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Original Research

International multicenter survey on screening and confirmatory testing in primary aldosteronism

Mitsuhide Naruse,[1,*†] Masanori Murakami,[2,*†] Takuyuki Katabami,[3] Tomaz Kocjan,[4,5] Mirko Parasiliti-Caprino,[6] Marcus Quinkler,[7] Matthieu St-Jean,[8] Sam O'Toole,[9] Filippo Ceccato,[10] Ivana Kraljevic,[11] Darko Kastelan,[11] Mika Tsuiki,[12] Jaap Deinum,[13] Edelmiro Menéndez Torre,[14] Troy Puar,[15] Athina Markou,[16] George Piaditis,[16] Kate Laycock,[17] Norio Wada,[18] Marianne Aardal Grytaas,[19] Hiroki Kobayashi,[20] Akiyo Tanabe,[21] Chin Voon Tong,[22] Nuria Valdés Gallego,[23] Sven Gruber,[24] Felix Beuschlein,[24] Lydia Kürzinger,[25] Norlela Sukor,[26] Elena A.B. Aisha Azizan,[26] Oskar Ragnarsson,[27] Michiel F. Nijhoff,[28] Giuseppe Maiolino,[29] Guido Di Dalmazi,[30] Valentina Kalugina,[31] André Lacroix,[32] Raluca Maria Furnica,[33] and Tomoko Suzuki[34]

[1]Endocrine Center and Clinical Research Center, Ijinkai Takeda General Hospital, Kyoto, 601-1495, Japan
[2]Department of Molecular Endocrinology and Metabolism, Tokyo Medical and Dental University, Tokyo, 113-8510, Japan
[3]Division of Metabolism and Endocrinology, Department of Internal Medicine, St. Marianna University Yokohama City Seibu Hospital, Yokohama, 241-0811, Japan
[4]Department of Endocrinology, Diabetes, and Metabolic Diseases, University Medical Centre Ljubljana, Ljubljana, 1000, Slovenia
[5]Faculty of Medicine, University of Ljubljana, Ljubljana, 1000, Slovenia
[6]Endocrinology, Diabetes, and Metabolism; Department of Medical Sciences; University of Turin, Turin, 10126, Italy
[7]Endocrinology in Charlottenburg, Berlin, 10117, Germany
[8]Division of Endocrinology, Department of Medicine, Centre intégré universitaire de santé et de services sociaux de l'Estrie - Centre hospitalier Universitaire de Sherbrooke, Sherbrooke, Quebec, J1H 5N4, Canada
[9]Department of Endocrinology, The Royal Hallamshire Hospital, Sheffield, S10 2JF, UK
[10]Endocrinology Unit, Department of Medicine DIMED, University of Padova, Padova, 35128, Italy
[11]Department of Endocrinology, University Hospital Centre Zagreb, Zagreb, 10 000, Croatia
[12]Department of Endocrinology and Metabolism, NHO Kyoto Medical Center, Kyoto, 612-8555, Japan
[13]Department of Internal Medicine, Radboud University Medical Center, Nijmegen, 6525 GA, Netherlands
[14]Servicio de Endocrinología, Hospital Universitario Central de Asturias, Oviedo, 33011, Spain
[15]Department of Endocrinology, Changi General Hospital, Singapore, 529889, Singapore
[16]Endocrinology Department and Diabetes Center, 'G. Gennimatas' General Hospital of Athens, Athens, 115 28, Greece
[17]Department of Diabetes & Metabolism, Barts Health NHS Trust, St Bartholomew's and Royal London Hospital, London, E1 1BB, UK
[18]Department of Diabetes and Endocrinology, Sapporo City General Hospital, Sapporo, 060-8604, Japan
[19]Department of Medicine, Haukeland University Hospital, Bergen, 5021, Norway
[20]Division of Nephrology, Hypertension, and Endocrinology, Department of Internal Medicine, Nihon University School of Medicine, Tokyo, 173-8610, Japan
[21]Department of Diabetes, Endocrinology, and Metabolism, National Center for Global Health and Medicine, Tokyo, 162-8655, Japan
[22]Department of Medicine, Hospital Melaka, Melaka, 75400, Malaysia
[23]Department of Internal Medicine, Section of Endocrinology and Nutrition, Hospital Universitario de Cabueñes, Gijón, 33394, Spain
[24]Department of Endocrinology, Diabetology and Clinical Nutrition, Clinic for Endocrinology and Diabetology, University Hospital Zurich, CH-8091, Switzerland
[25]Department of Internal Medicine, Division of Endocrinology and Diabetes, University Hospital Würzburg, Würzburg, 97082, Germany
[26]Department of Medicine, The National University of Malaysia (UKM) Medical Centre, Kuala Lumpur, 59200, Malaysia
[27]Department of Endocrinology, Sahlgrenska University Hospital, Göteborg,413 45, Sweden
[28]Department of Internal Medicine, Division of Endocrinology and Diabetes, Leiden University Medical Center, Leiden, 2333 ZA, The Netherlands
[29]Clinica Medica 3, Department of Medicine - DIMED, University of Padova, Padova, 35126, Italy
[30]Unit of Endocrinology and Diabetes Prevention and Care, University of Bologna, Bologna, 40126, Italy
[31]Endocrinology Department, North-Western State Medical University named after I.I. Mechnikov, St Petersburg, 191015, Russia
[32]Endocrine Division, Department of Medicine, Centre hospitalier de l'Université de Montréal (CHUM), Montréal, H2X 3E4, Canada
[33]Department of Endocrinologie, Cliniques Universitaires Saint Luc, Bruxelles, 1200, Belgium
[34]Department of Public Health, International University of Health and Welfare School of Medicine, Chiba, 286-8686, Japan

***Corresponding author:** Endocrine Center and Clinical Research Center, Ijinkai Takeda General Hospital, 28-1 Ishida moriminami-cho, Fushimi-ku, Kyoto, 601-1495 Japan. Email: mtsnaruse@nifty.com (M.N.); Department of Molecular Endocrinology and Metabolism, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8510, Japan. E-mail: mmurakami.mem@tmd.ac.jp (M.M.)

[†]M. Naruse and M. Murakami contributed equally as corresponding authors.

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Abstract

Objective: Primary aldosteronism (PA) is one of the most frequent causes of secondary hypertension. Although clinical practice guidelines recommend a diagnostic process, details of the steps remain incompletely standardized.

Design: In the present SCOT-PA survey, we have investigated the diversity of approaches utilized for each diagnostic step in different expert centers through a survey using Google questionnaires. A total of 33 centers from 3 continents participated.

Results: We demonstrated a prominent diversity in the conditions of blood sampling, assay methods for aldosterone and renin, and the methods and diagnostic cutoff for screening and confirmatory tests. The most standard measures were modification of antihypertensive medication and sitting posture for blood sampling, measurement of plasma aldosterone concentration (PAC) and active renin concentration by chemiluminescence enzyme immunoassay, a combination of aldosterone-to-renin ratio with PAC as an index for screening, and saline infusion test in a seated position for confirmatory testing. The cutoff values for screening and confirmatory testing showed significant variation among centers.

Conclusions: Diversity of the diagnostic steps may lead to an inconsistent diagnosis of PA among centers and limit comparison of evidence for PA between different centers. We expect the impact of this diversity to be most prominent in patients with mild PA. The survey raises 2 issues: the need for standardization of the diagnostic process and revisiting the concept of mild PA. Further standardization of the diagnostic process/criteria will improve the quality of evidence and management of patients with PA.

Keywords: aldosterone, renin, screening, confirmatory tests, primary aldosteronism

Significance

The international multicenter survey of **S**creening and **C**onfirmatory **T**esting in **P**rimary **A**ldosteronism demonstrated significant diversity in various aspects of diagnosing primary aldosteronism. Prominent diversity was identified in the conditions of blood sampling, assay methods for aldosterone and renin, and the methods and diagnostic cutoff for screening and confirmatory tests. It is conceivable that diagnostic diversity has an enormous impact on patients with mild PA. Further standardization of the diagnostic process is needed to improve the quality of evidence and clinical practice of PA.

Introduction

Primary aldosteronism (PA) is caused by the primary excess aldosterone production from the adrenals and is the most frequent cause of secondary hypertension.[1-4] While PA causes various cardiovascular and renal complications and treatment-resistant hypertension,[5-7] its specific treatment can result in clinical and biochemical cures, prevent the development of target organ damage, and improve prognosis.[2-4] Therefore, diagnosing PA correctly in the daily clinical practice of hypertension is of utmost importance. Algorithms for diagnosing PA have been published in various clinical practice guidelines.[8-12] The basic process of the diagnosis is (1) screening of at-risk hypertensive patients, (2) confirmation of aldosterone hypersecretion by confirmatory testing, and (3) subtype diagnosis, primarily relying on adrenal venous sampling (AVS).

Clinical practice guideline recommends using the plasma aldosterone-to-renin ratio (ARR) to detect cases with possible PA followed by confirmatory testing to confirm or exclude the diagnosis. However, details of the screening (index, cutoff) and confirmatory test (number, type, and cutoff) seem to vary among physicians, centers, and countries. The diversity of the diagnostic methods and approaches employed may affect the standardization of PA's clinical practice and research from a global perspective.[10,11,13-15] For example, previous studies suggested that the apparent prevalence of PA is highly dependent on the diagnostic thresholds for screening and confirmatory testing.[16,17] Therefore, investigation of the actual conditions in which screening and confirmatory tests are undertaken would help further improve the standardization of clinical practice and research in PA.

The primary objective of the present survey was to investigate the details of the present conditions under which screening and confirmatory testing are undertaken in various referral centers; conditions of blood sampling, assay methods for aldosterone and renin, the main index (plasma aldosterone concentrations [PAC], plasma renin activity [PRA], ARC [active renin concentration], ARR), and their cutoff values for the screening test, the choice and numbers of confirmatory tests and their cutoff values, protocol of the confirmatory tests, and conditions to bypass the confirmatory tests, respectively. The survey was conducted by summarizing the experts' opinions from referral centers in the European Network for the Study of Adrenal Tumors (ENS@T) and Japan (International Multicenter Study on **S**creening and **C**onfirmatory testing in **P**rimary **A**ldosteronism, SCOT-PA).

Methods

We conducted the SCOT-PA survey as a multinational, multicenter questionnaire-based study. A total of 33 centers (6 centers in Japan that were part of the Japan PA Study (JPAS) and Japan Rare Adrenal Diseases Study (JRAS) and 27 centers in Europe, Canada, and South-east Asia that were part of the ENS@T network participated. We conducted the first round with 15 questions related to the screening and confirmatory tests listed below in October 2021 with a 1-month survey period using a Google Forms-Questionnaire-based survey following the AVSTAT Study, which assessed the rates of AVS, implementation, AVS success rate, and adrenalectomy rate.[18] After collecting the first responses, we conducted data cleaning through e-mail correspondence. We performed data aggregation from December 2021 to January 2022. We conducted the second round with 6 questions on the feasibility of future standardization of the assay methods, screening, and confirmatory tests between February 2, 2022 and February 16, 2022.

First-round questionnaire:

Q1 Who do the answers to this questionnaire apply to?
Q2 What is the primary guideline you comply with?
Q3 What are the conditions of blood sampling for screening?

Q4 What is the assay method of PAC?
Q5 Which PRA or ARC do you use for the renin assay?
Q6 What is the assay method of PRA?
Q7 What is the assay method of ARC?
Q8 What is the screening method and its cutoff for positive results?
Q9 Which confirmatory test do you prioritize as the first-line test?
Q10 Which confirmatory test do you conduct as the second-line test?
Q11 How many confirmatory tests do you conduct for routine practice?
Q12 Please check all the tests you perform and specify the parameters and cutoff with units in the "Comments."
Q13 Please specify the protocol of each test
Q14 Do you think you can bypass the confirmatory testing under certain conditions?
Q15 If your answer is 'Yes' to Q14, what are the conditions to bypass the confirmatory testing?

Second-round questionnaire on the feasibility of future standardization with a choice of answers of easy, possible, difficult, impossible, and not needed.

Q1 Assay method of PAC
Q2 Assay method of renin (PRA, ARC)
Q3 Screening methods (ARR, PAC, PRA, combination, etc.)
Q4 Cutoff for screening test
Q5 Type of confirmatory test
Q6 Cutoff for the confirmatory test

Measurement parameters

Measures investigated in the present survey are as follows: the conditions of blood sampling for screening, the assay method of PAC, assay methods of renin (PRA and ARC), the screening method and its cutoff for positive results, the first and second line confirmatory test, numbers of confirmatory test used in routine practice, the indices and cutoff with units, the protocol of each test, possibility and the conditions for bypassing the confirmatory testing.

We also investigated the experts' opinions concerning the standardization of the screening and the confirmatory tests. Measures investigated were as follows: assay method of PAC and renin, screening methods (ARR, PAC, PRA, combination, etc.), a screening cutoff, and the type and cutoff of the confirmatory test.

Statistical analysis

The collected data of the answer to the questionnaire were thoroughly reviewed. The data were cross-checked for missed values, irregularities, inconsistencies, and corrective measures were taken as required. Descriptive statistics were used to analyze data of interest. Categorical variables were presented as percentages to show diversity among the centers. Microsoft Excel was used for all calculations.

Ethics

We conducted the survey according to the guidelines for clinical studies published by the Ministry of Health and Labor, Japan. The survey was conducted according to the Declaration of Helsinki. The ethics committee approved the survey of the Ijinkai Takeda General Hospital as the project lead center (no. 2021010), and individual centers participated. The project was a survey of the experts' opinions affiliated with the specialized referral centers of the clinical practice of PA. The agreement of the experts was confirmed by their participation in the survey and answering the questionnaire. No clinical information about patients was used.

Results

Thirty-six members from 33 referral centers in 17 countries participated in the survey. Most of the survey participants were representative of the affiliated centers/departments (84.9%). Although 5 participants chose to answer the questionnaires as individuals rather than representing a standardized approach in their department, the results were analyzed center-based as appropriate. In cases where 2 experts from the same center participated in the survey, they were counted as 1 center. Most of the participating centers from the ENS@T group comply with the Endocrine Society clinical guideline on PA[8] (75.8%), while Centers in Japan follow Japan Guideline for PA.[9,12] One center in the ENS@T follows European Society of Hypertension (ESH) guidelines.[11]

Conditions of blood sampling for screening

The conditions before the blood sampling were categorized into 6: antihypertensive medication, posture, time of the day, fasting, potassium repletion, and unrestricted salt intake. About 90% of the centers adopt pre-specified conditions of blood sampling for screening. Moderately defined conditions (1 or 2 conditions adopted) were used in 50.0% and strictly defined conditions (more than 3 conditions adopted) were used in 40.6%. Modifying the antihypertensive medication was the most frequently defined condition to avoid possible influence on the screening results. Antihypertensive medication was changed to calcium channel blockers (CCBs) and alpha-blockers. Posture was the second most common condition designated before blood sampling. Most of the centers (80%) used the seated position rather than the supine position (15%) or upright (5%). Blood sampling was conducted mainly in the morning, especially between 8 AM and 9 AM (Figure 1).

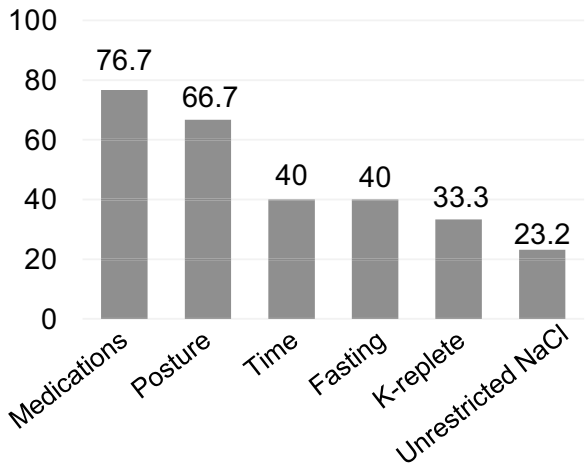

Figure 1. Types and frequency of regulated conditions of blood sampling for screening of PA.

Table 1. Assay methods of PAC, ARC, and PRA.

	RIA	CLEIA	LC-MS/MS	EIA	IRMA
PAC	6/33(18.2)	22/33(66.7)	5/33(15.2)	–	–
ARC	1/22(4.5)	19/22(86.4)	–	–	2/22(9.1)
PRA	8/13(61.5)	–	2/13 (15.4)	3/13(23.1)	–

Numbers of lefts and percentages (in parenthesis) are shown.
Abbreviations: ARC, active renin concentration; CLEIA, chemiluminescent enzyme immunoassay; EIA, enzyme immunoassay; IRMA, Immunoradiometric assay; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PAC, plasma aldosterone concentration; PRA, plasma renin activity; RIA, radioimmunoassay.

Assay methods of PAC and renin

Most centers use chemiluminescent enzyme immunoassay (CLEIA) (66.7%) for PAC measurement, followed by radioimmunoassay (RIA) (18.2%). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) as an ideal method is not standard (Table 1). Various kits for CLEIA ($n=6$) and RIA ($n=4$) from different manufacturers are used. Most centers use ARC (60.6%) for renin measurement, while the rest use PRA (39.4%). Most centers use CLEIA for ARC, and limited centers use Immunoradiometric assay (IRMA) and RIA (Table 1). Various kits for CLEIA ($n=4$) and IRMA ($n=2$) from different manufacturers are used. Most centers use RIA for PRA, while others use EIA or LC-MS/MS (Table 1). Various kits for RIA ($n=3$) from different manufacturers are in use.

Screening method and its cutoff for positive results

Four types of screening methods (ARR with PAC, ARR alone, PAC with PRA or ARC, ARR with PAC, and PRA) are used. Most of the centers use ARR in combination with PAC or ARR alone. A combination of PAC and PRA or ARC and ARR, PAC, and PRA is not common (Figure 2). ARR was calculated by PAC and PRA in 12 centers (Table 2A) and by PAC and ARC in 19 centers (Table 2B). Units of ARR were expressed in either SI units or non-SI units. The ARR cutoff varied among centers. The variation is partly attributed to different assay methods of PAC as a numerator and renin as a denominator (PRA or ARC). The cutoff varied among centers even with the assay methods of the same principle of PAC and renin. Differences between centers were 4- to 5-fold in ARR with PRA (Table 2A) and ARC (Table 2B). Table 2C summarizes the PAC cutoff used for the screening. The PAC cutoff, which is used in combination with ARR for screening, varied among centers, ranging from 50 pg/mL (140 pmol/L) to 216 pg/mL (600 pmol/L), with a difference of more than 4-fold.

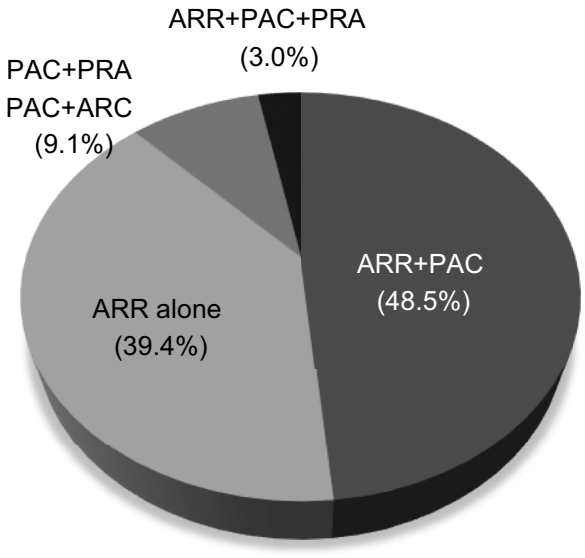

Figure 2. Proportion of screening methods. PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARC, active renin concentration; ARR, aldosterone-to-renin ratio.

Confirmatory tests

Most centers conduct only 1 confirmatory test (72.7%), while 21.2% of the centers routinely conduct 2 confirmatory tests. Most centers in the ENS@T use saline infusion test (SIT, 72.4%) as the first-line confirmatory test, followed by CCT (21.2%). All centers in Japan and 2 centers in Italy use CCT as the first-line test. Dexamethasone SIT or Dexamethasone captopril, valsartan test, and fludrocortisone dexamethasone suppression test (FDST)[19] was used in a limited number of centers (6%) (Table 3). Centers that use SIT for the first-line test used CCT for the second-line test. All centers in Japan use SIT as the second-line test. Other tests are used as the second-line test in a small number of centers (Table 3).

The protocol and the cutoff of the SIT

SIT is performed by loading 2 L of saline over 4 hours. Most centers perform SIT in a seated position,[20] while one-third of the centers perform it in a supine position. Most centers use 4 hours after saline loading as the timing for decision-making, while a small number of centers also use 2 hours (Figure 3A). The cutoff of PAC varied among centers, ranging from 50 pg/mL (140 pmol/L) to 100 pg/mL (280 pmol/L). The cutoff of PAC for SIT varied even with the same assay methods (CLEIA, RIA, and LC-MS/MS) (Table 4). Some centers set boundary areas with different terminology: 70–100 pg/mL (positive) by LC-MS/MS, 50–100 pg/mL (possible) by LC-MS/MS, 50–100 pg/mL (intermediate, likely, possible) by CLEIA, and 12–60 pg/mL (borderline) by CLEIA, respectively.

The protocol and the cutoff of the CCT

Most centers use 50 mg captopril for CCT, while limited centers use 25 mg. The timing of blood sampling for decision-making varied among centers: 60 min, 90 min, 120 min, or their combination (Figure 3B). Centers in Japan, where CCT is the first-line test, use ARR with the cutoff of 200 pg/mL/ng/mL/h (PRA) or 40 pg/mL/pg/mL (ARC), and 1 center in Italy uses ARR with the cutoff of 91 pmol/L/mIU/L. Centers in the ENS@T where CCT is the second line test define the result as positive when a decrease in PAC after CCT is less than 30% of the basal line.

Table 2. Current status of the ARR and PAC cutoff.

A. The ARR cutoff by PAC and PRA for screening in 12 centers		
pg/mL/ng/mL/h	**pmol/L/ng/mL/h**	**No. of centers**
100-200 (borderline)	277-555[a]	7[b]
198[a]	550	1
200	555[a]	7[b]
300	832[a]	1
360[a]	1000	2
400	1110[a]	1
Range: 100-400	Range: 277-1110	Total no. of centers:12
B. The cutoff of ARR by PAC and ARC for screening in 19 centers		
pg/mL/mIU/L	pmol/L/mIU/L	No. of centers
10.8[d]	30	1
11.5	31.9[d]	1
12	33.3[c,d]	3
12-24[d] (borderline)	33.3-66.6[c,d]	1[e]
12.6[d]	35	2
13.0[d]	36	1
17.3[d]	47.9[c]	1
21	58.3[d]	1
21.6[d]	60	2
23.1[d]	64	1
23.8[d]	65.9[c]	1
24	66.6[d]	3[e]
27.0-36.0[d]	75-100[d] (possible)	1[f]
36.0[d]	100	1[f]
49.0	135.9[d]	1
Range: 10.8–49.0	Range: 30–135.9	Total no. of centers: 19
C. The PAC cutoff for screening		
PAC[g]	No.	Details
50–100 pg/mL (140–280 pmol/L)	$N = 7$	50 pg/mL (140 pmol/L) ($n = 1$) 60 pg/mL (170 pmol/L) ($n = 6$)
100–150 pg/mL (280–420 pmol/L)	$N = 8$	100 pg/mL (280 pmol/L) ($n = 3$) 108 pg/mL (300 pmpl/L) ($n = 2$) 120 pg/mL (330 pmol/L) ($n = 1$) 150 pg/mL (420 pmol/L) ($n = 3$)
>200 pg/mL (>550 pmol/L)	$N = 3$	200 pg/mL (550 pmol/L) ($n = 2$) 216 pg/mL (600 pmol/L) ($n = 1$)
Range: 50–200< pg/mL (140–550<)		Min: 50 pg/mL (140 pmol/L) Max: 216 pg/mL (600 pmol/L)

[a]PAC unit was converted from pmol/L to pg/mL or vice versa (1000 pmol/L = 360 pg/mL) (http://unitslab.com/).
[b]Seven centers use 200 pg/mL/ng/mL/h as the ARR cutoff and 100-200 pg/mL/ng/mL/h as the borderline range.
[c]ARC unit converted from ng/L to mIU/L (mIU/L = ng/L × 1.67).
[d]PAC unit converted from pmol/L to pg/mL (1000 pmol/L = 360 pg/mL).
[e]One center use 24 pg/mL/mIU/L as the ARR cutoff and 12-24 pg/mL/mIU/L as the borderline range.
[f]One center use 36 pg/mL/mIU/L as the ARR cutoff and 27-36 pg/mL/mIU/L as the borderline range.
[g]PAC unit was converted from pmol/L to pg/mL or vice versa (1000 pmol/L = 360 pg/mL) (http://unitslab.com/).

Bypassing the confirmatory test before adrenalectomy

Most centers (78.8%) think they can bypass the confirmatory test under certain conditions.[8,9,11,12] In contrast, one-fifth of the centers never bypassed the test, considering the high variability of the PAC and renin and the benefit of obtaining information on the severity of the hyperaldosteronism. Most centers selected hypokalemia (<3.5 mEq/L: 68.8%; <3.0 mEq/L: 25%; <3.2 mEq/L: 6.3%), high PAC (>200 pg/mL: 68.8%; others [<150-<1000]: 31.2%), high ARR, and suppressed renin (below the detection limit) as the essential factor for bypassing the test. Young age (definitions varied between centers from <30, <35, <40, and <50 years old, respectively) and adrenal tumor on CT (>1.0-1.5 cm) were also selected as conditions (Figure 4).

Table 3. Current status of the confirmatory tests.

Confirmatory test	SIT	CCT	DSIT or DCVT	FDST	OSLT	FUT	Posture test
First line	24/33 (72.4)	7/33 (21.2)	1/33 (3.0)	1/33 (3.0)	-	-	-
Second line	7/33 (25.0)	14/33 (50.0)	-	2/33 (7.2)	3/33 (10.7)	1/33 (3.5)	1/33 (3.5)

Numbers and percentages (in parenthesis) are shown.
Abbreviations: CCT, captopril challenge test; DSIT, dexamethasone saline infusion test; DCVT, dexamethasone, captopril and valsartan test; FDST, fludrocortisone suppression test; FUT, furosemide upright test; OSLT, oral salt loading test; SIT, saline infusion test.

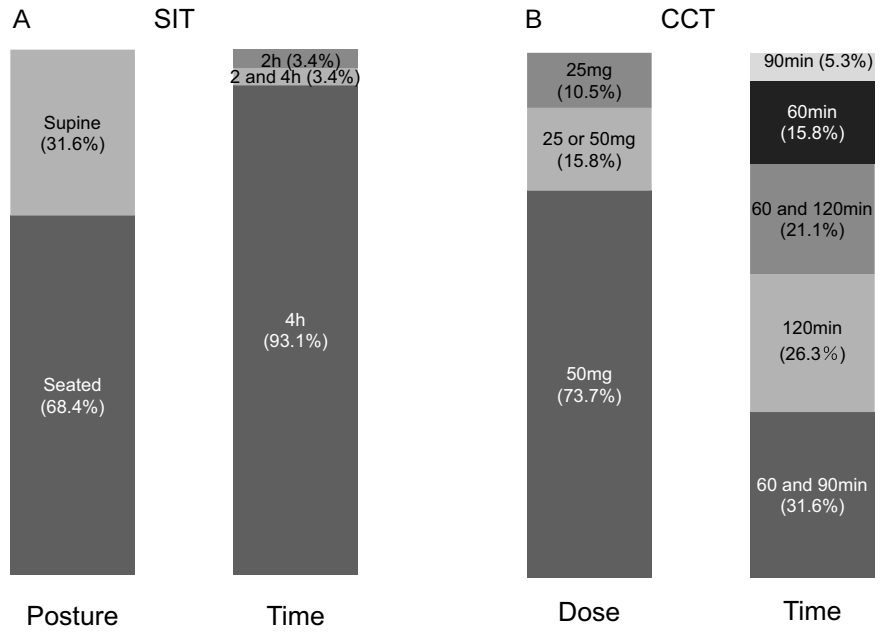

Figure 3. (A) The methods and the timing of the decision-making of the SIT and (B) CCT. Saline infusion test; CCT, captopril challenge test.

Expert opinion on the feasibility of standardization of the screening and confirmatory tests

Since the first-round questionnaire demonstrated the significant diversity of the screening and confirmatory tests, we have conducted the second-round questionnaire on the feasibility of future standardization in the diagnostic process of screening and confirmatory tests. The questionnaire included PAC and renin assay methods, the screening methods and their cutoff values, and the confirmatory test type and cutoff type (Table 5). The answer of "possible" was most common in all the items of the questions. Feasibility, including the answer "easy" and "possible," occupied most of the answers, with the screening methods as the highest (80.0%) and the cutoff values of the screening methods as the lowest (53.3%). No experts chose the answer of 'impossible' to standardize the screening and confirmatory tests.

Discussion

We have demonstrated a significant diversity of screening and confirmatory testing in the various aspects of diagnosing PA: conditions of blood sampling, assay methods for aldosterone and renin, and the methods of screening and confirmatory testing (Figure 5). This is the first report detailing the heterogeneity of approaches to PA diagnosis based on a large international survey. The heterogeneity of diagnostic methods for screening and confirmation of PA was partly due to the lack of specific documentation in the current guidelines.[8-12] The cutoff value of ARR was not specified in the Endocrine Society clinical guideline[8] and ESH guideline,[11] in which 20–40 (ng/dL/ng/mL/h) was shown as the range of cutoff value when PRA was used for calculation. This vague documentation reflects the current unstandardized situation of the PA screening method. The lack of a golden standard for confirmatory tests was also evident. For example, the Endocrine Society clinical guideline[8] recommends 4 confirmatory tests: the CCT, SIT, OSLT, and FDST. However, the guidelines of the Japan Endocrine Society recommend the CCT, SIT, OSLT, and FUT.[9,12] In addition, the cutoff values of CCT, SIT, and OSLT diverge between both guidelines.[8,9,12] Our survey helped to understand which confirmatory tests were chosen and performed in the daily clinical activity in the referral centers.

Table 4. Current status of the SIT cutoff.

Assay methods of PAC[a]			Cutoff	
LC-MS/MS	CLEIA	RIA	pg/mL	pmol/L
2	7	1	100	280
	1		75	210
1		1	68.4	190
2	9	2	60	170
	3	1	50	140

[a]No. of centers.
Abbreviations: CLEIA, chemiluminescent enzyme immunoassay; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PAC, plasma aldosterone concentration; RIA, radioimmunoassay.

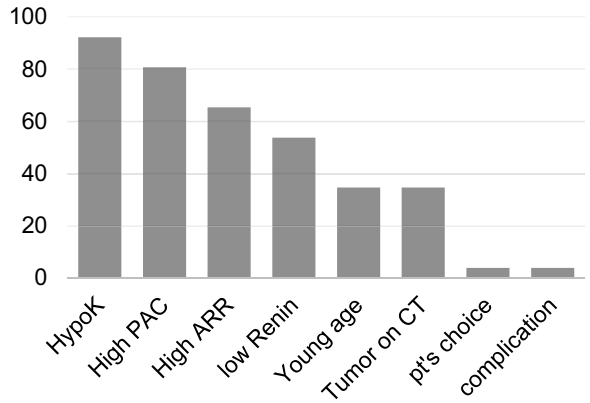

Figure 4. Conditions to bypass the confirmatory test. PAC, plasma aldosterone concentration; ARR, aldosterone-to-renin ratio.

Table 5. Expert opinion on the feasibility of harmonization of the assay methods, screening, and confirmatory test.

	Assay methods		Screening		Confirmatory test	
Expert opinion on the feasibility	PAC	Renin	Methods	Cutoff	Type	Cutoff
(1) Easy	6.6%	3.2%	13.3%	10.0%	16.7%	13.3%
(2) Possible	56.7%	53.3%	66.7%	43.3%	43.3%	53.3%
(3) Difficult	36.7%	43.3%	20.0%	43.3%	36.7%	30.0%
(4) Impossible	0%	0%	0%	0%	0%	0%
(5) Not needed	0%	0%	0%	3.4%	3.3%	3.4%
Feasibility[a]	63.3%	56.5%	80.0%	53.3%	60.0%	66.6%

[a]Expressed as the sumup of (1) and (2).

Strictly defined conditions have been recommended for blood sampling of PAC and renin to avoid false-negative and false-positive results. However, half of the centers adopted moderate definitions which include modifying antihypertensive medications and posture as the major conditions. Antihypertensive drugs were changed to CCBs and/or alpha-blockers as recommended by the guidelines.[8-12]

Most centers used the seated position rather than the supine position for basal ARR samples. Blood sampling was conducted mainly in the morning, especially between 8 AM and 9 AM. In addition, fasting conditions, potassium repletion if hypokalemic, and unrestricted salt intake were also included as additional conditions. Considering the feasibility of routine clinical practice and many patients potentially needing the screening, a moderate definition of these conditions seems realistic and achievable. However, the comprehensive effects of the diverse conditions on the screening results cannot be simplified. Whether there is any significant difference in the clinical outcome between the strict and moderate definitions of the conditions of blood sampling remains to be elucidated.

Although the determination of PAC and renin is critical in diagnosing PA, assay methods are diverse. RIA, the primary method during the last quarter-century, has been replaced by non-RIA methods such as ELISA and CLEIA in line with the global trend of reducing the use of radioactive materials. As expected, the present survey demonstrated that CLEIA is PAC's most common assay method, while various kits (CLEIA kits: $n=6$; RIA: $n=4$) were used. Any immunoassay could have some limitations related to the non-specific interaction of the antibodies with plasma-interfering substances, resulting in an overestimation of the true values. Accordingly, measured values by RIA[21] and CLEIA[22] have been demonstrated to be significantly higher than those by LC-MS/MS, an ideal method for approximating the real value. However, the widespread usage of LC-MS/MS has challenges regarding available facilities and is expensive. Pretreatment of

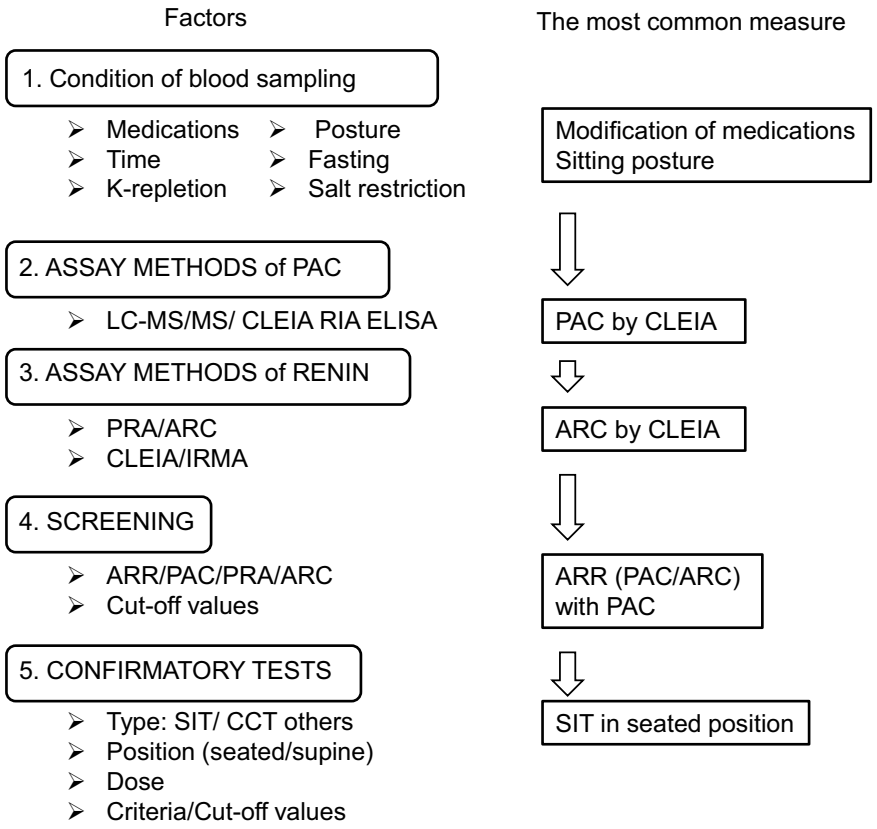

Figure 5. Summary of the factors underlying the diversity of the diagnosis of PA and the most common steps from the present survey.

plasma to remove interfering macromolecules before immunoassay could be a simple solution,[22] although its feasibility in routine laboratory practice remains to be established. The recently developed CLEIA kits have shown good traceability to certified reference materials of aldosterone (NMIJ CRM 64026402, the National Metrology Institute of Japan)[23] and good correlation with LC-MS/MS results.[24] Establishing the traceability to the certified reference material of aldosterone and correlation with LC-MS/MS will reduce the disparity between different assay methods and diversity among centers.

Although PRA measured by RIA has been commonly used, it has recently been replaced by the ARC in many centers. ARC can save time by bypassing the plasma incubation step. In addition, most centers use CLEIA which enables simultaneous measurement of PAC and ARC, leading to efficient diagnosis. Although various kinds of CLEIA kits and IRMA kits from different manufacturers are used in the centers, the correlation between these different kits remains unknown.

The methods of screening included the judgment index and their cutoff values. ARR reflected the typical endocrine feature of PA, increased aldosterone, and a suppressed renin status and contributed substantially to the effective screening of PA.[25,26] However, since accurate quantification of renin is difficult in the low range, false-positive results could be derived from a significant influence of renin as the denominator. To avoid the caveat of possible false-positive results, combining ARR with PAC has been advocated as the screening criteria.[8,9,11,12,27] Accordingly, it was demonstrated in the present survey that most centers use ARR in combination with PAC over the ARR alone. However, it should be noted that the combination of ARR and PAC could be considered redundant because of the duplicate use of PAC in the same index. Since PAC and renin measurements' sensitivity, specificity, and accuracy have improved dramatically in recent years, it could be one possibility that using optimal cutoffs for each of PAC and renin instead of ARR may help to simplify and standardize the screening method. In addition, the variety of the cutoff of the screening methods was prominent. The first factor was the different methods evaluating renin as the denominator, PRA, and ARC, respectively. Although provisional conversion factor from ARC to PRA has been used in clinical practice, these 2 parameters are based on qualitatively different assay principles; therefore, exact conversion and direct comparison between ARRs by PRA and ARC are not feasible.[28,29] The second factor was that the ARR cutoffs used for screening differed 4-fold regardless of ARC or PRA as a denominator.

Confirmatory tests play an essential role in diagnosing PA by proving renin-independent aldosterone secretion. Clinical practice guidelines recommend at least 1 positive test.[8,9,11,12] Accordingly, most centers conduct only 1 confirmatory test, while 30% of the centers routinely conduct 2 or 3 tests, including when the first test is negative. Of the confirmatory tests, most centers use the SIT as the first-line test, followed by CCT. CCT is used as the second-line test in patients when the SIT is negative, and SIT is used as the second-line test when CTT is negative. Other tests, including FDST, posture test, and FUT, are used in a limited number of centers. Most centers conduct SIT in a seated position to avoid a false-negative result,[20] while 30% of the centers perform this test in a supine position. The cutoff of PAC 4 hours after 2 L saline loading for judgment found a 2-fold difference from 50 pg/mL to 100 pg/mL, regardless of assay methods.

In contrast to the SIT and CCT, no centers participated in the present survey used the oral salt loading test (OSLT) with urine aldosterone excretion as the index. The results are quite in contrast to the conditions in the United States, where OSLT is commonly used in Mayo Clinic[30] and many other centers. Although the reason underlying the difference remains unknown, it may be related to difficulties in accurately collecting urine for 24 hours and measuring urine aldosterone concentration in addition to the different medical insurance systems and the experts' opinions. Whatever the reasons, establishing a single confirmatory test that is easy to conduct, has fewer complications, and has better specificity is highly desirable.

The diversity of the various aspects of screening methods (conditions of blood sampling, assay methods, indices) and the confirmatory test (type and condition of test, indices, and cutoff values) is expected to result in a quantitatively and qualitatively heterogeneous diagnosis of PA between centers. Although the diversity may not significantly impact individual patients as far as the clinical practice is completed in 1 center, it may result in an inconsistent diagnosis, PA or non-PA, in patients with hypertension between centers. The impact of this diversity could be more significant, especially in PA patients with the mild phenotype (mild PA) with normal serum potassium concentrations, a mild degree of PAC elevation, no nodules on CT, and bilateral subtype by AVS.[12] PAC levels were shown to vary considerably in patients with PA and may require repeated sampling for diagnosis.[31] A strict definition of blood sampling, the combination of ARR with PAC, and a higher cutoff of the positive results in the screening and the confirmatory test increase the specificity and decrease the sensitivity of the diagnosis. If the case of the opposite conditions, the opposite effect will be obtained. Better specificity may risk missing PA patients, especially those with mild PA. In contrast, better sensitivity may increase the number of patients with suspected PA followed by confirmatory testing and imaging studies. Which condition, index, and cutoff are superior in clinical practice remains unknown.

In addition, the diversity of the diagnosis potentially impacts the results of clinical studies and their wider applicability. It may interfere with comparing the results from different centers and clinical practice guidelines based on the manuscripts as evidence. Therefore, standardization of the diagnostic process of the screening and the confirmatory test is highly desirable from the global point of view of the clinical practice of PA. We, therefore, conducted the second round of questionnaires of the experts' opinions on the feasibility of the future standardization of the screening and confirmatory testing. Feasibility combining the answer "easy" and "possible" occupied most of the answers, with the screening methods being the highest and the cutoff values of the screening methods the lowest. Following these positive perspectives, we suggest large-scale prospective and comparative international studies with outcome data to standardize the screening and confirmatory methods for PA diagnosis in the near future.

Even mild PA has been suggested to result in excess morbidity.[32] Until the clinical entity and concept of the pathology of bilateral PA with mild aldosterone excess are established, however, the cutoff of the screening and confirmatory tests should focus on the specificity and sensitivity of the lateralized PA. Quantitative investigation of the post-surgical outcomes based on the PASO criteria is essential.[33] In addition, those in the range between the dissociated cutoff are recommended

to be designated as borderline PA,[12] treated with MR antagonist, and subjected to long-term follow-up.

Limitation

Our survey has some limitations. First, the present survey is based on a questionnaire to clinicians in referral centers for PA in several European countries, Canada, south-east Asia, and Japan. The number of experts and centers was limited and may not necessarily represent all the experts and centers. In addition, the selection of the experts and centers was not randomized but selected by the hand-raising method among the ENS@T centers. All these could potentially bias all the estimates. Second, the results are cross-sectional and do not reflect changes over time. Details of the clinical practice of PA by the experts and centers are predicted to change with time. The results only reflect the expert's opinion at the time of the survey and do not always reflect a permanent state. However, the diversity demonstrated in the present survey is significant and notable for the clinical practice of PA. Besides the importance and feasibility of potential standardization, a periodical survey of the diagnostic process of screening and confirmatory testing would be helpful.

Conclusion

In the present SCOT-PA survey, we have demonstrated a significant diversity in the various aspects of diagnosing primary aldosteronism. The prominent diversity was recognized in the conditions of blood sampling, assay methods of aldosterone and renin, and the methods and the cutoff of screening and confirmatory tests. The most common measure was modifying antihypertensive drugs and sitting posture for blood sampling, PAC, and ARC by CLEIA, a combination of ARR with PAC as an index for screening and SIT in a seated position for a confirmatory test (Figure 5). The cutoff values for screening and SIT showed significant variation among centers. Diagnostic diversity might have an enormous impact in patients with mild PA. However, most experts were optimistic about a possible future standardization of each step. Further step-by-step standardization of the diagnostic process will help improve the quality of evidence and clinical practice of PA.

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Author Contributions

Planning of research and preparation of protocol: M.N., M.M., M.Q., J.D., A.L. Providing expert's opinion and information about the current condition of diagnosis of primary aldosteronism: M.N., M.M., T.K., T.Ko., M.P.C., M.Q., M.S., S.O., F.C., I.K., D.K., M.T., J.D., E.M.T., T.P., A.M., G.P., K.L., N.W., M.A.G., H.K., A.T., C.V.T., N.V.G., S.G., F.B., L.K., N.S., E.A.A., O.R., M.F.N., G.M., G.D.D., V.K., A.L., and R.M.F. Guiding the methods of survey and handling the statistics: T.S. Drafting the manuscript: M.N., M.M., T.Ko., M.Q., S.O., and A.L. Writing the manuscript and approval of the submitted version: M.N., M.M., T.K., T.Ko., M.P.C., M.Q., M.S., S.O., F.C., I.K., D.K., M.T., J.D., E.M.T., T.P., A.M., G.P., K.L., N.W., M.A.G., H.K., A.T., C.V.T., N.V.G., S.G., F.B., L.K., N.S., E.A.A., O.R., M.F.N., G.M., G.D.D., V.K., A.L., R.M.F., and T.S.

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Data Availability

The data sets generated during and/or analyzed during the current study are not publicly available but from the corresponding author on reasonable request.

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