.4%, $p = 0.013$) than those of the APA group. There were no significant differences in age, heart rate, systolic blood pressure, random blood glucose level, serum creatinine level, serum chloride

Table 2 Clinical characteristics of seven patients with discordant PA diagnoses between PAC (RIA) and ePAC (CLEIA)

		Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Sex		Female	Female	Female	Female	Female	Female	Female
Age (year)		71	46	61	56	45	35	60
BMI (kg/m^2)		21.7	22.9	22.4	24.8	19.6	26.0	28.2
sBP (mmHg)		136	128	140	133	136	140	126
dBP (mmHg)		76	70	80	88	72	86	70
HR (/min)		72	76	82	76	75	60	80
RBG (mg/dL)		92	86	87	83	83	—	150
HbA1c (%)		5.7	5.1	6.5	5.3	5.5	5.3	8.4
Cre (mg/dL)		0.63	0.53	0.57	0.66	0.48	0.60	0.53
Na (mEq/L)		141	139	141	143	137	137	140
K (mEq/L)		3.5	3.7	4.1	3.8	3.7	3.9	4.0
Cl (mEq/L)		107	105	108	107	103	107	107
Urinary protein		–	–	–	–	–	–	±
Adrenal nodules		–	–	–	–	–	+	+
Subtype		IHA	IHA	IHA	IHA	IHA	IHA	IHA
SC	CS/ GL2021	Positive/ Positive	Positive/ Positive	Positive/ Positive	Positive/ Provisionally	Negative/ Positive	Positive/ Provisionally	Negative/ Provisionally
CCT	CS/ GL2021	Positive/ Negative	Positive/ Negative	Positive/ Negative	Positive/ Negative	Negative/ Negative	Negative/ Provisionally	Positive/ Provisionally
SIT	CS/ GL2021	–/–	Negative/ Negative	Negative/ Negative	Negative/ Negative	Positive/ Provisionally	Negative/ Negative	Positive/ Provisionally
Diagnosis	CS/ GL2021	Positive/ Negative	Positive/ Negative	Positive/ Negative	Positive/ Negative	Negative/ Provisionally	Negative/ Provisionally	Negative/ Provisionally

BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure; HR, heart rate; RBG, random blood glucose; HbA1c, glycated hemoglobin; Cre, serum creatinine; Na, serum sodium; K, serum potassium; Cl, serum chloride; CCBs, calcium channel blockers; ARBs, angiotensin receptor blockers; MRBs, mineralocorticoid receptor antagonists; IHA, idiopathic hyperaldosteronism; SC, screening; CCT, captopril challenge test; SIT, Saline infusion test; CS, the Consensus Statement on the Clinical Practice of Primary Aldosteronism in Japan [14]; GL2021, Japan Endocrine Society clinical practice guideline for the diagnosis and management of primary aldosteronism 2021 [13].

level, qualitative test for urinary protein, or number and type of antihypertensive medications between the APA and IHA groups. According to GL2021, using ePAC(CLEIA) for SC, 85.2% of APA cases were positive, compared to 57.7% of IHA cases. For CCT, 84.6% of APA cases were positive, compared to 29.0% of IHA cases, with an additional 29.0% provisionally positive and 42% negative ($p < 0.001$). For SIT, 63.6% of APA cases were positive and 18.2% were provisionally positive, compared with 16.5% of IHA cases that were positive, 50.6% provisionally positive, and 32.9% negative.

Table 3 Clinical characteristics of patients with APA and IHA

	APA	IHA	*p* value
Number	27	163	
Male (%)	15 (55.6)	55 (33.7)	0.034
Age (year)	49 (41–60)	49 (42–60)	0.83
Body mass index (kg/m^2)	23.5 (22.8–26.8)	26.7 (23.4–29.4)	0.020
Systolic blood pressure (mmHg)	130 (125–140)	136 (124–144)	0.18
Diastolic blood pressure (mmHg)	74 (68–82)	80 (70–89)	0.043
Heart rate (/min)	74 (66–80)	76 (68–82)	0.99
HbA1c (%)	5.4 (5.3–5.7)	5.7 (5.3–6.0)	0.013
Cre (mg/dL)	0.68 (0.54–0.80)	0.65 (0.56–0.78)	0.64
Na (mEq/L)	141 (140–144)	140 (139–141)	<0.001
Hypokalemia (%)	19 (70.4)	25 (15.3)	<0.001
K (mEq/L)	3.3 (2.7–3.6)	3.8 (3.6–4.0)	<0.001
Adrenal nodules (%)	20 (74.1)	75 (46.0)	0.012
Classification of antihypertensive drugs (%)			
Calcium channel blockers	22 (81.5)	123 (75.5)	0.63
Alpha-blockers	4 (14.8)	8 (4.9)	0.072
Angiotensin receptor blockers	3 (11.1)	5 (3.1)	0.088
Mineralocorticoid receptor antagonists	0	3 (1.8)	1.0
Beta-blockers	0	2 (1.2)	1.0
Diuretics	0	1 (0.6)	1.0
Potassium supplements (%)	13 (48.1)	14 (8.6)	<0.001
Screening (%)			0.025
Positive	23 (85.2)	94 (57.7)	
Provisionally positive	3 (11.1)	40 (24.5)	
Negative	1 (3.7)	29 (17.8)	
Captopril challenge test (%)			<0.001
Positive	22 (84.6)	47 (29.0)	
Provisionally positive	2 (7.7)	47 (29.0)	
Negative	2 (7.7)	68 (42.0)	
Saline infusion test (%)			0.003
Positive	7 (63.6)	14 (16.5)	
Provisionally positive	2 (18.2)	43 (50.6)	
Negative	2 (18.2)	28 (32.9)	
Decision (%)			<0.001
Diagnosis	23 (85.2)	52 (31.9)	
Provisionally positive	2 (7.4)	49 (30.1)	
Negative	2 (7.4)	62 (38.0)	

Data are expressed as the median (*IQR*) or *n* (%). The *p* values were calculated using the Mann–Whitney *U* test. HbA1c, glycated hemoglobin; Cre, serum creatinine; Na, serum sodium; K, serum potassium; APA, aldosterone-producing adenoma; IHA, idiopathic hyperaldosteronism.

Clinical characteristics of PA diagnosis, provisional diagnosis, and negative groups according to GL2021 using ePAC(CLEIA)

The clinical characteristics of the PA diagnosis, provisional diagnosis, and negative groups are shown in Table 4. No significant differences in age were observed among the groups. In the *post-hoc* analysis, the PA diagnosis group contained significantly more male patients than in the negative group ($p = 0.0088$) (Fig. 2A). Serum potassium levels were significantly lower in the PA diagnosis group (3.6 mEq/L) than in the provisional diagnosis group (3.9 mEq/L) and the negative group (3.8 mEq/L) (Fig. 2B). The prevalence of APA was significantly higher in the PA diagnosis group (30.7%) than in the provisional diagnosis group (3.9%) and the negative group (3.1%) (Fig. 2C). Subdivision of the PA diagnosis group based on the presence of hypokalemia revealed a significantly higher prevalence of APA in patients with hypokalemia (63.3% *vs.* 8.9%, $p < 0.001$) (Table 5A). When categorized according to the presence of adrenal nodules, the prevalence of APA was significantly higher in patients with adrenal nodules (39.6% *vs.* 14.8%, $p = 0.036$) (Table 5B). The clinical characteristics of patients provisionally diagnosed or not diagnosed with PA, including confirmed APA cases ($n = 4$), are presented in Supplementary Table S4. None of these patients exhibited hypokalemia, and among the four cases, one had adrenal nodules.

Discussion

To our knowledge, the present study is the first to apply ePAC(CLEIA) to the GL2021 to examine how changes in the measurement of PAC and, thus, the diagnostic criteria for PA affect its diagnosis. The transition from RIA to CLEIA has had a considerable impact on the diagnosis of PA, as evidenced by the results of the present study.

Although GL2021 defines provisional positive criteria for SC, CCT, and SIT, evidence for these criteria remains insufficient. We observed a non-negligible number of cases initially identified as positive for SC, CCT, and SIT using PAC(RIA) based on CS, which were then classified as provisionally positive or negative upon re-evaluation with ePAC(CLEIA) based on GL2021. Assuming that there were no criteria for provisionally positive results, patients originally considered provisionally positive would have been considered negative and cases that should have been diagnosed with PA would have been misdiagnosed as having essential hypertension. This highlights the challenge of determining appropriate cutoff values for diagnosing PA. Moreover, certain cases considered negative under the CS guidelines were identified as provisionally positive or positive using the new criteria, emphasizing the need to reassess the validity of provisionally positive cutoffs.

The significant impact of the transition from RIA to CLEIA on the diagnosis of PA can be attributed to the inherent limitations of RIA and the advantages of CLEIA. PAC measurements using RIA have limitations, particularly in terms of sensitivity and reproducibility [9, 10]. In contrast, PAC measurement using CLEIA offers improved traceability to the CRM for aldosterone and excellent linearity across a broad range of concentrations, including lower levels. Moreover, PAC values determined by CLEIA align well with those determined by LC-MS/MS [11, 23, 24]. The advantages of CLEIA over RIA underscore its potential as a standard PAC assay in routine clinical practice. In addition, the strong correlation between PAC measurements obtained using CLEIA and LC-MS/MS facilitated an international comparison of PA diagnoses.

Typical PA with a phenotype of hypertension, hypokalemia, and unilateral adrenal nodules was first described by Conn [25]. This classic form of PA, which is often associated with APA, responds well to laparoscopic adrenalectomy [26-28]. However, the broad implementation of screening for PA using the ARR has led to a considerable increase in milder forms of PA [29], which are predominantly IHA manageable with MRAs. AVS has been recommended as the gold standard for distinguishing APA from IHA, thereby determining indications for surgery. Over the past decade, despite a marked increase in the number of diagnosed PA cases and the introduction of AVS, only 20% of PA patients undergoing AVS have undergone adrenalectomy in Japan [30]. The diagnostic efficiency of AVS needs to be enhanced given its invasive nature, technical challenges, cost, and radiation exposure.

A retrospective cohort study within the Japan Rare/Intractable Adrenal Diseases Study (JRAS) of 3,689 patients with PA before changing PAC measurement methods revealed that patients positive for CCT or SIT showed higher APA rates than those who were provisionally positive [31]. The study reported that the percentages of APA in positive *vs.* provisionally positive cases were 44% *vs.* 7% for CCT and 49% *vs.* 5% for SIT, respectively [31]. However, it should be noted that the diagnostic criteria for SIT in the JRAS differed from those used in the present study.

According to the JRAS, patients with hypokalemia diagnosed with PA using ePAC(CLEIA) seem to be ideal AVS candidates because of the high prevalence of surgically treatable APA. However, the study showed that approximately 5% of cases with a provisional diagnosis of PA were APA [31], which is similar to the percentage

Table 4 Clinical characteristics of patients in the PA diagnosis, provisional diagnosis, and negative group

	Diagnosis	Provisional diagnosis	negative	*p* value
Number	75	51	64	
Male (%)	36 (48.0)	19 (37.4)	15 (23.4)	0.011
Age (year)	53 (43–63)	45 (38–58)	49 (45–60)	0.064
Body mass index (kg/m^2)	25.1 (23.2–28.2)	27.7 (24.1–30.0)	25.9 (22.7–30.6)	0.092
Systolic blood pressure (mmHg)	132 (123–142)	132 (120–140)	136 (128–148)	0.16
Diastolic blood pressure (mmHg)	78 (70–84)	78 (70–89)	80 (76–90)	0.13
Heart rate (/min)	74 (70–80)	75 (68–83)	76 (66–84)	0.89
Random blood glucose (mg/dL)	94 (89–102)	91 (87–102)	95 (88–102)	0.75
HbA1c (%)	5.6 (5.3–5.9)	5.6 (5.3–6.1)	5.7 (5.3–5.9)	0.84
Cre (mg/dL)	0.69 (0.55–0.86)	0.65 (0.56–0.78)	0.62 (0.56–0.74)	0.058
Na (mEq/L)	141 (140–142)	140 (139–141)	140 (139–141)	0.002
Hypokalemia (%)	30 (40.0)	5 (9.8)	9 (14.1)	<0.001
K (mEq/L)	3.6 (3.3–3.9)	3.9 (3.7–4.0)	3.8 (3.6–4.0)	<0.001
Semi-qualitative test for urinary protein (%)				0.76
–	61 (84.7)	45 (88.2)	51 (82.3)	
±	7 (9.7)	5 (9.8)	6 (9.7)	
1+	4 (5.6)	1 (2.0)	4 (6.5)	
2+	0	0	1 (1.6)	
Adrenal nodules (%)	48 (64.0)	20 (39.2)	27 (42.2)	0.007
Number or antihypertensive drugs (%)				0.008
0	11 (14.7)	19 (37.3)	12 (18.8)	
1	51 (68.0)	28 (54.9)	48 (75.0)	
2	13 (17.3)	3 (5.9)	3 (4.7)	
3	0	1 (2.0)	1 (1.6)	
Classification of antihypertensive drugs (%)				
Calcium channel blockers	63 (84.0)	31 (60.8)	51 (79.7)	0.008
Alpha-blockers	8 (10.7)	3 (5.9)	1 (1.6)	0.088
Angiotensin receptor blockers	5 (6.7)	1 (2.0)	2 (3.1)	0.38
Mineralocorticoid receptor antagonists	1 (1.3)	2 (3.9)	0	0.24
Beta-blockers	0	0	2 (3.1)	0.14
Diuretics	0	0	1 (1.6)	0.37
Potassium supplements (%)	20 (26.7)	2 (3.9)	5 (7.8)	<0.001
Aldosterone-producing adenoma (%)	23 (30.7)	2 (3.9)	2 (3.1)	<0.001

Data are expressed as the median (*IQR*) or *n* (%). The *p* values were calculated using one-way ANOVA. HbA1c, glycated hemoglobin; Cre, serum creatinine; Na, serum sodium; K, serum potassium.

in the present study (2 of 51 cases, 3.9%). Thus, APA cannot be ruled out, even with a provisional diagnosis of PA.

In the provisional diagnosis group in the present study, only two patients (3.9%) were diagnosed with APA, neither of whom exhibited hypokalemia, and adrenal nodules were observed in just one case. The low proportion of patients with APA in the provisional diagnosis group suggests a reduced need for AVS. The limited number of APA cases in this group precluded the identification of additional clinical indicators to distinguish APA from IHA. Therefore, to identify cases with a high pre-diagnostic probability of APA within the group and to guide appropriate AVS indications, further studies are necessary to establish clinical findings beyond hypokalemia and adrenal nodules.

In contrast, a higher proportion of patients with APA in the PA diagnosis group had hypokalemia and adrenal nodules in the present study, consistent with a previous

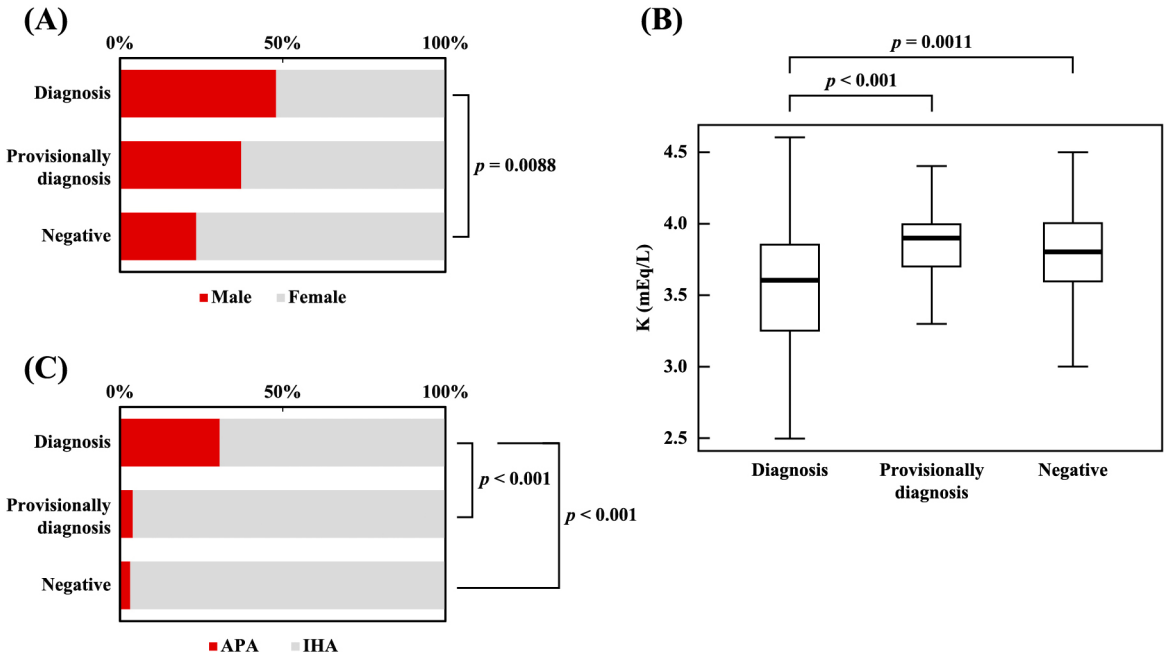

Fig. 2 (A) Comparison of male-to-female ratio among the PA diagnosis, provisional diagnosis, and negative groups. (B) Box plot of serum potassium levels among the PA diagnosis, provisional diagnosis, and negative groups. (C) Comparison of APA to IHA ratio among the PA diagnosis, provisional diagnosis, and negative groups.

(A) The percentages of male and female cases are shown in the red band and the gray band, respectively. (B) Within each box, horizontal black lines denote median values; boxes extend from the 25th to the 75th percentile of each group's distribution of values; vertical extending lines denote adjacent values (*i.e.*, the most extreme values within 1.5 interquartile range of the 25th and 75th percentile of each group). (C) The percentages of APA and IHA cases are shown in the red band and the gray band, respectively. PA: Primary aldosteronism; APA: Aldosterone-producing adenoma; IHA: idiopathic hyperaldosteronism.

Table 5A Comparison of APA prevalence based on the presence of hypokalemia in the PA diagnosis group

	Hypokalemia	Normokalemia	*p* value
N	30	45	
APA (%)	19 (63.3)	4 (8.9)	<0.001
IHA (%)	11 (36.7)	41 (91.1)	

Table 5B Comparison of APA prevalence based on the presence of adrenal nodule in the PA diagnosis group

	Adrenal nodule +	Adrenal nodule –	*p* value
N	48	27	
APA (%)	19 (39.6)	4 (14.8)	0.036
IHA (%)	29 (60.4)	23 (85.2)	

Data are expressed as *n* (%). *p* values were calculated using Fisher's exact test. Hypokalemia was defined as a serum potassium level <3.5 mEq/L; adrenal nodules were defined as a tumor diameter ≥10 mm on abdominal CT; PA, primary aldosteronism; APA, aldosterone-producing adenoma; IHA, idiopathic hyperaldosteronism.

report [31]. Positivity rates for SC, CCT, and SIT using ePAC(CLEIA) were considerably higher for APA than for IHA (85.2% *vs.* 57.7% for SC, 84.6% *vs.* 29.0% for CCT, and 63.6% *vs.* 16.5% for SIT). This suggests that SC and confirmatory tests may help distinguish APA from IHA. As the number of cases was too small to establish an appropriate cutoff, further studies are required to resolve this issue.

Based on our findings, we propose that provisionally positive cases should be considered a criterion for initiating medical therapy with MRAs because of the high likelihood of IHA. In contrast, positive cases should be regarded as a criterion for considering AVS because of the increased possibility of APA.

A critical limitation of the present study was the estimation of PAC(CLEIA) based on PAC(RIA) measurements using a conversion formula. Given the variability in PAC(RIA) [32] among patients, this approach could hinder the interpretation of results, rendering the study speculative. Direct comparison of PAC measured using both CLEIA and RIA in the same patient is essential for accurate evaluation. Moreover, because only patients with PA who underwent AVS were included in the present study, there might have been a selection bias, potentially overestimating the prevalence of APA. Additionally, the diagnosis was based on Japanese clinical guidelines, which may have limited the applicability of this study to other countries.

In conclusion, the present study demonstrated that the transition from PAC(RIA) to PAC(CLEIA) resulted in changes in the diagnostic criteria for PA, leading to a considerable number of cases previously classified as positive by CS being reclassified as provisionally positive or negative according to GL2021. These findings highlight the necessity of establishing appropriate cutoff values and validating provisionally positive criteria to guide management decisions, particularly concerning AVS, in patients with a high likelihood of surgically remediable APA.

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Disclosure

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Author's contribution

All the authors contributed to this study. Data were collected by K.H. The draft of the manuscript was written by K.H., Y.N., A.T., M.U., T.T., T.Y., Y.I., K.Y., R.C., T.U., and S.O. and H.M. reorganized the final form of the manuscript. All the authors have read and approved the final version of the manuscript.

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