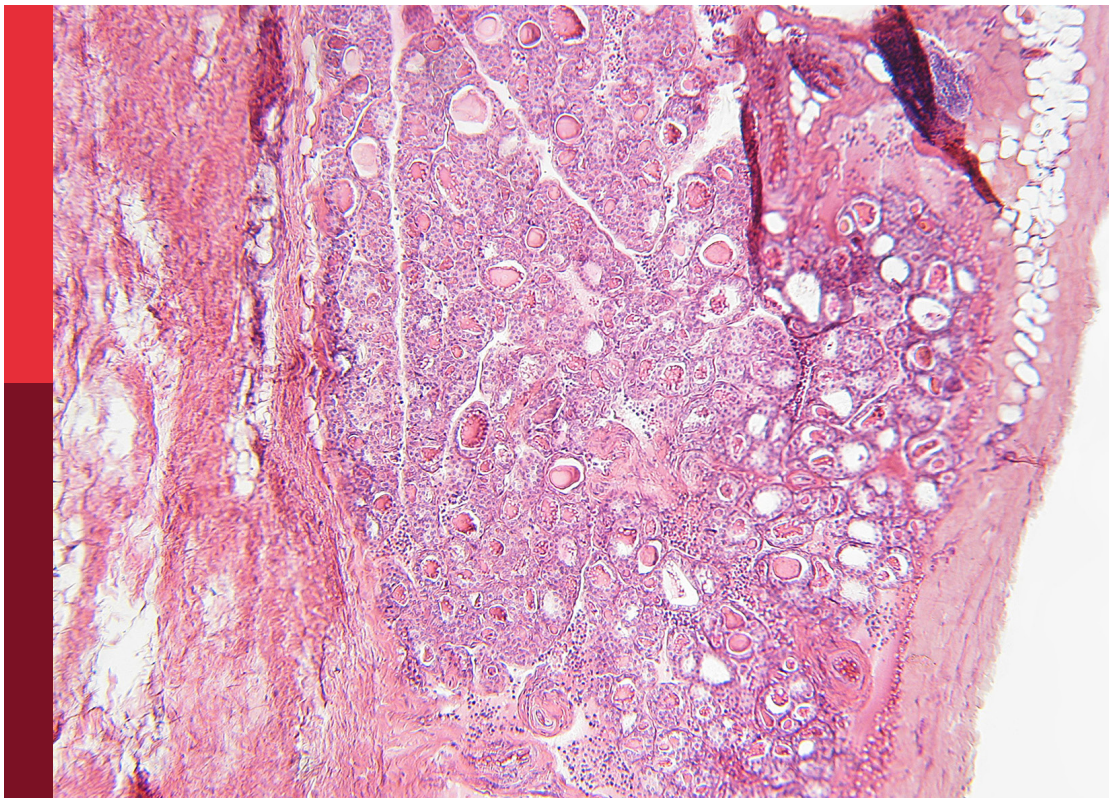
frontiers | Research Topics

Adrenal related hypertension: from bench to bedside, volume II

Edited by
Elena Aisha Azizan, Norlela Sukor, Carmela Maniero and Brasilina Caroccia

Published in
Frontiers in Endocrinology



ISSN 1664-8714
ISBN 978-2-8325-6623-7
DOI 10.3389/978-2-8325-6623-7

Generative AI statement
Any alternative text (Alt text) provided alongside figures in the articles in this ebook has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Adrenal related hypertension: from bench to bedside, volume II

Topic editors

Elena Aisha Azizan — National University of Malaysia, Malaysia
Norlela Sukor — Universiti Kebangsaan Malaysia Medical Center (UKMMC), Malaysia
Carmela Maniero — Barts Health NHS Trust, United Kingdom
Brasilina Caroccia — University of Padua, Italy

Citation

Azizan, E. A., Sukor, N., Maniero, C., Caroccia, B., eds. (2025). *Adrenal related hypertension: from bench to bedside, volume II*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-6623-7

Table of contents

frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 03 July 2025
DOI 10.3389/fendo.2025.1647120

OPEN ACCESS

EDITED AND REVIEWED BY
Henrik Falhammar,
Karolinska Institutet (KI), Sweden

*CORRESPONDENCE
Elena Aisha Azizan
elena.azizan@ukm.edu.my

RECEIVED 14 June 2025
ACCEPTED 18 June 2025
PUBLISHED 03 July 2025

CITATION
Aminuddin A and Azizan EA (2025) Editorial: Adrenal related hypertension: from bench to bedside, volume II. *Front. Endocrinol.* 16:1647120. doi: 10.3389/fendo.2025.1647120

Editorial: Adrenal related hypertension: from bench to bedside, volume II

Amnani Aminuddin[1] and Elena Aisha Azizan[1,2]*

[1]Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia, [2]Research Center, Hospital Tunku Ampuan Besar Tuanku Aishah Rohani, Universiti Kebangsaan Malaysia Specialist Children's Hospital, Kuala Lumpur, Malaysia

KEYWORDS

endocrine hypertension, adrenal, aldosterone, primary aldosterone, hypertension, secondary hypertension

Editorial on the Research Topic
Adrenal related hypertension: from bench to bedside, volume II

Hypertension secondary to adrenal disorders remains a major risk factor for cardiovascular diseases and death (1, 2). Primary aldosteronism (PA), the most common form of adrenal related hypertension, is frequently asymptomatic and underdiagnosed, highlighting the need for improved screening and diagnostic strategies (3). Furthermore, understanding the causes is key to improving its management (4, 5). This Research Topic compiles 4 review articles and 8 original research articles that explore new diagnostic and prognostic approaches, as well as new insights into the pathogenesis of adrenal related hypertension.

While current PA guidelines recommend measuring the plasma ARR, the prevalence of positive screening varies due to unstandardized protocols (6). The discovery of aldosterone driver mutations in both normotensive (in aldosterone-producing micronodules of normotensive adrenal glands that increase with age) and hypertensive individuals, suggest that PA exists along a continuum of disease progression rather than being limited to distinct subtypes (7). In light of these, Kitamoto et al. reviews the need to refine current strategies for screening PA to improve detection and address underdiagnosis. They correlate low renin levels with increased cardiovascular risk from dysregulated aldosterone production. Accordingly, they propose that optimal screening should be conducted in all hypertensive patients and begin with plasma renin activity (PRA) evaluation, using a low PRA (<1.0 ng/mL/h) and high plasma aldosterone concentration (PAC; >12 ng/dL).

Our review (Aminuddin et al.) summarizes current knowledge on adrenal cortex homeostasis regulation, offering insights into PA pathogenesis and the rationale for using aldosterone synthase (CYP11B2) inhibitors as treatments for patients who are not candidates for surgery or have experienced surgical failure. The review identifies key factors disrupting adrenocorticoids homeostasis, including aldosterone driver mutations, contributing to the development of CYP11B2-positive adrenal cortical neoplasms. Given the key role of CYP11B2 in PA, the review compiles pharmacological strategies targeting this enzyme and explores how CYP11B2 inhibition impacts adrenal cell fate to ensure the safety and efficacy of the treatments.

Xiang et al.'s comprehensive review, discusses key factors that may predict the prognosis following adrenalectomy. This is especially important for closer monitoring in patients with a poorer prognosis. Preoperative factors such as lower body mass index, female sex, younger age, shorter duration of hypertension, larger nodule on imaging, and low periadrenal adipose tissue volume are associated with clinical success. Variant adrenal venous anatomy complicates adrenalectomy. The classical type of unilateral PA (UPA) had a better prognosis compared to the non-classical type. Lastly, genetic features may have variable effects on prognosis.

In the systematic review McCarthy et al., the authors selected three single-center studies from Japan, Singapore, and China to determine the prevalence of PA among patients with stroke or transient ischemic attack (TIA). From the meta-analysis, the pooled PA prevalence in adults with stroke or TIA is not uncommon with 5.8%. Though, the statistical heterogeneity was high, with an I^2 statistic of 87.6%. This heterogeneity could be due to differences in patient age, the timing of PA testing after acute cerebral event, aldosterone-to-renin ratio (ARR) thresholds, and confirmatory testing methods, as well as the confounding effects of antihypertensive drugs.

Interestingly, Jiang et al. reported that UPA with cortisol co-secretion is not uncommon in the Chinese population. Notably, the combination of excess aldosterone and cortisol increases the risk of cardiovascular diseases compared to UPA without cortisol secretion, underlining the need for corticol co-secretion screening and appropriate management of UPA with cortisol co-secretion to optimize patient outcomes. They found that UPA patients with cortisol co-secretion had distinct clinical characteristics and had a lower chance of achieving complete clinical success. They were older, had a longer history of hypertension, larger adrenal tumors, and were more responsive to ACTH.

Meanwhile, Sun et al. compared the diagnostic efficacy of the saline infusion test and captopril challenge test (CCT) for PA. Their findings suggest that CCT had higher diagnostic value based on post-CCT PAC suppression. Importantly, the optimal cutoff for post-CCT PAC suppression differed for patients under and over 50 years old in the Chinese population. This supports the need for personalized diagnostic approaches in PA.

Whereas the original research by Yin et al. reported that the non-invasive ^{68};Ga-Pentixafor Positron Emission Tomography/Computed Tomography (PET/CT) procedure was an efficient method for diagnosing PA compared to adrenal vein sampling (AVS) with sensitivity 89% vs. 79%. Moreover, the PET/CT identified 94% of patients who achieved complete biochemical and clinical success after adrenalectomy, compared to 78% identified by AVS, suggesting enhanced predictive accuracy of PET/CT. Of note, cases with unclear subtyping diagnoses based on AVS results were included in this study. Yin et al.'s findings is supported by another study in a Chinese cohort, that found ^{68}Ga-pentixafor PET/CT to identify the dominant side of aldosterone secretion in PA with an accuracy rate similar to that achieved by AVS (85.7% vs. 71.4%) (8).

In a retrospective study, ter Haar et al. suggested a clinical decision model to subtype PA, particularly when right adrenal vein cannulationin AVS is unsuccessful. They proposed a decision index with a specificity exceeding 90% by using the ratio of aldosterone to cortisol from the left adrenal vein (LAV) and the inferior vena cava (IVC). According to their model: (1) an LAV/IVC index <1.2 suggests unilateral right-sided PA and supports right adrenalectomy; (2) a ratio between 1.2 and 2.4 indicates bilateral disease, thus mineralocorticoid receptor antagonist (MRA) therapy is advised; (3) an index ≥4.4 suggests unilateral left-sided PA, indicating left adrenalectomy; and (4) a resampling is advised only when the index is between 2.4 and 4.4.

In context of pathophysiological of PA, Nanba et al. investigated the potential association of double *CTNNB1* and *GNA11/Q* mutations in aldosterone-producing adenomas with pregnancy, menopause or puberty through a case study of a Japanese female patient with UPA, who also had high mRNA expression of luteinizing hormone/choriogonadotropin receptor (*LHCGR*) and gonadotropin-releasing hormone receptor (*GNRHR*). Despite experiencing menopause-like symptoms, the patient had regular menstrual cycles and no history of pregnancy-induced hypertension. They concluded that the disease can occur without a clear association with pregnancy or menopause.

Recent studies support the need to revisit the concept of mild PA (6). Herein, between 2017 – 2022, Makhnov et al. performed a large-scale screening for PA in an unselected cohort of primary care patients with hypertension in Sweden, aged 18–65 years according to the Endocrine Society guidelines (9). Among 1181 recruited patients, the PA prevalence among hypertensive patients was 4.5%, consistent with range reported in the Endocrine Society Guidelines (~ 5 – 13%). Importantly, they observed that the ARR≥50 pmol/mIU as an optimal diagnostic cut-off and recommended routine PA screening in hypertensive patients to improve patient management and reduce risk of morbidity and mortality.

The significant role of non-defining adrenal steroids in the metabolic alterations and comorbidities observed in endocrine hypertension, including pheochromocytoma/paraganglioma (PPGL), Cushing's syndrome, and PA was investigated by Knuchel et al. study. The retrospective analysis of 263 patients revealed that metabolomic profiles are not only influenced by disease-defining hormones, but also by other adrenal steroids. In PPGL, metabolomic changes were driven by catecholamine excess. In Cushing's syndrome, cortisol along multiple non-defining adrenal hormones contributed to metabolomic variation. In PA, non-aldosterone steroids like cortisol, cortisone, and dehydroepiandrosterone showed stronger associations compared to aldosterone.

Finally, Raber et al.'s study involving 303 patients with PPGL assessed long-term and survival outcomes. They found that overall survival and disease-specific survival (DSS) were generally favourable, especially in non-metastatic cases. Only 5% of all deaths were directly attributed to PPGL, with the remaining deaths caused by cardiovascular disease and other malignancies. Patients with metastases at diagnosis had the poorest outcomes, while those with non-metastatic recurrences had much longer

survival. Major adverse cardiovascular events occurred before diagnosis in 15% of patients and were strongly associated with shorter survival. Predictors of shorter DSS include older age, male sex, history of major adverse cardiovascular events, and primary metastatic disease.

In summary, this Research Topic highlights the evolving adrenal hypertension research, focusing on precise diagnostics, molecular analysis, and physiologically-informed screening to improve clinical care. The need for more personalized, evidence-based approaches is crucial. Whereas multi-center studies are needed to validate emerging screening strategies and therapies.

Author contributions

EA: Conceptualization, Supervision, Writing – review & editing, Resources. AA: Data curation, Conceptualization, Writing – original draft, Investigation.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. AA is supported by the Ministry of Higher Education (MOHE) Malaysia, a Long-term Research Grant Scheme-Jejak Sarjana Ulung (LRGS-JSU), LRGS/1/2021/SKK15/UKM/02/2 of which EAA is the principal investigator.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

References

1. Meng Z, Dai Z, Huang K, Xu C, Zhang Y-G, Zheng H, et al. Long-term mortality for patients of primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Front Endocrinol (Lausanne).* (2020) 11:121. doi: 10.3389/fendo.2020.00121

2. Patil S, Rojulpote C, Amanullah A. Primary aldosteronism and ischemic heart disease. *Front Cardiovasc Med.* (2022) 9:882330. doi: 10.3389/fcvm.2022.882330

3. Fernandez CJ, Nagendra L, Alkhalifah M, Pappachan JM. Endocrine hypertension: the urgent need for greater global awareness. *touchREVIEWS Endocrinology.* (2023) 19:11. doi: 10.17925/EE.2023.19.2.11

4. Tetti M, Gong S, Veglio F, Reincke M, Williams TA. Primary aldosteronism: pathophysiological mechanisms of cell death and proliferation. *Front Endocrinol (Lausanne).* (2022) 13:934326. doi: 10.3389/fendo.2022.934326

5. Azizan EAB, Drake WM, Brown MJ. Primary aldosteronism: molecular medicine meets public health. *Nat Rev Nephrol.* (2023) 19:788–806. doi: 10.1038/s41581-023-00753-6

6. Naruse M, Murakami M, Katabami T, Kocjan T, Parasiliti-Caprino M, Quinkler M, et al. International multicenter survey on screening and confirmatory testing in primary aldosteronism. *Eur J Endocrinol.* (2023) 188:125–34. doi: 10.1093/ejendo/lvac002

7. Omata K, Anand SK, Hovelson DH, Liu C-J, Yamazaki Y, Nakamura Y, et al. Aldosterone-producing cell clusters frequently harbor somatic mutations and accumulate with age in normal adrenals. *J Endocr Soc.* (2017) 1:787–99. doi: 10.1210/js.2017-00134

8. Zheng G, Ding J, Gao Y, Liu S, Yan X, Wang W, et al. 68Ga-pentixafor PET/CT in guiding surgical management of primary aldosteronism. *J Clin Transl Endocrinol.* (2025) 39:100384. doi: 10.1016/j.jcte.2025.100384

9. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* (2016) 101:1889–916. doi: 10.1210/jc.2015-4061

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 14 February 2024
DOI 10.3389/fendo.2024.1291775

OPEN ACCESS

EDITED BY
Valentina Morelli,
Istituto Auxologico Italiano, Italy

REVIEWED BY
Peter James Fuller,
Hudson Institute of Medical Research, Australia
Fabrizio Buffolo,
University of Turin, Italy

*CORRESPONDENCE
Yuan Li
yuanlixy@csu.edu.cn
Yunhua Wang
wangyunhua0801@csu.edu.cn
Xin Su
suxin71@csu.edu.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 10 September 2023
ACCEPTED 24 January 2024
PUBLISHED 14 February 2024

CITATION
Yin X, Ai K, Luo J, Liu W, Ma X, Zhou L, Xiang X, Su X, Wang Y and Li Y (2024) A comparison of the performance of ^{68}Ga-Pentixafor PET/CT *versus* adrenal vein sampling for subtype diagnosis in primary aldosteronism. *Front. Endocrinol.* 15:1291775. doi: 10.3389/fendo.2024.1291775

A comparison of the performance of ^{68}Ga-Pentixafor PET/CT *versus* adrenal vein sampling for subtype diagnosis in primary aldosteronism

Xuan Yin[1†], Kai Ai[2†], Jianguang Luo[3†], Wei Liu[4], Xiaowei Ma[1], Lianbo Zhou[1], Xin Xiang[1], Xin Su[4*], Yunhua Wang[1*] and Yuan Li[2*]

[1]Department of Nuclear Medicine, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China, [2]Department of Urology, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China, [3]Department of Diagnostic and Interventional Radiology, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China, [4]Department of Endocrinology, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China

Objective: To investigate the diagnostic efficiency and prognostic value of ^{68}Ga-Pentixafor PET/CT in comparison with adrenal vein sampling (AVS) for functional lateralization in primary aldosteronism (PA). Histology and long-term clinical follow-up normally serve as the gold standard for such diagnosis.

Methods: We prospectively recruited 26 patients diagnosed with PA. All patients underwent ^{68}Ga-Pentixafor PET/CT and AVS. Postsurgical biochemical and clinical outcomes of patients with unilateral primary aldosteronism (UPA), as diagnosed by PET/CT or AVS, were assessed by applying standardized Primary Aldosteronism Surgical Outcome (PASO) criteria. Immunohistochemistry (IHC) was performed to detect the expression of aldosterone synthase (CYP11B2) and CXCR4.

Results: On total, 19 patients were diagnosed with UPA; of these, 13 patients were lateralized by both PET/CT and AVS, four patients were lateralized by PET-only, and two by AVS-only. Seven subjects with no lateralization on AVS and PET received medical therapy. All patients achieved complete biochemical success except one with nodular hyperplasia lateralized by AVS alone. The consistency between PET/CT and AVS outcomes was 77% (20/26). Moreover, CYP11B2-positive nodules were all CXCR4-positive and showed positive findings on PET. Patients who achieved complete biochemical and clinical success had a higher uptake on PET as well as stronger expression levels of CXCR4 and CYP11B2.

Conclusion: Our analysis showed that ^{68}Ga-Pentixafor PET/CT could enable non-invasive diagnosis in most patients with PA and identify additional cases of unilateral and surgically curable PA which could not be classified by AVS. ^{68}Ga-Pentixafor PET/CT should be considered as a first-line test for the future classification of PA.

KEYWORDS

primary aldosteronism, ^{68}Ga-Pentixafor, PET/CT, CXCR4, endocrine hypertension

Introduction

Primary aldosteronism (PA), the most frequent cause of secondary arterial hypertension, exerts a significant impact on healthcare (1). Compared with equal grade essential hypertension, PA leads to a significantly higher extent of cardiovascular morbidity, with an increased risk of strokes, atrial fibrillation, and heart failure (2, 3). However, this condition can be cured with appropriate treatment. Adrenalectomy is recommended for patients with unilateral primary aldosteronism (UPA); this is associated with a higher rate of cure (4) while bilateral primary aldosteronism (BPA) is usually treated with mineralocorticoid-receptor antagonists (MRAs) (5). Therefore, it is essential to classify PA accurately for individually optimized therapy.

Nevertheless, only a minority of patients receive appropriate management because the diagnostic process remains challenging (6). At present, adrenal venous sampling (AVS) is widely accepted as the reference standard for functional assessment of adrenal masses (2, 7). However, not only is it an invasive and technically challenging approach, AVS also carries a risk of serious complications, thus limiting its application in clinical practice on PA subtyping (5, 8, 9).

Functional imaging in nuclear medicine, known as molecular imaging technology, has been proven to have significant potential. 11C-metomidate, as a positron emission tomographic (PET) imaging tracer, has been used for the diagnosis of primary aldosteronism in several small studies (10–17). Nevertheless, the low selectivity for CYP11B2 (aldosterone synthase) as well as CYP11B1 (11β-hydroxylase) and the short radio half-life of 11C presents challenges in this procedure (18).

The C-X-C chemokine receptor type 4 (CXCR4) is a transmembrane G protein-coupled receptor; the expression of this protein has been reported to be upregulated in aldosterone-producing tissue but is almost negligible in non-functional adenoma(NFA) (19–21). The PET tracer gallium-68 Pentixafor (^{68}Ga-Pentixafor) is a specific ligand for CXCR (22–24). Recent research demonstrated that ^{68}Ga-Pentixafor PET/CT possesses unprecedented accuracy for the detection of aldosterone-producing adenoma (APA), mainly based on histopathology (25–30). However, there are only few reports assessing the concordance of ^{68}Ga-Pentixafor PET/CT and AVS, the current gold standard. As complete biochemical success could define the correct diagnosis and appropriate treatment (31–33), in the present study, we compared PET with AVS by using biochemical outcomes as a primary quality measure of diagnosis to evaluate its efficacy for identifying functional adrenal adenomas. This study aimed to explore the potential of ^{68}Ga-Pentixafor PET as a noninvasive alternative to AVS to help guide clinical management decisions.

Abbreviations: APA, aldosterone-producing adenoma; AVS, adrenal venous sampling; BPA, bilateral primary aldosteronis; CXCR4, C-X-C chemokine receptor type 4; LCR, lesional SUVmax to the contralateral adrenal tissue SUVmean; LI, lateralization index; LLR, lesional SUVmax to the normal liver SUVmean; MAPN/MAPM multiple aldosterone-producing nodules/micronodules; MRAs, mineralocorticoid-receptor antagonists; NFA, non-functional adenoma; PA, primary aldosteronism; UPA, unilateral primary aldosteronism.

Methods

Study design and participants

The study protocol was approved by Ethics Committee of National Medical Research Center, Second Xiangya Hospital, Central South University. All patients provided written informed consent prior to ^{68}Ga-Pentixafor PET/CT.

This was a prospective clinical trial that used both ^{68}Ga-Pentixafor PET/CT and AVS to subtype PA. We prospectively recruited patients with a clinical diagnosis of PA at The Second Xiangya Hospital from the 1st of July 2022 to the 1st of December 2022. The patients were referred to us by a certified panel of specialists in clinical endocrinology. Patients were eligible if they had confirmed PA (according to guidelines published by the Endocrine Society, details are provided in the Supplementary Materials) and were scheduled for adrenalectomy (33–36). The exclusion criteria were as follows: 1) a suspicion of adrenocortical carcinoma; 2) familial PA due to germline mutations, and 3) contraindications to isotope scanning, for example pregnancy or claustrophobia. Finally, 26 patients were included in our analysis (Figure 1).

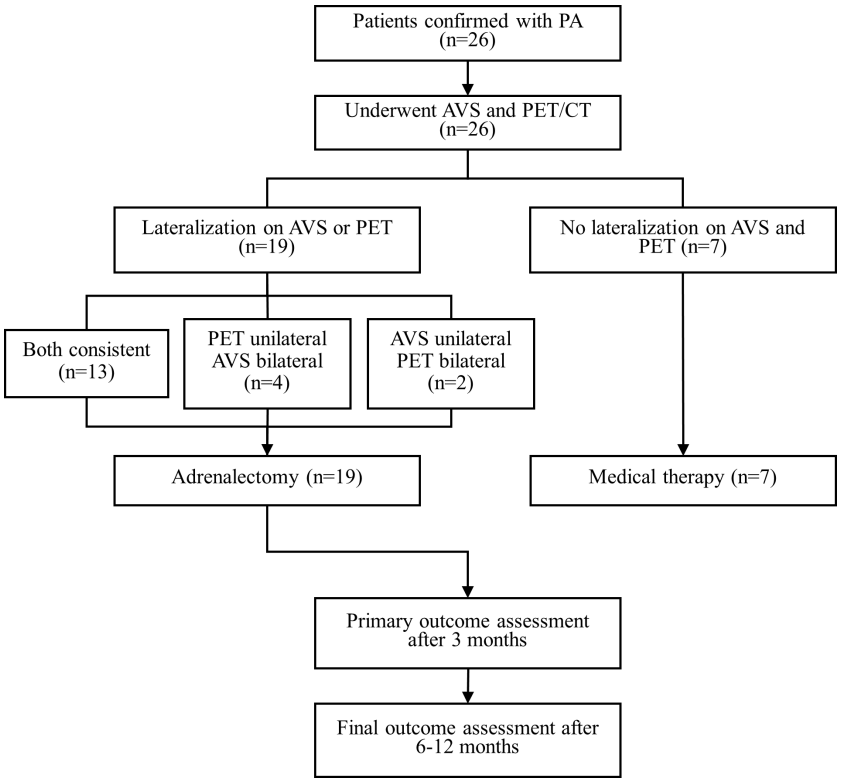


FIGURE 1
Results of AVS, ^{68}Ga-Pentixafor PET/CT and treatment of all patients. AVS, adrenal vein sampling; CT, computed tomography; PET, ^{68}Ga-Pentixafor PET/CT.

Adrenal vein sampling

Patients with confirmed PA underwent adrenal CT scanning and AVS. Prior to the AVS procedure, medications that may influence the levels of renin and aldosterone (such as potassium sparing diuretics, aldosterone receptor antagonists, and angiotensin receptor-blockers) were withdrawn at least for 2 to 6 weeks. We corrected hypokalemia as early as possible, if present, to reach a serum potassium level that was ≥3.5 mmol/L. Detailed methods and criteria are provided in the Supplementary Materials.

^{68}Ga-Pentixafor synthesis

^{68}Ga-Pentixafor was synthesized in a sterile environment following reported labelling approach (30, 37). Details are provided in the Supplementary Materials.

PET/CT imaging and analysis

^{68}Ga-Pentixafor PET/CT scans were acquired by a dedicated PET/CT scanner (Siemens Biograph mCT 64; Siemens Medical Solutions, Erlangen, Germany). Prior to the acquisition of ^{68}Ga-Pentixafor PET/CT images, the patients received a normal diet with no special preparation. After 25 min of the intravenous administration of ^{68}Ga-Pentixafor (mean 88 ± 15 MBq), static images were collected from the head to the mid-thigh for 10 min. Corresponding CT scans for attenuation correction were acquired over the adrenal glands using a low-dose protocol using specific parameters (35 mAs, 120 keV, a 512 × 512 matrix, a 5-mm slice thickness, an increment of 5 mm/s, a rotation time of 0.5 s, and a pitch index of 0.8). Dynamic images were reconstructed using the following scheme: 1 × 40 s, 10 × 5 s, 3 × 10 s, 2 × 15 s,5 × 30 s, 5 × 120 s, 5 × 300 s and 2 × 600 s. Fused PET and low-dose CT images were obtained to evaluate the uptake of ^{68}Ga-Pentixafor.

All PET scans were independently analyzed by two nuclear medicine physicians experienced in ^{68}Ga-Pentixafor PET interpretation. These physicians were blinded to clinical data and AVS results. Disagreements were decided by mutual consensus. A positive adrenal lesion detection by PET/CT was defined by visual analysis as exhibiting a higher uptake than the ipsilateral or contralateral normal adrenal glands. A negative detection was considered if there was an equal or reduced uptake of ^{68}Ga-Pentixafor when compared with the contralateral adrenal glands. Normal adrenal glands were defined as those with no morphological changes in the contralateral to unilateral lesions of PA patients who achieved complete biochemical success after adrenalectomy.

Quantitative analyses were performed with PMOD 4.3 software (Zurich, Germany: PMOD Technologies). We also calculated the maximum standardized uptake values (SUVmax) of the adrenal lesions, specific uptake value ratios such as the lesional SUVmax to the normal liver SUVmean (LLR), and the lesional SUVmax to the contralateral adrenal tissue SUVmean (LCR).

Management of patient therapy and outcomes

Patients underwent AVS and then ^{68}Ga-Pentixafor PET/CT; the mean time interval between these tests were 3 ± 1 days (range, 2–4 days). The management of patients was co-determined at a multidisciplinary meeting of endocrinologists, radiologists and urologists, based on clinical and imaging presentations. If one of the AVS or PET/CT images displayed lateralization, the patient would undergo unilateral adrenalectomy. If neither of the images were lateralized, medication was administered.

The outcome was first evaluated approximately three months after adrenalectomy in accordance with the Primary Aldosteronism Surgical Outcome (PASO) consensus (Supplementary Table 1) (31). For those treated by medical therapy, we collected medicine and blood pressure data for comparison. At least six months post-treatment, patients were reassessed for biochemical and clinical outcomes. The primary outcome was the lateralization accuracy of PET in comparison with AVS by considering the biochemical cure rate post-surgery as the reference. Secondary outcome was the accuracy of each diagnostic test compared to final histology and clinical follow-up.

Immunohistochemistry

Immunohistochemical analyses were performed using paraffin-embedded specimens from 19 subjects who underwent unilateral adrenal excision. CXCR4 and CYP11B2 antibodies were used as primary antibodies. Immunohistochemical staining was performed on adrenal sections with an automatic immunostaining system. Detailed semi-quantitative analysis are provided in the Supplementary Materials. Classical (unilateral aldosterone-producing adenoma, APA) and non-classical (multiple aldosterone-producing nodules/micronodules, MAPN/MAPM or aldosterone-producing diffuse hyperplasia, APDH) PA were diagnosed according to the Histology of Primary Aldosteronism (HISTALDO) consensus (37, 38).

Statistical analysis

This study was designed to have a power of 80% to detect a sensitivity of 0.8 when comparing to a non-significant diagnosis (a sensitivity of 0.5) using a two-sided test at a significance level of 0.05. First, normality was assessed using the S-W test. Nonnormally distributed data were expressed as median (interquartile range, IQR) and compared using the Mann-Whitney U-test, while normally distributed data were reported as mean ± SD and compared using unpaired t test. The Chi-squared test was used for categorical variables. The correlation between the uptake and uptake ratio of ^{68}Ga-Pentixafor in adrenal lesions as well as other characteristics of patients were assessed using Pearson's or Spearman's correlation coefficient. Receiver-operating characteristic (ROC) curves were also constructed to determine the threshold for semi-quantitative parameters and the diagnostic accuracy of ^{68}Ga-Pentixafor PET/CT for the diagnosis of UPA, with complete biochemical success as the gold standard. Statistical significance was defined as $P < 0.05$.

Results

Baseline characteristics

In total, 26 patients (eight females and 18 males) were included in this investigation. The mean age of the patients was 50.3 ± 2.0 years and the median duration of hypertension was 8.5 years. All patients with PA (100%, 26/26) suffered from hypertension; four of these patients (15%, 4/26) had refractory hypertension. Twenty-four patients with PA (92%, 24/26) had hypokalemia. Table 1 shows detailed characteristics. Individual data of all 26 patients recruited into study is provided in Supplementary Table 2.

Clinical management

All 26 patients underwent ^{68}Ga-Pentixafor PET/CT and AVS. Nineteen patients were diagnosed with UPA and underwent adrenalectomy. Of these, 15 cases of APA and four cases of MAPN/MAPM were definitively diagnosed by histology and immunohistochemistry. The remaining seven subjects with no lateralization on AVS and PET were considered to have BPA and subsequently received medical therapy.

Comparisons between AVS and ^{68}Ga-Pentixafor PET/CT

Using the PASO consensus, of the 19 patients who underwent adrenalectomy, 18 patients achieved complete biochemical success and only one patient achieved partial biochemical success. This patient had a diagnosis of right-sided dominant aldosterone secretion by AVS but not PET and was confirmed to have nodular hyperplasia by pathological examination. All subjects showed an improvement of blood pressure; 15 had complete clinical success and four showed partial success (two were diagnosed by PET and AVS, one by PET-only, and one by AVS-only). None of the patients showed an absent biochemical or clinical success.

The consistency between PET and AVS findings was 77% (20/26). Both ^{68}Ga-Pentixafor PET/CT and AVS examinations consistently and accurately identified 13 cases of UPA and seven cases of BPA. None of the patients showed AVS-determined lateralization that was contralateral to the lesion on PET/CT. Of the 19 UPA patients, 17 cases were lateralized correctly by ^{68}Ga-Pentixafor PET while 15 were identified by AVS. Four patients showed no dominant secretion on initial AVS but PET imaging showed unilateral lateralization (three on the left and one on the right), including one patient with concurrent hypercortisolism; all patients achieved biochemical success following unilateral adrenal resection. In addition, two patients had only AVS-determined lateralization. One of the patients with bilateral nodules on CT showed comparable ^{68}Ga-Pentixafor uptake on both

TABLE 1 Characteristics of patients recruited in study.

Characteristics	Total (n=26)	UPA (n=19)		BPA (n=7)	
		Baseline	Post-treatment	Baseline	Post-treatment
Age (years)	50.3±2.0	49.6±2.6	–	52.0±3.0	–
Gender, Female/Male	8/18	7/12	–	1/6	–
BMI (kg/m2)	25.1±1.0	25.3±0.9	–	26.2±0.8	–
Duration of hypertension (years)	8(3-12)	10(3-15)	–	7(6-8)	–
Systolic BP (mmHg)	183±8	181±6	127±4 ****	172±7	131±2 ***
Diastolic BP (mmHg)	110±5	108±4	80±2 ****	98±3	82±2 **
Duration of hypokalemia (years)	0.5(0.02-2)	0.5(0.02-2)	–	0.5(0.08-1)	–
Serum potassium (mmol/l)	2.7±0.1	2.6±0.2	4.2±0.1 ****	2.9±0.2	4.1±0.1 ***
PAC (ng/dl)	33.3(21.9-47.8)	33.5(21.6-57.0)	3.7(3.1-8.2) ****	34.2±4.9	8.3±1.7 **
PRA (ng/ml/h)	0.1(0.07-0.3)	0.2±0.04	0.4(0.2-1.8) **	0.1 (0.04-0.2)	0.6(0.4-0.7)
ARR ([ng/dl]/[ng/ml/h])	176.3(112.3-417.7)	167.4(103.1-415.0)	10(4.6-18.8) *	468.9±182.4	12.5±2.1 *
Long diameter on CT (cm)	1.2(0.7-2.5)	1.4±0.1	–	1.0±0.1	–
AVS-LI	11.6±3.4	7.5(3.8-38.9)	–	1.3±0.1	–

****P <0.0001; ***P <0.001; **P <0.01; *P <0.05. (Asterisks indicate significant differences between baseline vs post treatment).
UPA, unilateral primary aldosteronism; BPA, bilateral primary aldosteronism; BMI, body mass index; BP, blood pressure; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, aldosterone-renin ratio; AVS-LI, lateralization index based on AVS.

sideswhile AVS only identified uptake on the right (Figure 2). Of the 10 patients presenting with bilateral adrenal gland lesions, ^{68}Ga-Pentixafor PET/CT successfully identified functional lateralization in nine patients, whereas AVS lateralized eight subjects (Figure 3). Characteristics of six patients with discordant lateralization in PET/CT and AVS was shown as Supplementary Table 3.

An optimal cutoff SUVmax value of 5.71 was calculated by ROC analysis yielding a sensitivity of 78.95%, a specificity of 100%. The area under the ROC curve (AUC) was 0.94 (95% CI, 0.87–1.00). A cutoff value for LCR of 1.39 yielded a sensitivity of 89.47% and a specificity of 100%, whereas the cutoff value for LLR at 3.05 yielded a sensitivity of 94.74% and a specificity of 100%. The AUC for LCR and LLR were 0.91 (95% CI, 0.79–1.00) and 0.99 (95% CI, 0.97–1.00), respectively. Moreover, the AUC for AVS-LI was 0.89 (95% CI, 0.75–1.00). To diagnose UPA, the LLR had a higher AUC than other uptake values of PET/CT and AVS-LI (Figure 4).

Correlation of ^{68}Ga-Pentixafor PET/CT with clinical management and outcomes

Patients in the surgery group had a high ^{68}Ga-Pentixafor SUVmax on the dominant side compared with those who received medications (13.2 ± 1.9 vs 3.9 ± 0.5, $p < 0.01$). Similarly, significantly higher LCR and LLR values were evident in the surgery group when compared with the medication group (3.2 ± 0.5 vs 1.3 ± 0.1; 7.7 ± 1.0 vs 2.4 ± 0.3, respectively, $p < 0.05$).

Among the surgery group, patients who achieved both complete biochemical and clinical success had higher uptake values for resected adrenal lesions than those who achieved partial success (Figure 5). The SUVmax, LCR, and LLR of ^{68}Ga-Pentixafor were 14.7 ± 2.2 versus 7.5 ± 1.7 ($p = 0.12$), 3.6 ± 0.5 versus 1.6 ± 0.2 ($p = 0.07$), and 8.6 ± 1.1 versus 4.2 ± 0.6 ($p = 0.06$), respectively. However, the difference was not statistically significant.

Pathological and immunohistochemical analysis

Immunohistochemical analysis was performed for CXCR4 and CYP11B2 in sections of postoperative adrenal tissue from 19 patients with UPA. Immunohistochemical tests for CYP11B2 were used to identify functional nodules. All lesions (19/19, 100%) had CYP11B2-positive nodules. Based on this data, 15 subjects were classified as having classical unilateral primary aldosteronism–APA, of whom 11 were lateralized by both PET/CT and AVS; three patients had PET-only lateralization and one had AVS-only lateralization. All patients showed complete biochemical success post-surgery. Furthermore, MAPN/MAPM were discovered in the remaining four lesions. Of these, two cases were consistently lateralized by PET/CT and AVS and one was lateralized by PET and achieved complete biochemical success. One patient with bilateral multiple adrenal nodules was lateralized by AVS alone and demonstrated bilateral comparable radioactive uptake on PET/CT; this patient achieved partial biochemical success after surgery. Based on the adrenal glands, the highest uptake value for ^{68}Ga-Pentixafor uptake was detected in typical APA lesions (Supplementary Figure 1).

Moreover, CYP11B2-positive nodules were all CXCR4-positive and showed positive findings on ^{68}Ga-Pentixafor PET/CT. The h score of CXCR4 and CYP11B2 showed a significant relationship with the SUVmax of ^{68}Ga-Pentixafor (r = 0.56, 0.54, respectively,

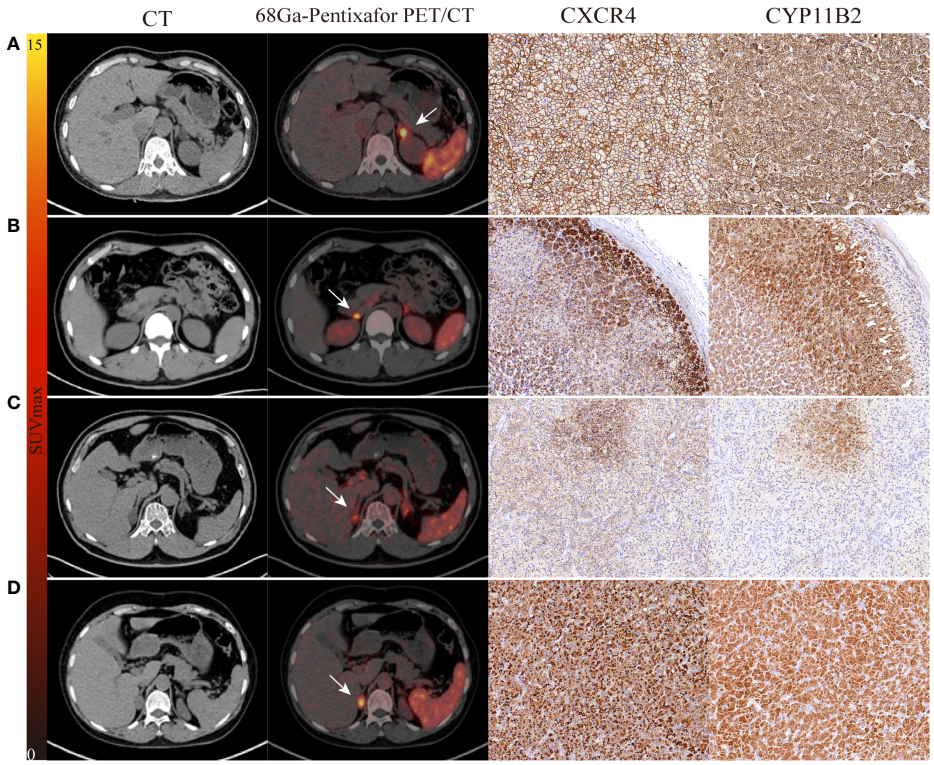


FIGURE 2
The performance of ^{68}Ga-Pentixafor PET/CT imaging in PA patients. **(A, B)** show strong and moderate expression separately, as determined by immunohistochemistry with CXCR4 and CYP11B2; positive findings were detected on both AVS and PET/CT scanning (LI, SUVmax; 21.27, 13.01 vs. 8.10, 5.54). **(C)** A 54-year-old male with bilateral adrenal gland lesions on CT. AVS lateralized the right (LI of 6.73) while PET/CT showed comparable uptake on both sides (SUVmax of R-8.81 and L-7.86). Postoperative pathological examination identified MAPM with weak expression of CXCR4 and CYP11B2. Partial biochemical and clinical success were observed during follow-up. **(D)** A 60-year-old female with concurrent hypercortisolism. AVS was indefinite (LI of L-1.07 and R-0.93) while PET/CT showed positive finding (SUVmax of 14.42); there was positive expression of CXCR4 and CYP11B2. White arrows indicate the tumor lesion. Magnification ×20 for immunohistochemical staining.

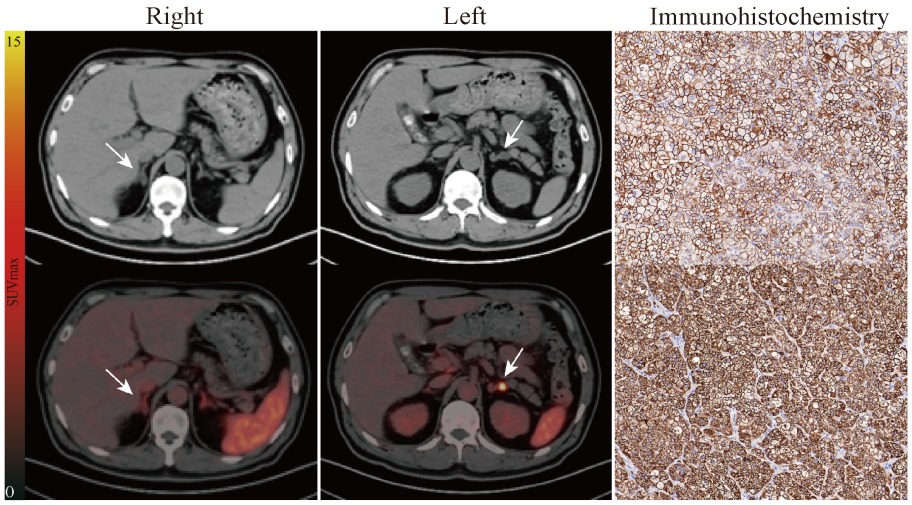


FIGURE 3
Representative pathological and imaging findings from a 54-year-old male with bilateral adrenal nodules. CT (up) and fusion image (down) in the "Right" column showed slight radioactivity uptake in the right adrenal lesion (1.5 cm × 1.0 cm, SUVmax of 2.86). Left lateralization was identified by ^{68}Ga-Pentixafor PET/CT as is shown in the "Left" column (1.5 cm × 1.1 cm, SUVmax of 5.13, LCR of 1.79, LLR of 3.25). AVS showed the same judgment (LI of 3.80). The patient subsequently underwent left adrenalectomy. Immunohistochemistry for CXCR4 (up) and CYP11B2 (down) showing high levels of expression. Follow-up confirmed complete biochemical success. White arrows indicate the tumor lesion. Magnification ×20 for immunohistochemical staining. LCR, ratio of lesional SUVmax to contralateral adrenal SUVmean; LLR, ratio of lesional SUVmax to normal liver SUVmean.

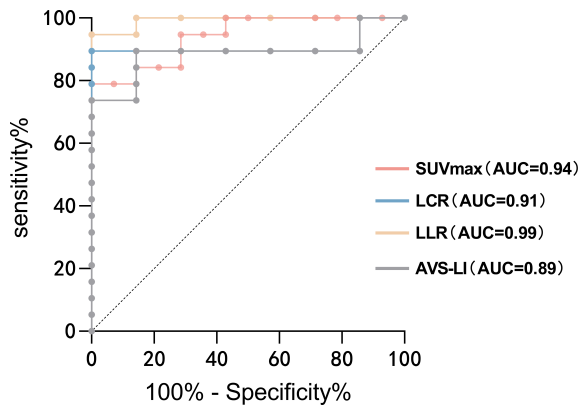


FIGURE 4
ROC analysis for identifying the dominant side of PA. The AUCs of ROC curves for SUVmax, LCR, LLR and AVS-LI were 0.94 (95% CI, 0.87–1.00), 0.91 (95% CI, 0.79–1.00), 0.99 (95% CI, 0.97–1.00) and 0.89 (95% CI, 0.75–1.00), respectively. To diagnose UPA, the LLR had a higher AUC than other uptake values of PET/CT and AVS-LI. AUC, the area under the ROC curve; AVS-LI, lateralization index based on AVS.

p< 0.05). Furthermore, patients with complete biochemical and clinical success had a higher h score for CYP11B2 by immunohistochemistry than those with partial success ($p < 0.001$). The higher h score for the CXCR4 group indicated a significantly higher rate of complete success in patients than the low score group (Figure 6).

Correlation between ^{68}Ga-Pentixafor PET/CT and clinical characteristics

The results of the correlation analyses for SUVmax for ^{68}Ga-Pentixafor uptake with lesions and patient clinical features among the 26 patients are shown in Table 2 and Supplementary Figure 2. The long diameter of nodules exhibited a moderate positive association with SUVmax (Spearman $\rho = 0.47$, $p < 0.05$). A moderate correlation was detected between PAC and SUVmax (Spearman $\rho = 0.42$; $p < 0.05$). Furthermore, LI based on AVS was significant and positively correlated with the SUVmax (Spearman $\rho = 0.74$; $p < 0.01$) of dominant adrenal glands in patients diagnosed with UPA. The relationships of other ^{68}Ga-Pentixafor uptake values with clinical features are shown in Supplementary Table 4.

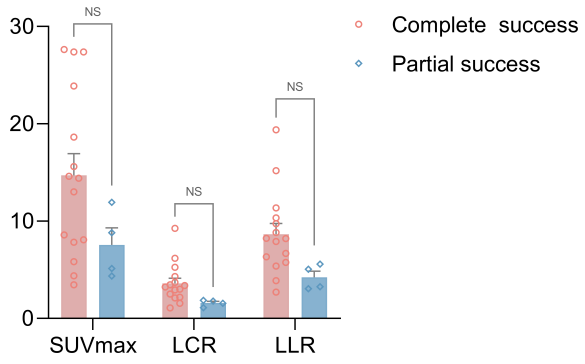


FIGURE 5
A comparison of ^{68}Ga-Pentixafor SUVmax, LCR and LLR values between the complete biochemical and clinical success group and the partial success group for surgery patients (SUVmax of 14.7 ± 2.2 vs. 7.5 ± 1.7; LCR of 3.6 ± 0.5 vs. 1.6 ± 0.2; LLR of 8.6 ± 1.1 vs. 4.2 ± 0.6, respectively, $p > 0.05$). ns, no significance.

Discussion

In this prospective clinical study, non-invasive ^{68}Ga-Pentixafor PET/CT demonstrated comparable functional detection capabilities for subtyping PA when compared with AVS using biochemical and clinical follow-up as the gold standard. Notably, both methods independently detected PA patients who could be cured by unilateral adrenal resection, while the other was not successfully lateralized. The sensitivity and accuracy of ^{68}Ga-Pentixafor PET for the functional lateralization of PA patients were 89% (17/19) and 92% (24/26) respectively, while those of AVS were 79% (15/19) and 85% (22/26), respectively.

Our results showed that the optimum SUVmax cut-off for the identification of functional nodules based on ^{68}Ga-Pentixafor was 5.71; at this cut-off value, the sensitivity was 78.95% while the specificity was 100%. The AUC was 0.94 (95% CI, 0.87–1.00). However, the absolute values of SUVmax among functional lesions varied widely from patient to patient; this may be attributed to different CXCR4 expression levels between individuals. Referring to the dominant side diagnostic principle of AVS, the uptake value ratios should play a greater role in classification. As shown by our data, the LCR and LLR performed better than SUVmax. Notably, when the threshold of LLR was 3.05, the sensitivity and specificity were 94.74% and 100%, respectively; the AUC was 0.99. This finding is in agreement with a previous prospective study involving 33 PA and 3 NFA patients which demonstrated the superior detectability of LLR than other uptake values (29). The consistency between this quantitative criteria and our initial "visual assessment" was 96% (25/26). Thus, LLR might be considered as the best index of ^{68}Ga-Pentixafor PET/CT for the identification of functional lesions.

During follow-up, 18 patients who underwent adrenalectomy achieved complete biochemical cure; of these, 17 patients (94%, 17/18) were identified by PET/CT and 14 patients (78%, 14/18) were identified by AVS. The consistency of these two methods was 77% (20/26). We found that four UPA patients presented with false-negative results on AVS while true-positive lesions were identified by PET/CT scanning. All of these achieved biochemical cure after excision of the dominant adrenal gland on PET, including one patient with concurrent hypercortisolism and another who had adrenal poly-nodular hyperplasia post-surgery. Furthermore, three of these patients showed bilateral adrenal gland lesions on CT. These results showed that ^{68}Ga-Pentixafor could be used to diagnose UPA cases where AVS results are not definite or non-identifiable. Nevertheless, two UPA patients with a true-positive result on AVS showed a comparable uptake value on both sides in PET/CT. Postoperative pathology from one of these patients confirmed nodular hyperplasia; subsequent immunohistochemical staining of CXCR4 and CYP11B2 indicated

10.3389/fendo.2024.1291775

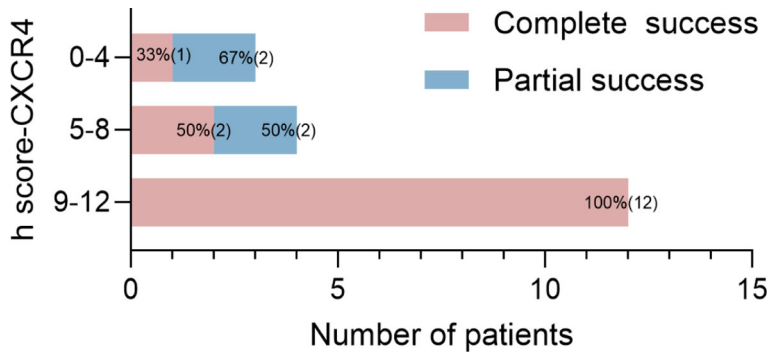


FIGURE 6
Proportions and absolute numbers (in parentheses) of patients with different prognoses for the three groups by h score (0–4, 5–8, 9–12).

low levels of MAPM expression. Partial biochemical success was observed. The other patient had an adenoma that was < 8 mm in size; the LI was close to the threshold.

There were six patients with discordant lateralization in PET/CT and AVS. Though few in number, we found that 50% (3/6) patients presented with bilateral adrenal gland lesions (nodules or hyperplasia) on CT. Of the remaining patients with unilateral adrenal disease, nobody has classical single nodule except for one with concurrent hypercortisolism. Compared to consistent cases, the proportion of multiple nodules and hyperplasia is significantly higher. We speculate the reason for discordance cases may be that these patients have asymmetrical bilateral disease, and the two methods detect different levels of lesions functionality in different way, leading to inconsistent lateralization results. Besides, for adrenal glands that appear "normal" on CT, functional changes may precede morphological changes. The smallest nodule size that can be detected is 0.7cm in our study. And there is a moderate positive association between SUVmax of PET/CT and the long diameter of adrenal nodules (Spearman $\rho = 0.47$, $p < 0.05$), suggesting that the sensitivity of this method may be more ideal in patients with bigger lesions.

TABLE 2 Correlation coefficients between SUVmax of PA patients and clinical features.

Clinical features	Correlation coefficients	P value
Age	-0.31	0.13
BMI	-0.08	0.71
Systolic pressure	0.12	0.57
Diastolic pressure	0.33	0.01
The long diameter of nodule	0.47*	0.02
AVS-LI#	0.74**	0.001
Serum potassium	-0.03	0.89
PAC	0.42*	0.04
ARR	-0.09	0.66

**P <0.01; *P <0.05. #: among patients who were diagnosed with UPA.

In our study, all four hyperplastic adrenal lesions with positive uptake on ^{68}Ga-Pentixafor PET/CT showed increased expression levels of CXCR4 and CYP11B2. Even if the h score was lower than classical APA, it can be inferred that the hyperplastic adrenal tissue in patients with PA may also be functional and could be recognized by ^{68}Ga-Pentixafor PET/CT. Moreover, our data are supported by the latest World Health Organization (WHO) Classification of Adrenal Cortical Tumors, which recommends using CYP11B2 immunohistochemistry to identify the functional sites of aldosterone production rather than simply distinguishing functional adenomas from non-functional hyperplasia (34).

In addition, all 19 UPA cases undergoing immunohistochemical staining were positive for CYP11B2 and all CYP11B2-positive lesions were also CXCR4-positive. In semi-quantitative analysis, SUVmax was correlated with the h score for both CXCR4 and CYP11B2 ($r = 0.56, 0.54$, respectively, $p < 0.05$). Furthermore, we found patients who achieved both complete biochemical and clinical success had higher h scores for CXCR4 and CYP11B2, as determined by immunohistochemistry of resected adrenals, when compared with those who achieved partial success ($p < 0.001$). CYP11B2 is involved in the terminal steps of aldosterone biosynthesis and, allows the localization of aldosterone synthesis. However, the precise relationship between radioactive uptake and the secretion of aldosterone remains unclear and further studies are now required.

An important consideration is whether non-invasive functional imaging ^{68}Ga-Pentixafor PET/CT, as a first differential diagnostic step, might enable non-invasive diagnosis in most patients with PA. According to our data, 17 patients (89%, 17/19) were successfully diagnosed as UPA by PET, thus avoiding invasive AVS. Only two patients with UPA missed the opportunity for surgery. When considering patients without obvious PET lateralization, it was evident that these patients could choose to receive medication or proceed with subsequent AVS as a second-line examination. Unlike the high technical requirements of AVS, ^{68}Ga-Pentixafor PET/CT is readily available at most centers where PET scans can be performed. And none of patients reported adverse events associated with ^{68}Ga-Pentixafor. Furthermore, as an outpatient procedure, PET/CT can save considerable amounts of time and economic costs than AVS for patients. In the healthcare centers of China, the average cost of AVS was about ¥12000, while that of PET/CT is ¥5500. According to the

published literature, the average price of PET/CT in Europe is also about half the average price of AVS (≈€1200). The application of new technology can save patients about half of the previous cost while eliminating the pain of surgery. In our patient satisfaction survey, all subjects expressed a positive attitude towards the replacement of invasive tests with non-invasive tests in the future. Based on these findings, we can conclude that ^{68}Ga-Pentixafor PET presents a new diagnostic approach that could influence future therapeutic decision-making for the management of patients with PA.

Some researchers have emphasized that the control of biochemical levels as essential as blood pressure control in order to achieve good cardiovascular outcomes, whether amongst cohorts treated by adrenalectomy or medication (38–40). Hence, we used histology and clinical follow-up as the reference criteria in our current research to evaluate the accuracy of both PET/CT and AVS more objectively. In particular, we included cases with inconclusive subtyping diagnosis based on AVS results; these cases have been rarely discussed previously. ^{68}Ga-Pentixafor PET/CT showed relatively high levels of sensitivity and specificity in the detection of functional lesions for most patients and played an important role in clinical treatment and prognostic prediction.

There are several limitations to our study that need to be considered. First, as a prospective study carried out in a single center, our study features inherent bias in terms of selection. For example, the proportion of unilateral lesions in our study was higher than that of bilateral lesions; one reason for this is that patients with a more severe phenotype were more likely to be referred to our centers. In addition, our sample size was small. Larger scale studies are now needed. Second, we cannot ascertain false negative test results since it would be unethical to perform operations on all patients. Third, the follow-up period was not long enough to compare the final probability of cardiovascular events in patients whose treatment was directed by ^{68}Ga-Pentixafor PET/CT or AVS.

Conclusion

Our prospective clinical trial found that ^{68}Ga-Pentixafor PET/CT functional imaging represents a novel and reliable tool for PA subtype diagnosis for both functional lateralization and follow-up outcomes. This method provides a means of non-invasive diagnosis for most patients with PA and offers a universal diagnostic alternative to AVS, thus reducing several complicated invasive operations. Furthermore, ^{68}Ga-Pentixafor PET/CT identified additional cases of unilateral surgically curable PA for which AVS failed to perform classification. Based on this, we recommend ^{68}Ga-Pentixafor PET/CT as a first-line test for future classification. Our findings should be verified in a larger population.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Ethics Committee of National Medical Research Center, Second Xiangya Hospital, Central South University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

XY: Data curation, Investigation, Writing – original draft, Writing – review & editing. KA: Data curation, Formal analysis, Writing – original draft. JL: Data curation, Formal analysis, Writing – original draft. WL: Data curation, Resources, Validation, Writing – review & editing. XM: Project administration, Supervision, Validation, Writing – review & editing. LZ: Investigation, Software, Visualization, Writing – review & editing. XX: Software, Visualization, Writing – review & editing. XS: Conceptualization, Project administration, Validation, Writing – review & editing. YW: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. YL: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by National Natural Science Foundation of China (82373435 and 82172878), the major grant of the Research and Development Program of Hunan Province of China (2019SK2252 and 2022SK2035), and the Natural Science Foundation of Hunan Province of China (2020JJ4801).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1291775/full#supplementary-material

References

1. Reincke M, Bancos I, Mulatero P, Scholl UI, Stowasser M, Williams TA. Diagnosis and treatment of primary aldosteronism. *Lancet Diabetes Endocrinol* (2021) 9:876–92. doi: 10.1016/S2213-8587(21)00210-2

2. Turcu AF, Yang J, Vaidya A. Primary aldosteronism — a multidimensional syndrome. *Nat Rev Endocrinol* (2022) 18:665–82. doi: 10.1038/s41574-022-00730-2

3. Zennaro M-C, Boulkroun S, Fernandes-Rosa FL. Pathogenesis and treatment of primary aldosteronism. *Nat Rev Endocrinol* (2020) 16:578–89. doi: 10.1038/s41574-020-0382-4

4. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Incidence of atrial fibrillation and mineralocorticoid receptor activity in patients with medically and surgically treated primary aldosteronism. *JAMA Cardiol* (2018) 3:768–74. doi: 10.1001/jamacardio.2018.2003

5. Lee FT, Elaraj D. Evaluation and management of primary hyperaldosteronism. *Surg Clin North Am* (2019) 99:731–45. doi: 10.1016/j.suc.2019.04.010

6. Vaidya A, Carey RM. Evolution of the primary aldosteronism syndrome: updating the approach. *J Clin Endocrinol Metab* (2020) 105:3771–83. doi: 10.1210/clinem/dgaa606

7. Rossi GP. Update in adrenal venous sampling for primary aldosteronism. *Curr Opin Endocrinol Diabetes Obes* (2018) 25:160–71. doi: 10.1097/MED.0000000000000407

8. Rossi GP, Auchus RJ, Brown M, Lenders JW, Naruse M, Plouin PF, et al. An expert consensus statement on use of adrenal vein sampling for the subtyping of primary aldosteronism. *Hypertension* (2014) 63:151–60. doi: 10.1161/HYPERTENSIONAHA.113.02097

9. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, et al. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. *Eur J Endocrinol* (2016) 175:G1–34. doi: 10.1530/EJE-16-0467

10. Burton TJ, Mackenzie IS, Balan K, Koo B, Bird N, Soloviev DV, et al. Evaluation of the sensitivity and specificity of (11)C-metomidate positron emission tomography (PET)-CT for lateralizing aldosterone secretion by Conn's adenomas. *J Clin Endocrinol Metab* (2012) 97:100–9. doi: 10.1210/jc.2011-1537

11. Mendichovszky IA, Powlson AS, Manavaki R, Aigbirhio FI, Cheow H, Buscombe JR, et al. Targeted molecular imaging in adrenal disease-an emerging role for metomidate PET-CT. *Diagnostics (Basel)* (2016) 6:42. doi: 10.3390/diagnostics6040042

12. Ouyang J, Hardy R, Brown M, Helliwell T, Gurnell M, Cuthbertson DJ. 11C-metomidate PET-CT scanning can identify aldosterone-producing adenomas after unsuccessful lateralisation with CT/MRI and adrenal venous sampling. *J Hum Hypertens* (2017) 31:483–4. doi: 10.1038/jhh.2017.9

13. O'Shea PM, O'Donoghue D, Bashari W, Senanayake R, Joyce MB, Powlson AS, et al. 11 C-Metomidate PET/CT is a useful adjunct for lateralization of primary aldosteronism in routine clinical practice. *Clin Endocrinol (Oxf)* (2019) 90:670–9. doi: 10.1111/cen.13942

14. Chen Cardenas SM, Santhanam P. 11C-metomidate PET in the diagnosis of adrenal masses and primary aldosteronism: a review of the literature. *Endocrine* (2020) 70:479–87. doi: 10.1007/s12020-020-02474-3

15. Puar TH, Khoo CM, Tan CJ, Tong AKT, Tan MCS, Teo AED, et al. 11C-Metomidate PET-CT versus adrenal vein sampling to subtype primary aldosteronism: a prospective clinical trial. *J Hypertension* (2022) 40:1179–88. doi: 10.1097/HJH.0000000000003132

16. Wu X, Senanayake R, Goodchild E, Bashari WA, Salsbury J, Cabrera CP, et al. [11C]metomidate PET-CT versus adrenal vein sampling for diagnosing surgically curable primary aldosteronism: a prospective, within-patient trial. *Nat Med* (2023) 29:190–202. doi: 10.1038/s41591-022-02114-5

17. Soinio M, Luukkonen A-K, Seppänen M, Kemppainen J, Seppänen J, Pienimäki J-P, et al. Functional imaging with 11C-metomidate PET for subtype diagnosis in primary aldosteronism. *Eur J Endocrinol* (2020) 183:539–50. doi: 10.1530/EJE-20-0532

18. Crimì F, Spimpolo A, Cecchin D, Rossi GP. Functional imaging by 11C-metomidate PET: a really useless technique for primary aldosteronism subtyping? *Eur J Endocrinol* (2021) 184:L9–10. doi: 10.1530/EJE-20-1402

19. Bluemel C, Hahner S, Heinze B, Fassnacht M, Kroiss M, Bley TA, et al. Investigating the chemokine receptor 4 as potential theranostic target in adrenocortical cancer patients. *Clin Nucl Med* (2017) 42:e29–34. doi: 10.1097/RLU.0000000000001435

20. Itcho K, Oki K, Kobuke K, Yoshii Y, Ohno H, Yoneda M, et al. Aberrant G protein-receptor expression is associated with DNA methylation in aldosterone-producing adenoma. *Mol Cell Endocrinol* (2018) 461:100–4. doi: 10.1016/j.mce.2017.08.019

21. Gomez-Sanchez CE, Kuppusamy M, Reincke M, Williams TA. Disordered CYP11B2 expression in primary aldosteronism. *Horm Metab Res* (2017) 49:957–62. doi: 10.1055/s-0043-122238

22. Herrmann K, Lapa C, Wester H-J, Schottelius M, Schiepers C, Eberlein U, et al. Biodistribution and radiation dosimetry for the chemokine receptor CXCR4-targeting probe ^{68}Ga-Pentixafor. *J Nucl Med* (2015) 56:410–6. doi: 10.2967/jnumed.114.151647

23. Philipp-Abbrederis K, Herrmann K, Knop S, Schottelius M, Eiber M, Lückerath K, et al. *In vivo* molecular imaging of chemokine receptor CXCR4 expression in patients with advanced multiple myeloma. *EMBO Mol Med* (2015) 7:477–87. doi: 10.15252/emmm.201404698

24. Demmer O, Dijkgraaf I, Schumacher U, Marinelli L, Cosconati S, Gourni E, et al. Design, synthesis, and functionalization of dimeric peptides targeting chemokine receptor CXCR4. *J Med Chem* (2011) 54:7648–62. doi: 10.1021/jm2009716

25. Ding J, Tong A, Zhang Y, Wen J, Zhang H, Hacker M, et al. Functional characterization of adrenocortical masses in nononcologic patients using ^{68}Ga-Pentixafor. *J Nucl Med* (2022) 63:368–75. doi: 10.2967/jnumed.121.261964

26. Gao Y, Ding J, Cui Y, Li T, Sun H, Zhao D, et al. Functional nodules in primary aldosteronism: identification of CXCR4 expression with ^{68}Ga-Pentixafor PET/CT. *Eur Radiol* (2022) 33:996–1003. doi: 10.1007/s00330-022-09058-x

27. Hu J, Xu T, Shen H, Song Y, Yang J, Zhang A, et al. Accuracy of gallium-68 pentixafor positron emission tomography-computed tomography for subtyping diagnosis of primary aldosteronism. *JAMA Netw Open* (2023) 6:e2255609. doi: 10.1001/jamanetworkopen.2022.55609

28. Zheng Y, Long T, Peng N, Zhen M, Ye Q, Zhang Z, et al. The value of targeting CXCR4 with ^{68}Ga-Pentixafor PET/CT for subtyping primary aldosteronism. *J Clin Endocrinol Metab* (2023) 109(1):171–82. doi: 10.1210/clinem/dgad421

29. Ding J, Zhang Y, Wen J, Zhang H, Wang H, Luo Y, et al. Imaging CXCR4 expression in patients with suspected primary hyperaldosteronism. *Eur J Nucl Med Mol Imaging* (2020) 47:2656–65. doi: 10.1007/s00259-020-04722-0

30. Heinze B, Fuss CT, Mulatero P, Beuschlein F, Reincke M, Mustafa M, et al. Targeting CXCR4 (CXC chemokine receptor type 4) for molecular imaging of aldosterone-producing adenoma. *Hypertension* (2018) 71:317–25. doi: 10.1161/HYPERTENSIONAHA.117.09975

31. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, et al. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol* (2017) 5:689–99. doi: 10.1016/S2213-8587(17)30135-3

32. Holler F, Heinrich DA, Adolf C, Lechner B, Bidlingmaier M, Eisenhofer G, et al. Steroid profiling and immunohistochemistry for subtyping and outcome prediction in primary aldosteronism—a review. *Curr Hypertens Rep* (2019) 21:77. doi: 10.1007/s11906-019-0985-0

33. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2016) 101:1889–916. doi: 10.1210/jc.2015-4061

34. Mete O, Erickson LA, Juhlin CC, De Krijger RR, Sasano H, Volante M, et al. Overview of the 2022 WHO classification of adrenal cortical tumors. *Endocrine Pathol* (2022) 33:155–96. doi: 10.1007/s12022-022-09710-8

35. Byrd JB, Turcu AF, Auchus RJ. Primary aldosteronism: practical approach to diagnosis and management. *Circulation* (2018) 138:823–35. doi: 10.1161/CIRCULATIONAHA.118.033597

36. Vaidya A, Mulatero P, Baudrand R, Adler GK. The expanding spectrum of primary aldosteronism: implications for diagnosis, pathogenesis, and treatment. *Endocr Rev* (2018) 39:1057–88. doi: 10.1210/er.2018-00139

37. Williams TA, Gomez-Sanchez CE, Rainey WE, Giordano TJ, Lam AK, Marker A, et al. International histopathology consensus for unilateral primary aldosteronism. *J Clin Endocrinol Metab* (2021) 106:42–54. doi: 10.1210/clinem/dgaa484

38. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. *Lancet Diabetes Endocrinol* (2018) 6:51–9. doi: 10.1016/S2213-8587(17)30367-4

39. Hundemer GL, Baudrand R, Brown JM, Curhan G, Williams GH, Vaidya A. Renin phenotypes characterize vascular disease, autonomous aldosteronism, and mineralocorticoid receptor activity. *J Clin Endocrinol Metab* (2017) 102:1835–43. doi: 10.1210/jc.2016-3867

40. Buffolo F, Monticone S, Williams T, Rossato D, Burrello J, Tetti M, et al. Subtype diagnosis of primary aldosteronism: is adrenal vein sampling always necessary? *IJMS* (2017) 18:848. doi: 10.3390/ijms18040848

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 05 March 2024
DOI 10.3389/fendo.2024.1286297

OPEN ACCESS

EDITED BY
Norlela Sukor,
Universiti Kebangsaan Malaysia Medical Center (UKMMC), Malaysia

REVIEWED BY
Andrea Gutierrez Maria,
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NIH), United States
Alexander Akhmedov,
University of Zurich, Switzerland

*CORRESPONDENCE
Kazutaka Nanba
knamba@umich.edu

RECEIVED 31 August 2023
ACCEPTED 21 February 2024
PUBLISHED 05 March 2024

CITATION
Nanba K, Blinder AR, Udager AM, Hirokawa Y, Miura T, Okuno H, Moriyoshi K, Yamazaki Y, Sasano H, Yasoda A, Satoh-Asahara N, Rainey WE and Tagami T (2024) Double somatic mutations in *CTNNB1* and *GNA11* in an aldosterone-producing adenoma. *Front. Endocrinol.* 15:1286297. doi: 10.3389/fendo.2024.1286297

Double somatic mutations in *CTNNB1* and *GNA11* in an aldosterone-producing adenoma

Kazutaka Nanba [1,2,3]*, Amy R. Blinder[3], Aaron M. Udager[4,5,6], Yuusuke Hirokawa[7], Takayoshi Miura[8], Hiroshi Okuno[8], Koki Moriyoshi[9], Yuto Yamazaki[10], Hironobu Sasano[10], Akihiro Yasoda[11], Noriko Satoh-Asahara[2], William E. Rainey[3,12] and Tetsuya Tagami[1,2]

[1]Department of Endocrinology and Metabolism, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [2]Department of Endocrinology, Metabolism, and Hypertension Research, Clinical Research Institute, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [3]Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, United States, [4]Department of Pathology, University of Michigan, Ann Arbor, MI, United States, [5]Michigan Center for Translational Pathology, University of Michigan, Ann Arbor, MI, United States, [6]Rogel Cancer Center, University of Michigan, Ann Arbor, MI, United States, [7]Department of Radiology, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [8]Department of Urology, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [9]Department of Diagnostic Pathology, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [10]Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan, [11]Clinical Research Institute, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [12]Division of Metabolism, Endocrinology, and Diabetes, University of Michigan, Ann Arbor, MI, United States

Double somatic mutations in *CTNNB1* and *GNA11/Q* have recently been identified in a small subset of aldosterone-producing adenomas (APAs). As a possible pathogenesis of APA due to these mutations, an association with pregnancy, menopause, or puberty has been proposed. However, because of its rarity, characteristics of APA with these mutations have not been well characterized. A 46-year-old Japanese woman presented with hypertension and hypokalemia. She had two pregnancies in the past but had no history of pregnancy-induced hypertension. She had regular menstrual cycle at presentation and was diagnosed as having primary aldosteronism after endocrinologic examinations. Computed tomography revealed a 2 cm right adrenal mass. Adrenal venous sampling demonstrated excess aldosterone production from the right adrenal gland. She underwent right laparoscopic adrenalectomy. The resected right adrenal tumor was histologically diagnosed as adrenocortical adenoma and subsequent immunohistochemistry (IHC) revealed diffuse immunoreactivity of aldosterone synthase (CYP11B2) and visinin like 1, a marker of the zona glomerulosa (ZG), whereas 11β-hydroxylase, a steroidogenic enzyme for cortisol biosynthesis, was mostly negative. CYP11B2 IHC-guided targeted next-generation sequencing identified somatic *CTNNB1* (p.D32Y) and *GNA11* (p.Q209H) mutations. Immunofluorescence staining of the

tumor also revealed the presence of activated β-catenin, consistent with features of the normal ZG. The expression patterns of steroidogenic enzymes and related proteins indicated ZG features of the tumor cells. PA was clinically and biochemically cured after surgery. In conclusion, our study indicated that *CTNNB1* and *GNA11*-mutated APA has characteristics of the ZG. The disease could occur in adults with no clear association with pregnancy or menopause.

KEYWORDS

primary aldosteronism, aldosterone-producing adenoma, CYP11B2, somatic mutation, CTNNB1, GNA11

Introduction

Aldosterone-producing adenoma (APA) is a major form of primary aldosteronism (PA). In the past decade, there has been significant progress in the determination of genetic causes of APA. The use of next-generation sequencing (NGS) in APA has resulted in the identification of somatic mutations responsible for excess aldosterone production. These affected genes include *KCNJ5* (1), *ATP1A1* (2), *ATP2B3* (2), *CACNA1D* (3, 4), *CACNA1H* (5, 6), and *CLCN2* (7–9). These aldosterone-driver genes encode ion channels or transporters. Mutations in these genes directly or indirectly increase intracellular calcium levels resulting in enhanced tumor cell aldosterone synthase (CYP11B2) expression and inappropriate aldosterone production (10). More recently, somatic mutations in *CADM1* (11) and *SLC30A1* (12) have also been identified as rare genetic causes of APA. An immunohistochemistry (IHC)-based sequencing approach that targets CYP11B2-expressing regions using formalin-fixed, paraffin-embedded (FFPE) tissue has enabled detection of these somatic mutations in the vast majority of APAs (13–16).

As in other adrenocortical tumors such as adrenocortical carcinoma and cortisol-producing adenoma, somatic activating mutations in exon 3 of the *CTNNB1* gene, that encodes β-catenin, have also been identified in 2-5% of APA (17–19). A recent study reported double somatic mutations of *GNA11* or *GNAQ* in *CTNNB1*-mutated APAs (20). As a possible pathogenesis of APA harboring these double mutations, an association with pregnancy, menopause, or puberty has been proposed based on the disease onset and increased tumor expression of luteinizing hormone/choriogonadotropin receptor (LHCGR) (20). However, due to its rare incidence, characteristics of APA with these double mutations have not been well characterized. Herein, we report the detailed clinical course of a Japanese woman with APA harboring somatic *CTNNB1* and *GNA11* mutations. Notably, the present case had no history of pregnancy-associated hypertension or irregular menstrual cycles at presentation.

Materials and methods

Immunohistochemistry

IHC was performed on 10% FFPE tissue sections as described previously (21). The following primary antibodies were used: CYP11B2 (MilliporeSigma, MABS1251; diluted 1:1250; RRID, AB_2783793), 17α-hydroxylase/17, 20 lyase (CYP17A1) (LSBio, LS-B14227; diluted 1:2000; RRID, AB_2857939), 11β-hydroxylase (CYP11B1) (clone 80-7-3; kindly provided by Dr. Celso Gomez-Sanchez; diluted 1:50; RRID, AB_2650563), and visinin like 1 (VSNL1) (MilliporeSigma, MABN762; diluted 1:1000; RRID, AB_2832208).

Immunofluorescence staining

Immunofluorescence (IF) was performed on FFPE sections of 5 μm thickness. After deparaffinization, the slides were boiled for 15 minutes in pH 6, citrate-based buffer (Vector Laboratories) for epitope retrieval followed by 10% normal goat serum (Abcam) blocking for 1 hour. The primary antibodies to β-catenin (Cell Signaling Technology, 9562; diluted 1:100; RRID, AB_331149) and KCNJ5 (G protein-activated inward rectifier potassium channel 4) (from Dr. Celso Gomez-Sanchez; clone 36-33-5; diluted 1:1000; RRID, AB_3086774) (22) were incubated overnight at 4°C. The fluorescent-conjugated secondary antibodies (Jackson ImmunoResearch, 111-545-144; diluted 1:100; RRID, AB_2338052 and Thermo Fisher Scientific, A-11032; diluted 1:100; RRID, AB_2534091) were then incubated for 1 hour at room temperature followed by autofluorescence quenching with TrueBlack® Lipofuscin Autofluorescence Quencher (Biotium) for 30 seconds. Finally, coverslips were mounted with 4',6-diamidino-2-phenylindole (DAPI).

DNA and RNA isolation

Genomic DNA (gDNA) and RNA from APA and adjacent normal adrenal tissue were isolated separately from serial FFPE

tissue sections using the AllPrep DNA/RNA FFPE kit (QIAGEN) as described previously (23). gDNA and RNA were used for targeted NGS and quantitative real-time RT-PCR (qPCR), respectively.

Targeted NGS

Ion Torrent™-based targeted NGS (Thermo Fisher Scientific) was used for sequencing analysis. The custom Ion AmpliSeq™ panel for targeted NGS included the full coding regions of following genes: *KCNJ5*, *ATP1A1*, *ATP2B3*, *CACNA1D*, *CACNA1H*, *CLCN2*, *CADM1*, *SLC30A1*, *CTNNB1*, *GNAS*, and *GNA11*. The methods for targeted NGS, including library preparation, sequencing, and variant calling, were performed as described previously (23).

Quantitative real-time RT-PCR

RNA was reverse transcribed using the high-capacity complementary DNA (cDNA) archive kit (Life Technologies). qPCR was performed using the StepOnePlus™ Real-Time PCR systems (Applied Biosystems) (23). The primer-probe sets for *CYP11B2* were designed in house and manufactured by IDT DNA (24). The following primer-probe sets were purchased from Thermo Fisher Scientific: *LHCGR* (Hs00174885_m1), *GNRHR* (gonadotropin-releasing hormone receptor) (Hs00171248_m1), and *ACTB* (β-actin) (Hs01060665_g1). *ACTB* transcript was used as an internal control for quantitative normalization. The delta-delta threshold cycle method was used to calculate fold changes in mRNA expression over adjacent normal adrenal.

This study was approved by the institutional review boards at the National Hospital Organization Kyoto Medical Center (20–038) and the University of Michigan (HUM00083056). The patient provided written consent for the use of specimen in this study and publication of this article.

Results

Case presentation

A 46-year-old Japanese woman was referred to us for the investigation of PA. She had been hypertensive at least for 4 months (her blood pressure was 216/105 mmHg at initial visit of the referring hospital). She had two pregnancies at the ages of 22 and 23 but had no history of pregnancy-associated hypertension or other complications according to her Maternal and Child Handbooks (25). Although she had menopause-like symptoms such as headaches, sweating, and fatigue, her menstrual cycle was regular at the time of presentation. She had urolithiasis at the age of 40. Computed tomography (CT) for the evaluation of urolithiasis detected a right adrenal tumor. However, no further investigation was performed at that time. She had no family history of endocrine disorders.

Laboratory testing showed hypokalemia and elevated plasma aldosterone concentration with suppressed renin (Table 1). She was diagnosed as having PA based on the results of captopril challenge test (Table 1) (26). Concomitant cortisol excess was not documented (Table 1). Adrenal CT revealed a 2 cm right adrenal mass (Figures 1A, B). Left adrenal was intact by imaging. Adrenal venous sampling indicated excess aldosterone production from the right adrenal gland (Table 2). ^{131}I-6β-iodomethyl-19-norcholesterol (NP-59) scintigraphy with dexamethasone suppression further demonstrated increased tracer uptake in the right adrenal lesion (Figure 1C). She underwent right laparoscopic adrenalectomy. The resected tumor was histologically diagnosed as adrenocortical adenoma according to the criteria of Weiss (27) and also harboring the foci of pseudoglandular formations (Figures 2A, B). Notably, Ki-67 labeling index was high (6% at hotspots) (Figure 2C). After surgery, her blood pressure and serum potassium were both normalized. Based on the primary

TABLE 1 Laboratory results of endocrine testing.

	Values
Baseline characteristics	
Serum creatinine (mg/dL)	0.61
Serum potassium (mEq/L)	2.9
PAC (ng/dL)	67.8
PRA (ng/mL/h)	0.3
ARR	226.0
Captopril challenge test[a]	
Baseline PAC (ng/dL)	101.2
Baseline PRA (ng/mL/h)	0.6
Baseline ARR	168.7
60 min PAC (ng/dL)	63.9
60 min PRA (ng/mL/h)	0.4
60 min ARR	159.8
90 min PAC (ng/dL)	52.4
90 min PRA (ng/mL/h)	0.5
90 min ARR	104.8
ACTH/cortisol circadian rhythm	
8:00 ACTH (pg/mL)	37.3
8:00 serum cortisol (μg/dL)	8.6
23:00 ACTH (pg/mL)	8.2
23:00 serum cortisol (μg/dL)	1.1
1 mg dexamethasone suppression test[b]	
ACTH (pg/mL)	<1.5
Serum cortisol (μg/dL)	0.9
PAC (ng/dL)	106.1

[a], ARR ≥ 20 at 60 or 90 minutes after 50 mg of captopril administration was considered as a positive result (26). [b], A cut-off cortisol level of ≥ 1.8 μg/dL was used to assess the presence of autonomous cortisol co-secretion (26). PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, aldosterone-to-renin ratio; ACTH, adrenocorticotropic hormone.

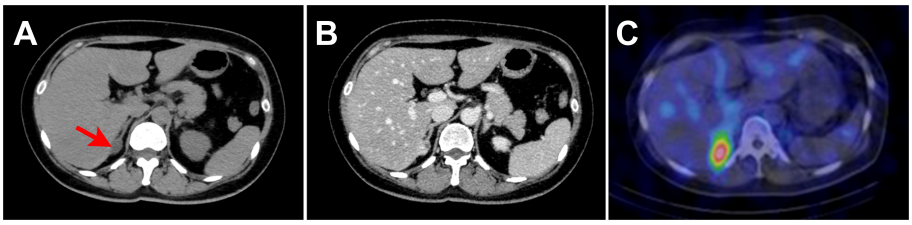


FIGURE 1
Imaging findings. **(A, B)**. Computed tomography (CT) revealed a 2 cm right adrenal mass (red arrow in **(A)**). The mean Hounsfield unit of the adrenal tumor on unenhanced CT was 14.0. **(A)**. Unenhanced CT. **(B)**. Contrast enhanced CT. **(C)**. NP-59 scintigraphy with dexamethasone suppression showed increased tracer uptake in the right adrenal lesion.

aldosteronism surgical outcome (PASO) study criteria (28), PA was clinically and biochemically cured after surgery (Table 3). No tumor recurrence was observed by imaging study performed at 2 years after surgery.

Histopathologic and genetic characteristics of the resected tumor

IHC revealed diffuse immunoreactivity of CYP11B2 in tumor cells suggestive of neoplastic production of aldosterone (Figures 3A, B). VSNL1, a marker for the normal zona glomerulosa (ZG) (29), was also abundant in the tumor (Figures 3C, D). Consistent with normal suppression of cortisol after 1 mg dexamethasone suppression test, immunoreactivity of CYP17A1 and CYP11B1, both required for cortisol biosynthesis, was markedly low (Figures 3E–H). The adjacent adrenal tissue demonstrated paradoxical hyperplasia of the ZG, a hyperplastic ZG with negative CYP11B2 immunoreactivity, and aldosterone-producing micronodules (30). There were no atrophic changes in the zona fasciculata (ZF) or zona reticularis (ZR) of the adjacent adrenal tissue (Supplementary Figure 1A). In the ZR, normal dehydroepiandrosterone sulfotransferase (DHEA-ST) immunoreactivity was observed (Supplementary Figure 1B).

TABLE 2 Results of adrenal venous sampling.

	Values
Right AV PAC (ng/dL)	1606.4
Right AV cortisol (μg/dL)	257
Left AV PAC (ng/dL)	257.3
Left AV cortisol (μg/dL)	498
IVC PAC (ng/dL)	115.1
IVC cortisol (μg/dL)	21.7
Selectivity index (right)	11.8
Selectivity index (left)	22.9
A/C (right AV)	6.25
A/C (left AV)	0.52
A/C (IVC)	5.3
Lateralized ratio	12.0
Contralateral ratio	0.10

Adrenal venous sampling was performed under cosyntropin stimulation. Selectivity index ≥ 5.0 was used as a cut-off for successful catheterization (26). Lateralized index > 4.0 was used as a cut-off for lateralized disease (26). AV, adrenal vein; PAC, plasma aldosterone concentration; IVC, inferior vena cava; A/C, aldosterone to cortisol ratio.

Targeted NGS identified double somatic *CTNNB1* (p.D32Y) and *GNA11* (p.Q209H) mutations with similar variant allele frequencies (Table 4). Using our method, these mutations were not detected in adjacent adrenal gDNA, suggesting their somatic origin. qPCR revealed high tumor expression of *CYP11B2* mRNA (599-fold over adjacent normal adrenal), confirming accurate sample collection. In agreement with previous studies (20, 31), *LHCGR* and *GNRHR* mRNA levels were also elevated within the tumor compared with those in adjacent normal adrenal (148-fold and 56-fold, respectively).

We further tested β-catenin protein localization using IF staining to assess Wnt/β-catenin activation status (Figures 4A–D). In IF staining, KCNJ5 was used as a plasma membrane marker. A subset of tumor cells revealed nuclear and/or cytoplasmic immunoreactivity of β-catenin, suggesting activated status, which is seen in the ZG of normal adrenal glands (32).

Discussion

The Wnt/β-catenin signaling pathway plays an important role in adrenocortical development, homeostasis, and regeneration (33). In the non-pathologic human adrenal cortex, activated β-catenin (nuclear and/or cytoplasmic expression) is restricted to the ZG, where physiologic aldosterone biosynthesis occurs. In contrast, non-activated β-catenin (cell membrane expression) is predominant in the ZF (32). Aberrant Wnt/β-catenin signaling was reported to lead to various adrenal disorders and dysregulated steroidogenesis (33). Although the prevalence of somatic *CTNNB1* mutation is relatively low in APA, activated β-catenin, i.e., nuclear and/or cytoplasmic localization of β-catenin, was reported in the majority of APA (34). A recent study investigating intra-tumor heterogeneity in APA demonstrated that β-catenin was activated mainly in CYP11B2-expressing regions of the tumor (16). The adrenal tumor from the present case showed diffuse CYP11B2 immunoreactivity. Like ZG cells, a subset of tumor cells demonstrated rosette-like structure and

10.3389/fendo.2024.1286297

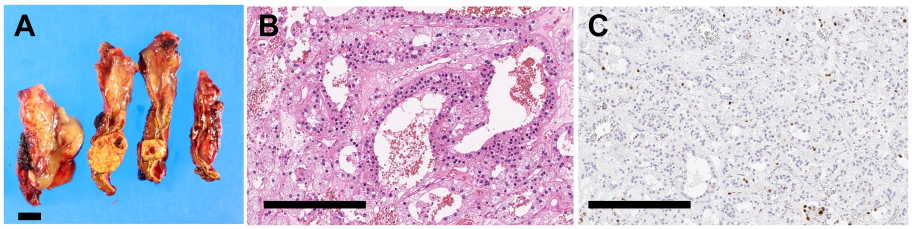


FIGURE 2
Histopathology of resected adrenal tumor. **(A).** Cut surfaces of resected adrenal tissue showing a yellow nodule with a diameter of 2.0 cm. Scale bar, 1 cm. **(B, C)**. High magnification photomicrographs of adrenal tumor. Scale bars, 300 μm. **(B)**. Hematoxylin and eosin staining. **(C)** Ki-67 staining.

activated β-catenin (Figure 4). The intense tumor expression of VSNL1, one of the ZG markers, also supports a ZG identity of the tumor (Figures 3C, D).

Zhou et al. (20) recently demonstrated the coexistence of gain-of-function mutations in *GNA11* or its close homolog, *GNAQ*, in 16 of 27 *CTNNB1*-mutated APAs (59%). The *GNA11* and *GNAQ* genes encode G-protein subunit alpha 11 (G11) and G-protein subunit alpha q (Gq), respectively. Gq/11 act as important modulators of angiotensin II receptor activation, which is one of the main physiologic regulators of aldosterone production in ZG cells (35). The mutations in *GNA11/Q* in APA have always been detected in the highly conserved p.Q209 residue that is crucial for GTPase activation. These mutations inhibit GTPase activity, resulting in constitutive activation of downstream signaling and enhanced aldosterone production (20). High tumor expression of LHCGR and GNRHR in APAs with *CTNNB1* (and *GNA11/Q*) mutations has been a rationale for the link between the disease onset and pregnancy, menopause, or puberty (20, 31). In Zhou's study above, double mutations of *CTNNB1* and *GNA11/Q* were more often seen in women than men (15 vs. 1) and the disease onset of 12 out of 16 cases (75%) was associated with pregnancy, menopause, or puberty (20). Our present case also showed elevated expression of *LHCGR*

TABLE 3 Post-operative clinical course.

	Post-operative data						
	1 month	3 months	6 months	12 months	18 months	24 months	30 months
Blood pressure (mmHg)	104/68	128/77	104/42	127/80	108/66	98/63	106/77
Serum creatinine (mg/dL)	0.66	0.68	0.73	0.66	0.72	0.66	0.67
Serum potassium (mEq/L)	4.4	4.2	4.9	4.0	4.6	4.3	4.3
PAC (ng/dL)	12.8	18.3	28.3	20.3	16.2[a]	13.2	7.9
PRA (ng/mL/h)	0.9	1.6	2.4	1.3	1.3	0.7	0.7
ARR	14.2	11.4	11.8	15.6	12.5	18.9	11.3

[a], Assay kit for PAC measurement (chemiluminescent enzyme immunoassay) was changed from the Accuraseed Aldosterone kit (FUJIFILM Wako Pure Chemical Corp, Japan) to the Accuraseed Aldosterone-S kit (FUJIFILM Wako Pure Chemical Corp, Japan) from this point. PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, aldosterone-to-renin ratio.

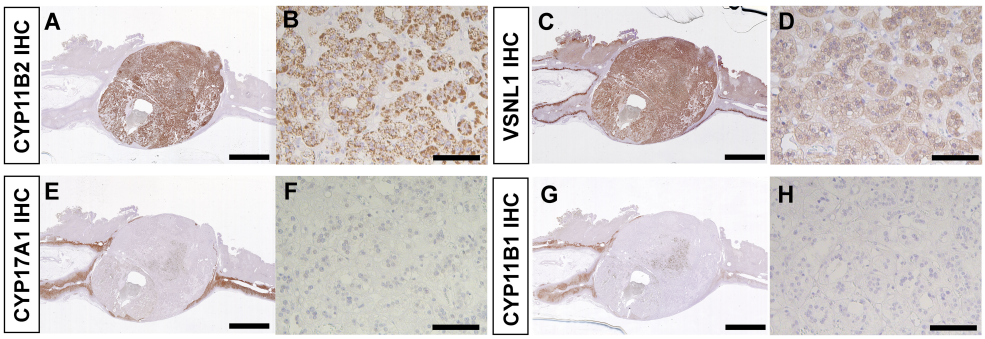


FIGURE 3
Immunohistochemistry of resected adrenal tumor. **(A, B)**. CYP11B2 IHC. **(C, D)**. VSNL1 IHC. **(E, F)**. CYP17A1 IHC. **(G, H)**. CYP11B1 IHC. **(A, C, E, G)**. Scanned images of stained slides. Scale bars, 5 mm. **(B, D, F, H)**. High magnification photomicrographs of adrenal tumor. Scale bars, 100 μm.

10.3389/fendo.2024.1286297

TABLE 4 Results of targeted NGS.

Gene	Exon	Nucleotide change	Amino acid change	FDP	VAF (%)	Reference sequence
CTNNB1	3	c.G94T	p.D32Y	1997	29.5	NM_001904
GNA11	5	c.G627C	p.Q209H	2000	29.7	NM_002067

FDP, flow-corrected read depth; VAF, variant allele frequency.

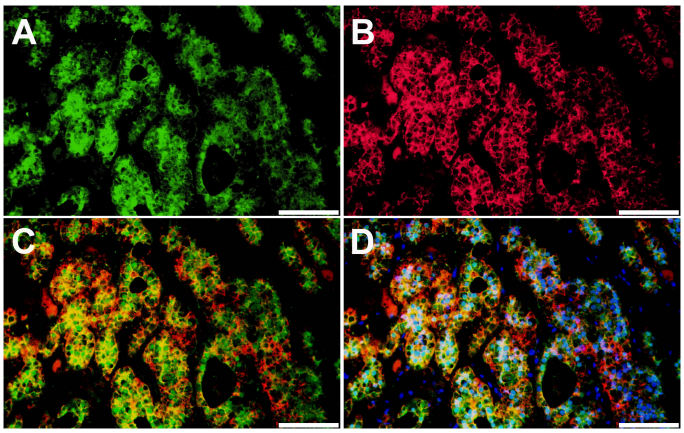


FIGURE 4
Localization of β-catenin protein in aldosterone-producing adenoma harboring somatic *CTNNB1* and *GNA11* mutations. β-catenin protein expression patterns in aldosterone-producing adenoma were assessed by immunofluorescence (IF) staining. **(A)**. IF for β-catenin (β-catenin, green). **(B)**. IF for KCNJ5 (KCNJ5, red). **(C)**. IF for β-catenin and KCNJ5. **(D)**. IF for β-catenin and KCNJ5 with DAPI (DAPI, blue). Scale bars, 100 μm.

and *GNRHR* mRNA in the tumor compared with that in adjacent adrenal. However, the pathophysiologic role of high tumor expression of *LHCGR* and *GNRHR* mRNA in our case is unclear since her disease onset was not directly associated with pregnancy or menopause. Of particular note, one of the 16 cases in Zhou's study had the same combination of mutations as our case (*CTNNB1* p.D32Y and *GNA11* p.Q209H) and the patient had no history of hypertension during her past 10 pregnancies (20).

Previous studies also reported aberrant expression of G protein-coupled receptors, including *LHCGR*, *GNRHR*, 5-hydroxytryptamine (serotonin) receptor 4 *(HTR4)*, and melanocortin 2 receptor *(MC2R)* in APAs (36, 37). In addition, some of patients with PA were reported to show enhanced aldosterone production in response to luteinizing hormone (LH), human chorionic gonadotropin (hCG), or gonadotropin-releasing hormone (GnRH) (38–42). Gagnon et al. (41) investigated genetic characteristics of GnRH/LH-responsive PA, including APA, bilateral macronodular adrenal hyperplasia, and other rarer forms. In their cohort, 17 patients with APA underwent *in vivo* GnRH and/or LH tests; 6, 10, and 1 had both, only GnRH, and only LH tests, respectively. Among 16 APAs tested for GnRH, 6 and 3 APAs showed positive and partial response, respectively. Positive response to LH was observed in 5 out of 7 APAs tested. Sequencing analysis of 15 APAs that had *in vivo* GnRH and/or LH tests revealed 3 *KCNJ5* (1 tested for GnRH and LH, no response; 1 tested for GnRH, partial response; 1 tested for LH, positive response), 1 *ATP1A1* (tested for GnRH, no response), and 1 *CACNA1D* mutations (tested for GnRH, no response). Of particular interest, there were no *CTNNB1*-mutated APAs in their cohort (41). Another study by Kishimoto et al. (40) demonstrated that *GNRHR* and *LHCGR* mRNA levels were higher and the response to GnRH was greater in APAs with no known mutations (mutation hotspots of *KCNJ5, ATP1A1, ATP2B3, CACNA1D*, and *CTNNB1* genes were screened) (n=9) compared with those with *KCNJ5* hotspot mutations (n=13). Genetic causes of GnRH/LH-responsive APAs appear to be heterogeneous and largely unknown. Further dedicated studies are needed.

Because of its rare incidence, clinical characteristics of the patients with APA harboring double *CTNNB1* and *GNA11/Q* mutations are not well characterized. Our case had typical clinical characteristics of PA with no excess cortisol co-secretion. Although the histologic findings were compatible with adrenocortical adenoma according to the criteria of Weiss (27), the tumor cells had unusually high Ki-67 labeling index for an adenoma (43). The present case was therefore closely followed up after surgery. Post-operative clinical course was indeed excellent with achievement of clinical and biochemical cure and no tumor recurrence was observed. Our present case also indicates that the occurrence of APA with double *CTNNB1* and *GNA11* somatic mutations is not always associated with pregnancy or menopause. In conclusion, we present a case of APA with double somatic mutations in *CTNNB1* and *GNA11*. Detailed clinical and histologic examination will provide useful information for better characterization of patients with PA caused by these rare mutations.

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by National Hospital Organization Kyoto Medical Center and the University of Michigan. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

All authors made individual contributions to authorship. KN and WR conceived the idea of molecular analysis. KN drafted the manuscript. AB and AU performed molecular analysis. KN, YH, TM, and HO were involved in the care of the patient. KM, YY, and HS were involved in histologic diagnosis. AY, NS-A, and TT provided input for the case and manuscript. All authors contributed to the article and approved the submitted version.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was supported by NIH grants R01DK106618 and R01DK043140 to WR. This work was also supported by grants from Japan Heart Foundation and Takeda Science Foundation to KN.

Acknowledgments

We would like to thank Dr. Celso E. Gomez-Sanchez at the University of Mississippi for providing the monoclonal antibodies against human CYP11B1 and KCNJ5. We also thank medical staff at National Hospital Organization Kyoto Medical Center.

Conflict of interest

KN received a research grant from AstraZeneca, which is unrelated to the current work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1286297/full#supplementary-material

References

1. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science*. (2011) 331:768–72. doi: 10.1126/science.1198785

2. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet*. (2013) 45:440–4, 444e1-2. doi: 10.1038/ng.2550

3. Scholl UI, Goh G, Stolting G, de Oliveira RC, Choi M, Overton JD, et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet*. (2013) 45:1050–4. doi: 10.1038/ng.2695

4. Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet*. (2013) 45:1055–60. doi: 10.1038/ng.2716

5. Scholl UI, Stolting G, Nelson-Williams C, Vichot AA, Choi M, Loring E, et al. Recurrent gain of function mutation in calcium channel CACNA1H causes early-onset hypertension with primary aldosteronism. *Elife*. (2015) 4:e06315. doi: 10.7554/eLife.06315

6. Nanba K, Blinder AR, Rege J, Hattangady NG, Else T, Liu CJ, et al. Somatic CACNA1H mutation as a cause of aldosterone-producing adenoma. *Hypertension*. (2020) 75:645–9. doi: 10.1161/HYPERTENSIONAHA.119.14349

7. Scholl UI, Stolting G, Schewe J, Thiel A, Tan H, Nelson-Williams C, et al. CLCN2 chloride channel mutations in familial hyperaldosteronism type II. *Nat Genet*. (2018) 50:349–54. doi: 10.1038/s41588-018-0048-5

8. Fernandes-Rosa FL, Daniil G, Orozco IJ, Goppner C, El Zein R, Jain V, et al. A gain-of-function mutation in the CLCN2 chloride channel gene causes primary aldosteronism. *Nat Genet*. (2018) 50:355–61. doi: 10.1038/s41588-018-0053-8

9. Dutta RK, Arnesen T, Heie A, Walz M, Alesina P, Soderkvist P, et al. A somatic mutation in CLCN2 identified in a sporadic aldosterone-producing adenoma. *Eur J Endocrinol*. (2019) 181:K37–41. doi: 10.1530/EJE-19-0377

10. Scholl UI. Genetics of primary aldosteronism. *Hypertension*. (2022) 79:887–897. doi: 10.1161/HYPERTENSIONAHA.121.16498

11. Wu X, Azizan EAB, Goodchild E, Garg S, Hagiyama M, Cabrera CP, et al. Somatic mutations of CADM1 in aldosterone-producing adenomas and gap junction-dependent regulation of aldosterone production. *Nat Genet*. (2023) 55:1009–21. doi: 10.1038/s41588-023-01403-0

12. Rege J, Bandulik S, Nanba K, Kosmann C, Blinder AR, Plain A, et al. Somatic SLC30A1 mutations altering zinc transporter ZnT1 cause aldosterone-producing adenomas and primary aldosteronism. *Nat Genet*. (2023) 55:1623–31. doi: 10.1038/s41588-023-01498-5

13. Nanba K, Omata K, Else T, Beck PCC, Nanba AT, Turcu AF, et al. Targeted molecular characterization of aldosterone-producing adenomas in white Americans. *J Clin Endocrinol Metab*. (2018) 103:3869–76. doi: 10.1210/jc.2018-01004

14. Nanba K, Omata K, Gomez-Sanchez CE, Stratakis CA, Demidowich AP, Suzuki M, et al. Genetic characteristics of aldosterone-producing adenomas in blacks. *Hypertension*. (2019) 73:885–92. doi: 10.1161/HYPERTENSIONAHA.118.12070

15. Nanba K, Yamazaki Y, Bick N, Onodera K, Tezuka Y, Omata K, et al. Prevalence of somatic mutations in aldosterone-producing adenomas in Japanese patients. *J Clin Endocrinol Metab*. (2020) 105:e4066–e4073. doi: 10.1210/clinem/dgaa595

16. De Sousa K, Boulkroun S, Baron S, Nanba K, Wack M, Rainey WE, et al. Genetic, cellular, and molecular heterogeneity in adrenals with aldosterone-producing adenoma. *Hypertension*. (2020) 75:1034–44. doi: 10.1161/HYPERTENSIONAHA.119.14177

17. Akerstrom T, Maharjan R, Sven Willenberg H, Cupisti K, Ip J, Moser A, et al. Activating mutations in CTNNB1 in aldosterone producing adenomas. *Sci Rep*. (2016) 6:19546. doi: 10.1038/srep19546

18. Scholl UI, Healy JM, Thiel A, Fonseca AL, Brown TC, Kunstman JW, et al. Novel somatic mutations in primary hyperaldosteronism are related to the clinical, radiological and pathological phenotype. *Clin Endocrinol (Oxf)*. (2015) 83:779–89. doi: 10.1111/cen.12873

19. Wu VC, Wang SM, Chueh SJ, Yang SY, Huang KH, Lin YH, et al. The prevalence of CTNNB1 mutations in primary aldosteronism and consequences for clinical outcomes. *Sci Rep*. (2017) 7:39121. doi: 10.1038/srep39121

20. Zhou J, Azizan EAB, Cabrera CP, Fernandes-Rosa FL, Boulkroun S, Argentesi G, et al. Somatic mutations of GNA11 and GNAQ in CTNNB1-mutant aldosterone-producing adenomas presenting in puberty, pregnancy or menopause. *Nat Genet*. (2021) 53:1360–72. doi: 10.1038/s41588-021-00906-y

21. Kitamura T, Blinder AR, Nanba K, Tsuiki M, Mishina M, Okuno H, et al. ACTH-independent production of 11-oxygenated androgens and glucocorticoids in an adrenocortical adenoma. *Eur J Endocrinol*. (2022) 187:K39–45. doi: 10.1530/EJE-22-0508

22. Yang Y, Gomez-Sanchez CE, Jaquin D, Aristizabal Prada ET, Meyer LS, Knosel T, et al. Primary aldosteronism: KCNJ5 mutations and adrenocortical cell growth. *Hypertension*. (2019) 74:809–16. doi: 10.1161/HYPERTENSIONAHA.119.13476

23. Nanba K, Chen AX, Omata K, Vinco M, Giordano TJ, Else T, et al. Molecular heterogeneity in aldosterone-producing adenomas. *J Clin Endocrinol Metab*. (2016) 101:999–1007. doi: 10.1210/jc.2015-3239

24. Pezzi V, Mathis JM, Rainey WE, Carr BR. Profiling transcript levels for steroidogenic enzymes in fetal tissues. *J Steroid Biochem Mol Biol*. (2003) 87:181–9. doi: 10.1016/j.jsbmb.2003.07.006

25. Takeuchi J, Sakagami Y, Perez RC. The mother and child health handbook in Japan as a health promotion tool: An overview of its history, contents, use, benefits, and global influence. *Glob Pediatr Health*. (2016) 3:2333794X16649884. doi: 10.1177/2333794X16649884

26. Naruse M, Katabami T, Shibata H, Sone M, Takahashi K, Tanabe A, et al. Japan Endocrine Society clinical practice guideline for the diagnosis and management of primary aldosteronism 2021. *Endocr J*. (2022) 69:327–59. doi: 10.1507/endocrj.EJ21-0508

27. Weiss LM. Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. *Am J Surg Pathol*. (1984) 8:163–9. doi: 10.1097/00000478-198403000-00001

28. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, et al. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol*. (2017) 5:689–99. doi: 10.1016/S2213-8587(17)30135-3

29. Seccia TM, Caroccia B, Gomez-Sanchez EP, Vanderriele PE, Gomez-Sanchez CE, Rossi GP. Review of markers of zona glomerulosa and aldosterone-producing adenoma cells. *Hypertension*. (2017) 70:867–74. doi: 10.1161/HYPERTENSIONAHA.117.09991

30. Williams TA, Gomez-Sanchez CE, Rainey WE, Giordano TJ, Lam AK, Marker A, et al. International histopathology consensus for unilateral primary aldosteronism. *J Clin Endocrinol Metab*. (2021) 106:42–54. doi: 10.1210/clinem/dgaa484

31. Teo AE, Garg S, Shaikh LH, Zhou J, Karet Frankl FE, Gurnell M, et al. Pregnancy, primary aldosteronism, and adrenal CTNNB1 mutations. *N Engl J Med*. (2015) 373:1429–36. doi: 10.1056/NEJMoa1504869

32. Boulkroun S, Samson-Couterie B, Golib-Dzib JF, Amar L, Plouin PF, Sibony M, et al. Aldosterone-producing adenoma formation in the adrenal cortex involves expression of stem/progenitor cell markers. *Endocrinology*. (2011) 152:4753–63. doi: 10.1210/en.2011-1205

33. Little DW 3rd, Dumontet T. LaPensee CR and Hammer GD. beta-catenin in adrenal zonation and disease. *Mol Cell Endocrinol*. (2021) 522:111120. doi: 10.1016/j.mce.2020.111120

34. Berthon A, Drelon C, Ragazzon B, Boulkroun S, Tissier F, Amar L, et al. WNT/beta-catenin signalling is activated in aldosterone-producing adenomas and controls aldosterone production. *Hum Mol Genet*. (2014) 23:889–905. doi: 10.1093/hmg/ddt484

35. Nogueira EF, Bollag WB, Rainey WE. Angiotensin II regulation of adrenocortical gene transcription. *Mol Cell Endocrinol*. (2009) 302:230–6. doi: 10.1016/j.mce.2008.08.024

36. Saner-Amigh K, Mayhew BA, Mantero F, Schiavi F, White PC, Rao CV, et al. Elevated expression of luteinizing hormone receptor in aldosterone-producing adenomas. *J Clin Endocrinol Metab*. (2006) 91:1136–42. doi: 10.1210/jc.2005-1298

37. Ye P, Mariniello B, Mantero F, Shibata H, Rainey WE. G-protein-coupled receptors in aldosterone-producing adenomas: a potential cause of hyperaldosteronism. *J Endocrinol*. (2007) 195:39–48. doi: 10.1677/JOE-07-0037

38. Zwermann O, Suttmann Y, Bidlingmaier M, Beuschlein F and Reincke M. Screening for membrane hormone receptor expression in primary aldosteronism. *Eur J Endocrinol*. (2009) 160:443–51. doi: 10.1530/EJE-08-0711

39. Albiger NM, Sartorato P, Mariniello B, Iacobone M, Finco I, Fassina A, et al. A case of primary aldosteronism in pregnancy: do LH and GNRH receptors have a potential role in regulating aldosterone secretion? *Eur J Endocrinol*. (2011) 164:405–12. doi: 10.1530/EJE-10-0879

40. Kishimoto R, Oki K, Yoneda M, Gomez-Sanchez CE, Ohno H, Kobuke K, et al. Gonadotropin-releasing hormone stimulate aldosterone production in a subset of aldosterone-producing adenoma. *Med (Baltimore)*. (2016) 95:e3659. doi: 10.1097/MD.0000000000003659

41. Gagnon N, Caceres-Gorriti KY, Corbeil G, El Ghoyareb N, Ludwig N, Latour M, et al. Genetic characterization of gnRH/LH-responsive primary aldosteronism. *J Clin Endocrinol Metab*. (2018) 103:2926–35. doi: 10.1210/jc.2018-00087

42. Castinetti F, Guerin C, Louiset E, Lacroix A. HCG-responsive aldosteronoma with transient secretion during pregnancy confirmed through HCG-stimulated adrenal venous sampling. *Front Endocrinol (Lausanne)*. (2023) 14:1153374. doi: 10.3389/fendo.2023.1153374

43. Iino K, Sasano H, Yabuki N, Oki Y, Kikuchi A, Yoshimi T, et al. DNA topoisomerase II alpha and Ki-67 in human adrenocortical neoplasms: A possible marker of differentiation between adenomas and carcinomas. *Mod Pathol*. (1997) 10:901–7. doi: 10.1097/00005392-199809010-00103

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 07 March 2024
DOI 10.3389/fendo.2024.1355398

OPEN ACCESS

EDITED BY
Silvia Monticone,
University of Turin, Italy

REVIEWED BY
Elisa Sconfienza,
University of Turin, Italy
Antonio Concistrè,
Sapienza University of Rome, Italy
Luigi Petramala,
Sapienza University of Rome, Italy

*CORRESPONDENCE
Josephine McCarthy
Josephine.mccarthy@monash.edu

†These authors have contributed equally to this work

RECEIVED 21 December 2023
ACCEPTED 21 February 2024
PUBLISHED 07 March 2024

CITATION
McCarthy J, Munnings M, Clissold B, Fuller PJ, Yang J and Phan TG (2024) Prevalence of primary aldosteronism in acute stroke or transient ischemic attack: a systematic review and meta-analysis. *Front. Endocrinol.* 15:1355398. doi: 10.3389/fendo.2024.1355398

Prevalence of primary aldosteronism in acute stroke or transient ischemic attack: a systematic review and meta-analysis

Josephine McCarthy[1,2,3,4]*, Mitchell Munnings[5], Benjamin Clissold[1,6], Peter J. Fuller[2,3], Jun Yang[1,2,3†] and Thanh G. Phan[1,6†]

[1]Department of Medicine, Monash University, Clayton, VIC, Australia, [2]Department of Endocrinology, Monash Health, Clayton, VIC, Australia, [3]Centre for Endocrinology and Metabolism, Hudson Institute of Medical Research, Clayton, VIC, Australia, [4]Department of Endocrinology, Eastern Health, Melbourne, VIC, Australia, [5]Department of Endocrinology, Launceston General Hospital, Launceston, TAS, Australia, [6]Department of Neurology, Monash Health, Clayton, VIC, Australia

Background and purpose: Primary aldosteronism (PA) is the most common endocrine cause of secondary hypertension with a prevalence of 14% in patients with newly diagnosed hypertension. Patients with PA experience a higher rate of cardiovascular events including stroke when compared to those with blood pressure matched essential hypertension. This systematic review and meta-analysis summarize current evidence on the prevalence of PA in patients with acute stroke or transient ischemic attack (TIA).

Methods: Two reviewers independently reviewed the literature for observational studies on the prevalence of PA in patients with acute stroke or TIA. MEDLINE and Embase were searched for studies up to December 13, 2023.

Results: Three single center studies conducted in Japan, Singapore and China were found to meet the inclusion criteria. The reported prevalence of PA in two cohort studies of adults with stroke or TIA were 3.1% and 4.0% and a third cross-sectional study in adults under 45 years old revealed a prevalence rate of 12.9%. Following a meta-analysis, the pooled prevalence of PA in adults with stroke or TIA is 5.8% [95% CI 1.6%-12.3%].

Conclusions: A considerable proportion of patients with stroke or TIA may have PA as the underlying cause of their hypertension. Given the increased risk of stroke associated with PA, clinicians should consider screening for PA in hypertensive patients with stroke or TIA. Further research is needed to evaluate the effect of timing and interfering medications on test results, which will inform an evidence-based approach to testing for PA following TIA or stroke.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42022328644.

KEYWORDS

primary aldosteronsim, hypertension, stroke, transient ischaemic attack, blood pressure

1 Introduction

Primary aldosteronism (PA), also known as Conn's syndrome, is a potentially curable form of secondary hypertension. PA is characterized by autonomous adrenal production of aldosterone, independent of renin production, resulting in a high aldosterone-to-renin ratio (ARR) (1). Studies in the general hypertensive population have found a PA prevalence of 5-15% (2–4). Hypertension due to PA results in a higher rate of cardiovascular events including stroke when compared with blood pressure (BP) matched essential hypertension. A meta-analysis of 31 studies, including 3838 patients with PA and 9284 patients with essential hypertension found that after a median of 8.8 years from hypertension diagnosis, compared to patients with essential hypertension, patients with PA had an increased risk of stroke with an odds ratio [OR] of 2.58 (95% CI 1.93-3.45) (5). Furthermore, we now know PA is more prevalent in patients with cardioembolic stroke and atrial fibrillation (AF) (6). PA is a treatable condition, either with curative surgery in the setting of a unilateral aldosterone producing adenoma or mineralocorticoid receptor antagonists (MRA) for bilateral adrenal disease. Targeted treatment leads to lower rates of cardiovascular events and stroke (7, 8). Despite the availability of targeted treatment, there is an absence of recommendations to screen for PA in stroke management guidelines (9–12). This systematic review and meta-analysis was performed to answer the clinical research question: what is the prevalence of PA in adults with acute stroke or transient ischaemic attack (TIA)? This evidence will inform future studies and may assist with guideline development.

2 Methods

For This systematic review adheres to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (13). The review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (URL: https://www.crd.york.ac.uk/PROSPERO/; unique identifier: CRD42022328644).

2.1 Search strategy

A comprehensive literature search was conducted for the prevalence of PA in acute stroke or (TIA). MEDLINE and Embase were searched using the Ovid platform from inception to December 13, 2023. Three concepts were included in the search strategy: PA, stroke, and TIA. Prevalence was used as a concept in the initial search strategy but then removed due to the few results returned when this term was included. No language or location limits were applied. The online database search was supplemented by a manual search of the reference lists of relevant articles which did not identify any further relevant records. The complete search strategy is provided in Supplementary Materials.

2.2 Eligibility criteria

2.2.1 Study types

The following study types were considered, cohort, cross-sectional and randomized controlled trials. Studies in languages other than English were considered.

2.2.2 Condition

Assessment of condition, context and population was used to formulate the clinical research question; what is the prevalence of PA in adult patients with acute stroke or TIA? (14) The condition under examination was PA, as diagnosed by a positive confirmatory test with centre-specific diagnostic thresholds (Table 1).

2.2.3 Context

The context was adult inpatients or outpatients with acute stroke or TIA.

2.2.4 Population

The population was adults 18 years old and over.

2.2.5 Exclusion criteria

Studies were excluded if 1) they were duplicates, 2) they were case series or case studies, 3) the participants were under the age of 18 years old, 4) it was the wrong patient population, or 4) there was no full text available.

2.3 Study selection

Duplicates were removed prior to importing articles into COVIDENCE. Two review authors (JM and MM) independently reviewed titles and abstracts against the eligibility criteria with translation assistance from a colleague fluent in French for one article published in French. Irrelevant articles were removed. The full texts of remaining articles were assessed against eligibility criteria. Any conflicts in study selection between the two review authors (JM, MM) were resolved by the senior review author (JY).

TABLE 1 Study Characteristics.

First author, Year, Country	Study design, sample size, n	Stroke, n (%)	TIA, n (%)	Age, y	Male (%)	Interfering medications	Positive ARR threshold	Screen test timing from stroke or TIA	Positive Confirmatory test threshold	PA prevalence in hypertensive patients, % (n)	PA prevalence, % (n)
Miyaji (15), 2016, Japan	Cohort, single center, 427	Ischemic 256 (60), ICH 106 (24.8), SAH 38 (8.9)	27 (6.3)	74*	56.7	Yes	ARR ≥200 pg/mL per ng/mL/hr (67 pmol/L per mU/L) and PAC ≥12 ng/dL (≥332 pmol/L)	Admission and 1 week	Rapid ACTH test: ratio of maximal PAC to cortisol ≥8.5	4.9 (14/288) 5.5‡	4.0 (17/427) 4.6 (17/373) ‡
Nguyen (6), 2022, Singapore	Cohort, single center, 192	Ischemic 156 (81.3), small artery occlusion 50 (26), large artery atherosclerosis 25 (13), cardioembolic 19 (10), undetermined 62 (32.3), hemorrhagic 20 (10.4)	16 (8.3)	58†	71	Yes	ARR >277 pmol/l per ng/ml/h (33.7 pmol/L per mU/L)	2-4 months	Seated SIT: post saline PAC >138 pmol/L OR hypokalemia with undetectable PRA and PAC >277 pmol/L	4.0 (95% CI: 0.9% –7.1%) (6/150)	3.1 (6/192) (95% CI: 1.2-6.7%)
Tang (16), 2020, China	Cross-sectional, single center, 116	Ischemic 71 (61), hemorrhagic 41 (35)	5 (4.3)	39*	75	No	ARR >1.00 ng/dl per μIU/ml (27.7 pmol/L per mU/L) and PAC >8 ng/dl (>221 pmol/L)	>3 months	Captopril challenge test: 2 hr PAC >11 ng/dl (55pmol/L)	21.2 (14/66)	12.9 (15/116)

ACTH, adrenocorticotropic hormone; ARR, aldosterone to renin ratio; ICH, intracerebral hemorrhage; PA, primary aldosteronism; PAC, plasma aldosterone concentration; PRA, plasma renin activity; SAH, subarachnoid hemorrhage; SIT, Saline Infusion Test; TIA, transient ischaemic attack. *Mean age, †Median age, †Prevalence in the 373 patients who completed both screening tests. Conversion factors: Aldosterone; 1ng/dL =27.7pmol/L, 1ng/dL = 10pg/mL. Renin: plasma renin activity 1 ng/mL/h = direct renin concentration 8.2 mU/L.

10.3389/fendo.2024.1355398

2.4 Data extraction

Two reviewers (JM, MM) independently performed two rounds of screening; (1) title and abstract and (2) full text of the remaining studies. COVIDENCE was used to record the extracted data. Data recorded included author, year of publication, study design, participant details, study setting, population characteristics, and results of PA screening and confirmatory tests. Any discrepancies in data recorded by the two reviewers (JM, MM) were resolved by a senior author (JY). The data extraction form is provided in Supplementary Materials.

2.5 Assessment of risk of bias

The University of Adelaide Joanna Briggs Institute's (JBI) Critical Appraisal Tool Checklist for Studies Reporting Prevalence Data was used by the two reviewers (JM and MM) to assess methodological quality of studies and risk of bias (14). The following areas of bias were assessed: sample frame, sampling, sample size, description subjects and setting, data analysis coverage, validity of methods to identify condition, statistical analysis and response rate. The complete data collection tool is provided in Supplementary Materials.

2.6 Statistical analysis

Statistical analyses were performed using Rstudio version 2022.12.0 Build 353 tidyverse and metaphor packages. To account for heterogeneity between studies a random effects model was used. Heterogeneity was assessed using the I^2 value. Heterogeneity interpretation was guided by the Cochrane Handbook; I^2 of 0-40% might not be important, 30-60% may represent moderate heterogeneity, 50-90% may represent substantial heterogeneity and 75-100% considerable heterogeneity (17).

2.7 Deviations from the protocol

There were no deviations from the systematic review protocol registered on PROSPERO.

2.8 Roles and responsibilities

JM developed the search strategy, performed the literature search, screened title and abstracts followed by full text review, data extraction and manuscript preparation. MM independently screened title and abstracts followed by full text review, data extraction and assisted with manuscript preparation. TP and JM performed the meta-analysis. JY assisted with the design of the systematic review and resolved conflicts in article eligibility and data extraction.

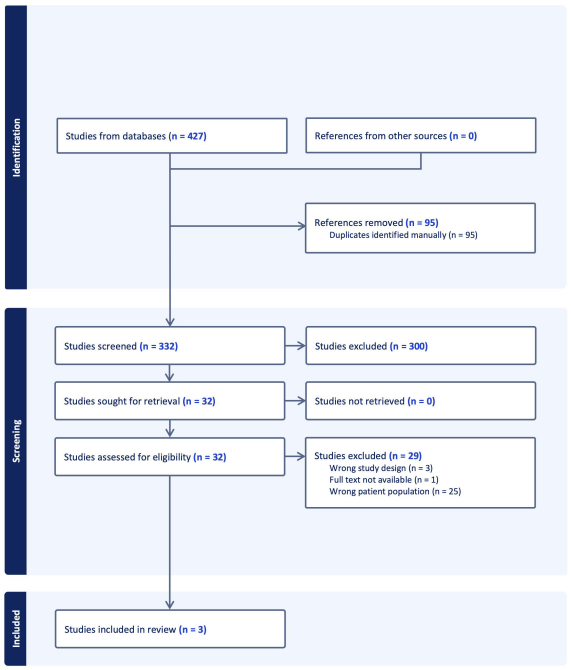


FIGURE 1
Preferred reporting items for systematic reviews and meta-analyses flowchart of included studies.

3 Results

3.1 Search results

The online databases yielded 427 articles; 95 duplicate articles were removed leaving 332 articles imported to COVIDENCE for screening. Following title and abstract screening, 32 full text articles remained for screening. Three articles fulfilled eligibility criteria for inclusion (Figure 1) (6, 15, 16).

3.2 Study characteristics

Two single center studies, one from Singapore and the other from Japan, were cohort studies of patients admitted with stroke or TIA (6, 15). The third, also a single center study undertaken in China, was a cross-sectional study in young adults with stroke or TIA (16). All studies assessed the prevalence of PA in patients with stroke or TIA. The characteristics of the three included studies are presented in Table 1.

3.3 Prevalence of PA in patients with stroke or TIA

The prevalence of PA in the prospective cohort study by Miyaji et al. was 4.0% (17 of 427) (15). This was based on two ARR screening tests at initial hospital presentation and 6.8+/- 3.5 days after hospitalization, and the ACTH confirmatory test (Table 1).

10.3389/fendo.2024.1355398

The prevalence of PA amongst patients with a history of hypertension was 4.9%. The mean cohort age was 74.3 years. The criteria for a positive screening test using plasma aldosterone concentration (PAC) and plasma renin activity (PRA) were ARR ≥200 pg/mL per ng/mL/hr (67 pmol/L per mU/L) and PAC ≥12 ng/dL (332 pmol/L) based on the Japan Endocrine Society guidelines. Confirmatory testing was performed if both screening tests were positive. Exceptions were made for some patients with only one positive screening test to proceed to confirmatory testing. This was due to the potential interference of medications given prehospital or during admission which may have resulted in a false negative screening test. Seven patients with an initial negative ARR and a follow up positive ARR went on to confirmatory testing of which two were diagnosed with PA. Five patients with an initial positive ARR and a follow-up negative ARR went on to confirmatory testing of which none were diagnosed with PA.

The prospective cohort study by Nguyen et al. used a single ARR screening test at two to four months post stroke or TIA and found a prevalence of PA of 3.1% (6 of 192) (6). Twenty six of 192 (14%) participants had a positive ARR. In patients with hypertension the prevalence of PA was 4.0% (95% CI: 0.9-7.1%). The median cohort age in this prospective study was 58 years [range 21-78 years]. The criteria for a positive screening test using PAC and PRA was an ARR cut-off >277 pmol/L per ng/ml/h (33.7 pmol/L per mU/L). Of the 192 participants who had an ARR screening test, 14 were positive and underwent a confirmatory saline suppression test of which 3 were positive. Another three patients were diagnosed with PA without confirmatory testing due to elevated aldosterone, undetectable renin and hypokalemia, or a cardiac/renal contraindication to saline suppression test.

In the cross-sectional study by Tang et al. of adults under the age of 45 years old with TIA or stroke, the prevalence of PA was 12.9% (15 of 116) (16). In patients who also had hypertension, the prevalence of PA was 21.2%. The mean age was 39.1 years. The ARR cut-off was 1.00 ng/dl per μIU/ml (27.7 pmol/L per mU/L) based on plasma aldosterone and renin concentrations. Duration between stroke or TIA and tests varied but no patients were screened within the first 3 months. Interfering medications were withdrawn prior to screening and confirmatory testing was performed with the captopril challenge test.

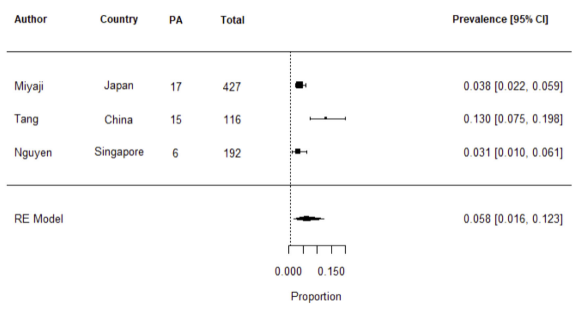


FIGURE 2
Meta-analysis of studies on the prevalence of PA in stroke or TIA using random effects model. Heterogeneity: Tau^2 = 0.0085; Q = 12.18; df = 2 (p = 0.0023); I^2 = 87.66%. Test for overall effect: Z = 4.36 (p = <0.0001).

3.4 Meta-analysis of prevalence of PA in stroke or TIA

The pooled prevalence of PA in patients with stroke or TIA across the three studies was 5.8% [95% CI 1.6%-12.3%], I^2 = 87.66% (Figure 2). In the presence of heterogeneity, one would normally proceed to a meta-regression analysis, however given there were only three studies, this was not pursued.

3.5 Risk of bias assessment of included studies

The three included studies used an appropriate sample frame of patients with TIA or stroke to address the target population. Tang et al. investigated prevalence only in adults <45 years and the smaller sample size may contribute to meta-analysis heterogeneity. All studies described the study subjects and setting in detail and data analysis was conducted with sufficient coverage of the identified sample. Miyaji et al. used the rapid Adrenocorticotrophic Hormone (ACTH) confirmatory test which is not well validated and may result in classification bias. Overall, using the University of Adelaide JBI Critical Appraisal Tool Checklist for Studies Reporting Prevalence Data, the three studies had a low risk of bias.

3.6 Management of interfering medications during PA testing in the setting of stroke or TIA

Two thirds of reported studies in this systematic review and meta-analysis (Miyaji et al. and Nguyen et al) continued interfering medications during screening and confirmatory testing. Interfering medications were withdrawn for screening and confirmatory testing in the study by Tang et al. where no patients were screened within the first three months of stroke or TIA (16). However, the prospective cohort studies where patients were assessed in the acute and subacute periods, interfering antihypertensive medications were continued during screening and/or confirmatory testing (6, 15). In Miyaji et al. 55.3% of all patients were on antihypertensive medications (15). In patients with PA, 52.9% (9 of 17) were on antihypertensive medication: 52.9% (9 of 17) were on calcium channel blockers, 11.8% (2 of 17) were on angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) and 5.9% (1 of 17) were on diuretics; no patients were on other antihypertensive medications including beta blockers (15). The total proportion of patients on antihypertensive medication in Nguyen et al. is not reported (6). However, of the 192 patients who completed screening 38% were on a calcium channel blocker, 35.9% were on an ACEI or ARB, 24% were on a beta-blocker, 2% on diuretics and 1% on alpha blockers (6).

3.7 Blood pressure in patients with PA and stroke or TIA

Both Miyaji et al. and Tang et al. found mean systolic blood pressure (SBP) during stroke or TIA admission was significantly higher in patients with PA compared to patients without PA (170.9 +/-26.1 mmHg vs 162.0 +/- 31.6 mmHg (*P* 0.012); and 180.0 +/- 30.9 mmHg vs 148.9 +/-29.6 mmHg respectively (*P* 0.002)) (See Supplementary Table 1) (15, 16). Miyaji et al. and Tang et al. also found acute admission mean diastolic blood pressure (DBP) was significantly elevated In PA compared to patients without PA (101.8 +/-15.7mmHg vs 89.2 +/- 20.3mmHg (*P* 0.003): and 125.9 +/-24.5mmHg vs 94.1 +/-20.5mmHg (*P* 0.000)) (15, 16).

The prevalence of hypertension in Miyaji et al's whole cohort (patients with and without PA) was 67.4%, and 82.4% in patients with PA compared to 68.8% in patients without PA. In patients with PA, mean initial BP was consistent with Stage 2 hypertension (mean BP 180/102 mmHg). Patients without PA also had a mean initial BP consistent with Stage 2 hypertension however the BP was not as elevated (mean BP 162/89 mmHg) compared to patients with PA.

Tang et al. recorded BP during initial admission in the cohort of young adults with stroke. The prevalence of hypertension in the whole cohort (patients with and without PA) was 57%. The prevalence of hypertension in patients with PA was 93.3% compared to 51.5% in patients without PA. Patients with PA also had higher a Grade of hypertension; 0% had Grade I hypertension (SBP 140-159 mmHg and/or DBP 90-99 mmHg), 14.3% had Grade II hypertension (SBP 160-179 mmHg and or DBP 100-109 mmHg), and 85.7% had Grade III hypertension (SBP ≥ 180 mmHg and/or DBP ≥ 110 mmHg) (18). Patients without PA had a lower Grade of hypertension; 23.1% Grade I hypertension, 28.8% Grade II hypertension and 48.1% Grade III hypertension (P = 0.00 when compared to patients with PA). This confirms that the grade of hypertension is positively associated with the presence of PA.

Nguyen et al. did not stratify initial admission SBP according to PA status but did so for three months. The prevalence of hypertension in the whole cohort (patients with and without PA) of Nguyen et al. was 78.1%. The prevalence of hypertension in patients with PA was 100%, compared to 77.4% in patients without PA. At three months when stratified by PA status, median SBP in patients with and without PA was not significantly different (145mmHg vs 137mmHg respectively (*P* 0.36)). On the other hand, at three months median DBP was significantly higher in patients with PA compared to patients without PA; 87.0 mmHg [range 84.3, 92.8] vs 80.0mmHg [74.0, 86.0] (*P* 0.011) (6). Patients with PA had a median BP consistent with American Heart Association Stage 2 hypertension (median BP 145/87 mmHg) versus Stage 1 hypertension in patients without PA (median BP 137/80 mmHg) (19). Resistant hypertension was defined as clinic systolic BP ≥140 mmHg, or diastolic BP ≥90 mmHg, while on three antihypertensive medications. Of the whole cohort 0.05% (9 of 192) had resistant hypertension. The prevalence of PA was higher in patients with resistant hypertension, 11.1% (1 of 9), 95% CI: 0.3%–48.3%.

3.8 Potassium levels in patients with PA and stroke or TIA

Miyaji et al. and Tang et al. both found that during admission for stroke or TIA, the mean potassium level in patients with PA were significantly lower compared to patients without PA (3.7 +/- 0.4 mmol/L vs 4.1 +/- 0.5 mmol/L (*P* 0.001); and 3.13 +/- 0.50 mmol/L vs 4.01 +/- 0.40 mmol/L (*P* 0.000) respectively) (15, 16). However, serum potassium was similar in Nguyen et al. where only two of six patients with PA had hypokalemia. Serum potassium was not significantly different when stratified by ARR positive versus ARR negative (potassium 3.9 mmol/L [range 3.6, 4.1] compared with 4.0 mmol/L [range 3.7, 4.2] (*P* 0.25 respectively) (6). Nguyen et al. did not stratify potassium results according to the presence or absence of PA (6).

3.9 Comorbidities in patients with PA and stroke or TIA

There were several patient groups in which there was a higher prevalence of PA. Nguyen et al. found a higher prevalence of PA amongst patients with both hypertension and atrial fibrillation (30%, 3 of 10), hypertension and hypokalemia (13.3%, 2 of 15), cardioembolic stroke (10.5%, 2 of 19), and age ≤50 years (6.1%, 3 of 49) (6). Tang et al. and Miyaji et al. examined for differences in the prevalence of PA among those with or without diabetes, but no significant difference was found (15, 16).

3.10 Possible origin of stroke or TIA

Nguyen et al. and Tang et al. both used the Trial of Org 10172 in Acute Stroke Treatment (TOAST) Classification for stroke aetiology with the five categories being 1) large-artery atherosclerosis, 2) cardioembolism, 3) small-vessel occlusion, 4) stroke of other determined etiology, and 5) stroke of undetermined aetiology (20). There was no significant difference between the stroke subtypes in patients with PA compared to patients without PA in the studies by Nguyen et al. and Tang et al. (P 1.0 and P 0.674 respectively). Miyaji et al. did not report on aetiology of stroke.

4 Discussion

Hypertension is a major modifiable risk factor for ischemic and hemorrhagic stroke (21). Despite PA being the most common endocrine cause of secondary hypertension with an increased risk of AF, stroke and TIA compared to patients with BP matched essential hypertension, there is a lack of research on its prevalence

and management in acute stroke or TIA (5). Studies over the last decade have reported a PA prevalence of 5-15% in the general hypertensive population (2–4). Based on 738 patients from three studies, this meta-analysis found a PA pooled prevalence of 5.8% in patients with stroke or TIA.

The sex distribution of PA in this meta-analysis was consistent with that previously reported, where approximately half were men (2). Nguyen et al. and Tang et al. both reported a similar sex distribution of PA in stroke or TIA (66.7% in both studies) (6, 16). Miyaji et al. had fewer men in the cohort (56%) than Nguyen et al. and Tang et al, which may explain the lower proportion of men with PA (29.4%). There were no sub analyses of the sex distribution of PA in patients with TIA.

Difference in patients with PA compared to patients without PA are striking for the higher systolic BP (170-180mmHg vs 148-162mmHg), and lower serum potassium (3.1-3.7mmol/L vs 4.0-4.1mmol/L) in patients with PA. This is consistent with PA being more likely to cause resistant hypertension and hypokalaemia (1). Blood pressure was taken 3 months post stroke with a trend for a higher number of antihypertensive agents in patients with PA compared to without PA (1.7 vs 1.0 respectively) which may account for the lack of difference in SBP between groups in Nguyen et al. (6) The markedly higher DBP in patients with PA compared to without PA (125.9mmHg vs 94.1mmHg) in the younger cohort of Tang et al. has previously been seen in PA when stratified by age (16, 22). There was also a higher prevalence of PA in people with a history of hypertension. Only a small proportion of people were diagnosed with PA but without hypertension (0% - 17.6%). Clinical presentation of stroke or TIA with elevated blood pressure may be a useful trigger for PA testing.

Meta-analyses of prevalence often have high statistical heterogeneity. This meta-analysis displayed high heterogeneity with an I^2 statistic of 87.6%. A study of 134 meta-analyses of prevalence revealed the median I^2 was 96.9% (IQR 90.5–98.7) (23) with larger I^2 in meta-analysis with a higher number of studies or extreme pooled estimates (defined as <10% or >90%). The high I^2 observed in the present meta-analysis is consistent with other prevalence studies with an extreme pooled prevalence <10%, in this case 5.8%. There are several prevalence modifying factors which contribute to the statistical heterogeneity in this study including differences in age of the populations sampled, hypertension prevalence, ARR threshold, confirmatory tests used and time from stroke to testing. Tang et al. had a younger population and higher prevalence of hypertension. Monticone et al. demonstrated in a prospective cohort study that the prevalence of PA increases with an increase in the stage of hypertension of the cohort (24). Uniformity in population sampling and testing methods may reduce heterogeneity of future meta-analyses.

Time from acute cerebral event to testing for PA differed in the three studies and is likely to impact reported prevalence. Miyaji et al. tested in the first week of the acute cerebral event which may lead to a false negative ARR (25). Miyaji et al. found the acute phase ARR was less reliable at predicting PA than the post stroke ARR, however the evidence is limited by the lack of confirmatory testing in every patient with either one or two positive ARRs (15). Nguyen et al. and Tang et al. tested ARR several months after the acute cerebral event, this subacute time frame for testing may be less prone to false negative ARR. A prospective human study on RAAS during acute ischaemic stroke found that angiotensin I, renin and aldosterone were significantly lower, angiotensin II was unchanged, and angiotensin converting enzyme activity was higher in the acute phase (within 48 hours) compared to post-stroke (8 months) (25). Acute stroke can affect ARR by various mechanisms including, dehydration which increases renin and aldosterone, high BP during the acute stroke phase with higher adrenaline and cortisol levels which lowers aldosterone and renin and interfering antihypertensive medications (25). Further research is required to establish the optimal ARR threshold for PA testing in the context of the acute hormonal changes during acute stroke.

The main limitation for PA testing in someone with established hypertension is the confounding effect of antihypertensive drugs. The Endocrine Society recommends a washout of all interfering antihypertensive medications with the use of substitute medications that have minimal effect on ARR but acknowledges that the ARR can be interpreted accordingly if medication switching is not feasible (1). In this systematic review, only Tang et al. adjusted antihypertensive agents for testing. To maintain stringent BP control during and shortly after stroke or TIA, both Miyaji et al. and Nguyen et al. continued interfering medications through the testing period. The frequent use of antihypertensive agents which can cause false negative screening results may contribute to the lower prevalence of PA in these two cohorts (6, 15). Of note, Nguyen et al. adopted a lower ARR threshold of >277 pmol/l per ng/ml/h (33.7 pmol/L per mU/L) to reduce the number of false negative results caused by interfering medications, although the evidence base for the threshold was not stated. However, only in the absence of medication interference, an ARR cut-off of >70 pmol/mU has a sensitivity and specificity greater than 95% (26). There is a paucity of data to provide any robust recommendation on ARR thresholds whilst on interfering medications (27–29).

Changing antihypertensive medications to non-interfering agents for PA screening can be challenging in the acute stroke period. For patients on ACEI's, the aldosterone to angiotensin II ratio may be a more reliable marker of aldosterone excess than ARR (30). However, the assay to measure angiotensin II is not widely available and angiotensin II concentration is not reported in any of the three articles. Further research is needed to understand the utility of angiotensin II for the diagnosis of PA in patients with stroke or TIA.

Although ARR was the screening test of choice for each of the three studies, the number of positive ARR required to proceed to confirmatory testing differed. The reported prevalence of PA in Miyaji et al. was 4.0% (17 of 427) however, 26 patients did not have initial blood sampling and 28 patients did not have follow up blood sampling leaving 373 patients with both screening tests. If only patients with both screening tests were considered, the prevalence becomes 4.6% overall and 5.5% in those with hypertension (15). Nguyen et al.'s prospective cohort study found a slightly lower prevalence of 3.1% amongst all patients despite a lower ARR cut-off than Miyaji's study (6, 15). Of note, in Nguyen et al, more than 50% of the participants with an abnormal ARR had hypokalemia (15 of 26). Previous studies show approximately 30% of patients with PA

have hypokalemia, therefore it may be possible that some patients with PA and normokalemia were missed, rendering the prevalence rate an underestimate (1).

The three included studies used different confirmatory tests. Tang et al. and Nguyen et al. used captopril challenge test and saline suppression test respectively, which are two of the four recommended confirmatory tests of the Endocrine Society (1, 6, 16). Miyaji et al. used the rapid adrenocorticotropic hormone (ACTH) test which is not one of the tests recommended by the Endocrine Society (1, 15). A recent systematic review and meta-analysis, limited to English, on the performance of PA confirmatory tests did not find any studies on the validity of the rapid ACTH test (31). Only one study, published in Japanese, has compared the validity of the rapid ACTH test against the captopril challenge test or furosemide plus upright test and found the rapid ACTH test had a sensitivity and specificity of >95% (32). Clinical practice differs internationally for the preferred confirmatory test; this will impact the reported prevalence in each study.

The link between mineralocorticoids and stroke has been established experimentally in rodents (33, 34). Rocha et al. studied Stroke Prone Spontaneously Hypertensive (SHRSP) rats treated with either placebo or the mineralocorticoid antagonist eplerenone for 19 weeks (33). Blood pressure was equally raised in both groups. Placebo treated rats showed clinical signs of stroke earlier and all died by 18 weeks. By comparison, only one eplerenone treated rat showed signs of stroke and died at 18 weeks. Histopathology revealed severe ischemic and hemorrhagic stroke lesions in the placebo treated rats compared with mild cerebral injury in the eplerenone treated rats. Dorrance et al. induced stroke experimentally (thread-occlusion technique) in SHRSP rats of which half were treated with spironolactone and Wistar-Kyoto (WKY) normotensive rats which were treated with placebo (34). Spironolactone reduced cerebral ischemic damage by 50% in SHRSP rats. Dorrance and Rocha both found that despite the reduction in cerebral ischemia following treatment with a mineralocorticoid antagonist, there was no effect on SBP. Limited human studies have established several mechanisms for aldosterone's adverse vascular effects including endothelial dysfunction, increased arterial wall stiffness, structural cardiac remodeling through atrial dilatation and fibrosis, and electrical remodeling through arrhythmogenicity (35–38). Nguyen et al. found patients with PA had a larger left atrial volume index, which predisposes to AF. Nguyen et al. also found the prevalence of PA in patients with stroke, hypertension and AF was 30%, similar to that found by Seccia et al. in patients with hypertension and AF (42%) (39). Milliez et al. found a 12-fold higher risk of AF in patients with PA compared to patients with essential hypertension (40). The results of these rodent studies confirm aldosterone has harmful actions on cerebral vasculature independent of its ability to increase BP and that treatment with a mineralocorticoid receptor antagonist such as spironolactone or eplerenone can reduce the frequency and severity of stroke.

To summarize, the underlying pathophysiological mechanisms leading to an increased risk of stroke has been explored in rodent studies and limited human studies, it goes beyond that purely due to aldosterone-mediated hypertension. These studies provide compelling evidence that timely diagnosis and targeted treatment can significantly reduce the severity of cerebral injury and the occurrence of stroke.

5 Conclusion

This systematic review and meta-analysis revealed that 3.1-12.9% of patients with acute stroke or TIA have PA, with a higher prevalence of up to 22% if only hypertensive patients are considered. However, there was significant variability between studies including the timing of the test, nature of confirmatory testing and medication use. A larger prospective study where patients are screened both in the acute and outpatient settings would help to inform the optimal timing and conditions of PA testing in patients with stroke and TIA. Furthermore, all three studies were in East Asian and South East Asian populations; data from other populations are needed. Additional research will facilitate the development of evidence-based guidelines for PA testing in patients with stroke or TIA so that this highly modifiable cardiovascular risk factor can be efficiently ameliorated.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

JM: Conceptualization, Writing – original draft, Writing – review & editing. MM: Writing – review & editing. BC: Writing – review & editing. PF: Writing – review & editing. JY: Conceptualization, Writing – review & editing. TP: Conceptualization, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. JM is supported by an Australian Government Research Training Program Scholarship, and an Endocrine Society of Australia Research Higher Degree Scholarship.

Acknowledgments

We thank the Director of Library and Online Services, Eastern Health, Ruth Lawrence and Clinical Librarian, Monash Health, Keren Moskal for assistance with the search strategy and obtaining articles.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1355398/full#supplementary-material

References

1. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. (2016) 101:1889–916. doi: 10.1210/jc.2015-4061

2. Libianto R, Russell GM, Stowasser M, Gwini SM, Nuttall P, Shen J, et al. Detecting primary aldosteronism in Australian primary care: A prospective study. *Med J Aust*. (2022) 216:408–12. doi: 10.5694/mja2.51438

3. Käyser SC, Dekkers T, Groenewoud HJ, van der Wilt GJ, Carel Bakx J, van der Wel MC, et al. Study heterogeneity and estimation of prevalence of primary aldosteronism: A systematic review and meta-regression analysis. *J Clin Endocrinol Metab*. (2016) 101:2826–35. doi: 10.1210/jc.2016-1472

4. Xu Z, Yang J, Hu J, Song Y, He W, Luo T, et al. Primary aldosteronism in patients in China with recently detected hypertension. *J Am Coll Cardiol*. (2020) 75:1913–22. doi: 10.1016/j.jacc.2020.02.052

5. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: A systematic review and meta-analysis. *Lancet Diabetes Endocrinol*. (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4

6. Nguyen V, Tu TM, Mamauag MJB, Lai J, Saffari SE, Aw TC, et al. Primary aldosteronism more prevalent in patients with cardioembolic stroke and atrial fibrillation. *Front Endocrinol (Lausanne)*. (2022) 13:869980. doi: 10.3389/fendo.2022.869980

7. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: A retrospective cohort study. *Lancet Diabetes Endocrinol*. (2018) 6:51–9. doi: 10.1016/S2213-8587(17)30367-4

8. Nasri A, Mansour M, Brahem Z, Kacem A, Hassan AA, Derbali H, et al. Stroke disclosing primary aldosteronism: report on three cases and review of the literature. *Annales d Endocrinol*. (2017) 78:9–13. doi: 10.1016/j.ando.2016.07.993

9. Stroke Foundation. Clinical Guidelines for Stroke Management. Available online at: https://Informme.Org.Au/En/Guidelines/Clinical-Guidelines-for-Stroke-Management (Accessed 2023, March 7).

10. Tony R. National Clinical Guideline for Stroke (2016). Available online at: https://www.strokeguideline.org/.

11. Organisation ES. Available online at: https://eso-stroke.org/guidelines/eso-guideline-directory.

12. Kleindorfer DO, Towfighi A, Chaturvedi S, Cockroft KM, Gutierrez J, Lombardi-Hill D, et al. 2021 Guideline for the prevention of stroke in patients with stroke and transient ischemic attack: A guideline from the American Heart Association/American Stroke Association. *Stroke*. (2021) 52:e364–e467. doi: 10.1161/STR.0000000000000375

13. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (Prisma-P) 2015 statement. *Syst Rev*. (2015) 4:1. doi: 10.1186/2046-4053-4-1

14. Aromataris E, Munn Z eds. *JBI Manual for Evidence Synthesis*. Adelaide, Australia: JBI (2020). Available at: https://synthesismanual.jbi.global. doi: 10.46658/JBIMES-20-01

15. Miyaji Y, Kawabata Y, Joki H, Seki S, Mori K, Kamide T, et al. Primary aldosteronism in patients with acute stroke: prevalence and diagnosis during initial hospitalization. *BMC Neurol*. (2016) 16:177. doi: 10.1186/s12883-016-0701-5

16. Tang Z, Chen H, He W, Ma L, Song Y, Mei M, et al. Primary aldosteronism in young adults with acute stroke: prevalence and clinical characteristics. *J Neurol Sci*. (2020) 413:116774. doi: 10.1016/j.jns.2020.116774

17. Deeks JJ HJ, Altman DG. Chapter 10: Analysing data and undertaking meta-analyses. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editors. *Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (Updated February 2022)*. Chicheste, UK: Cochrane (2022).

18. Joint Committee for Guideline Revision. 2018 Chinese guidelines for prevention and treatment of hypertension-a report of the revision committee of Chinese guidelines for prevention and treatment of hypertension. *J Geriatr Cardiol*. (2019) 16:182–241. doi: 10.11909/j.issn.1671-5411.2019.03.014

19. Whelton PK, Carey RM, Aronow WS, Casey DE Jr., Collins KJ, Dennison Himmelfarb C, et al. 2017 Acc/Aha/Aapa/Abc/Acpm/Ags/Apha/Ash/Aspc/Nma/Pcna guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: executive summary: A report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Hypertension*. (2018) 71:1269–324. doi: 10.1161/HYP.0000000000000066

20. Adams HP Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. Toast. Trial of org 10172 in acute stroke treatment. *Stroke*. (1993) 24:35–41. doi: 10.1161/01.str.24.1.35

21. Boehme AK, Esenwa C, Elkind MSV. Stroke risk factors, genetics, and prevention. *Circ Res*. (2017) 120:472–95. doi: 10.1161/CIRCRESAHA.116.308398

22. Ma L, Song Y, Mei M, He W, Hu J, Cheng Q, et al. Age-related cutoffs of plasma aldosterone/renin concentration for primary aldosteronism screening. *Int J Endocrinol*. (2018) 2018:8647026. doi: 10.1155/2018/8647026

23. Migliavaca CB, Stein C, Colpani V, Barker TH, Ziegelmann PK, Munn Z, et al. Meta-analysis of prevalence: I2 statistic and how to deal with heterogeneity. *Res Synthesis Methods*. (2022) 13:363–7. doi: 10.1002/jrsm.1547

24. Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, et al. Prevalence and clinical manifestations of primary aldosteronism encountered in primary care practice. *J Am Coll Cardiol*. (2017) 69:1811–20. doi: 10.1016/j.jacc.2017.01.052

25. Back C, Thiesen KL, Skovgaard K, Edvinsson L, Jensen LT, Larsen VA, et al. Raas and stress markers in acute ischemic stroke: preliminary findings. *Acta Neurol Scand*. (2015) 131:132–9. doi: 10.1111/ane.12298

26. Perschel FH, Schemer R, Seiler L, Reincke M, Deinum J, Maser-Gluth C, et al. Rapid screening test for primary hyperaldosteronism: ratio of plasma aldosterone to renin concentration determined by fully automated chemiluminescence immunoassays. *Clin Chem*. (2004) 50:1650–5. doi: 10.1373/clinchem.2004.033159

27. Mulatero P, Rabbia F, Milan A, Paglieri C, Morello F, Chiandussi L, et al. Drug effects on aldosterone/plasma renin activity ratio in primary aldosteronism. *Hypertension*. (2002) 40:897–902. doi: 10.1161/01.hyp.0000038478.59760.41

28. Jedrusik P, Symonides B, Lewandowski J, Gaciong Z. The effect of antihypertensive medications on testing for primary aldosteronism. *Front Pharmacol*. (2021) 12:684111. doi: 10.3389/fphar.2021.684111

29. Naruse M, Murakami M, Katabami T, Kocjan T, Parasiliti-Caprino M, Quinkler M, et al. International multicenter survey on screening and confirmatory testing in primary aldosteronism. *Eur J Endocrinol*. (2023) 188:125–34. doi: 10.1093/ejendo/lvac002

30. Guo Z, Poglitsch M, Cowley D, Domenig O, McWhinney BC, Ungerer JPJ, et al. Effects of ramipril on the aldosterone/renin ratio and the aldosterone/angiotensin ii ratio in patients with primary aldosteronism. *Hypertension (Dallas Tex: 1979)*. (2020) 76:488–96. doi: 10.1161/HYPERTENSIONAHA.120.14871

31. Leung AA, Symonds CJ, Hundemer GL, Ronksley PE, Lorenzetti DL, Pasieka JL, et al. Performance of confirmatory tests for diagnosing primary aldosteronism: A systematic review and meta-analysis. *Hypertension*. (2022) 79:1835–44. doi: 10.1161/hypertensionaha.122.19377

32. Omura M, Nishikawa T. [Screening tests and diagnostic examinations of hypertensives for primary aldosteronism]. *Rinsho Byori*. (2006) 54:1157–63.

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 22 March 2024
DOI 10.3389/fendo.2024.1343704

OPEN ACCESS

EDITED BY
Elena Aisha Azizan,
National University of Malaysia, Malaysia

REVIEWED BY
Chiara Lopez,
University of Turin, Italy
Akira Sugawara,
Tohoku University, Japan

*CORRESPONDENCE
Yinong Jiang
yinongjiang@126.com
Wei Song
songwei8124@163.com

RECEIVED 24 November 2023
ACCEPTED 04 March 2024
PUBLISHED 22 March 2024

CITATION
Sun K, Gong M, Yu Y, Yang M, Zhang Y, Jiang Y and Song W (2024) Comparison of saline infusion test and captopril challenge test in the diagnosis of Chinese with primary aldosteronism in different age groups. *Front. Endocrinol.* 15:1343704. doi: 10.3389/fendo.2024.1343704

Comparison of saline infusion test and captopril challenge test in the diagnosis of Chinese with primary aldosteronism in different age groups

Kaiwen Sun, Minghui Gong, Yang Yu, Minghui Yang, Ying Zhang, Yinong Jiang* and Wei Song*

Department of Cardiology, First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China

Background: To explore the diagnostic accuracy and the optimal cutoff value between the saline infusion test (SIT) and captopril challenge test (CCT) [including the value and suppression of plasma aldosterone concentration (PAC)] for primary aldosteronism (PA) diagnosing.

Methods: A total of 318 patients with hypertension were consecutively enrolled, including 126 patients with PA and 192 patients with essential hypertension (EH), in this observational study. The characteristics of patients and laboratory examinations were collected and compared. The comparison between SIT and CCT was carried by drawing the receiver operator characteristic curve (ROC) and calculating the area under the curve (AUC) to explore the diagnostic accuracy and the optimal cutoff value.

Results: The average age was 51.59 ± 10.43 in the PA group and 45.72 ± 12.44 in the EH group ($p<0.05$). The optimal cutoff value was 10.7 ng/dL for post-CCT PAC, 6.8 ng/dL for post-SIT PAC, and 26.9% for suppression of post-CCT PAC. The diagnostic value of post-CCT PAC was the highest with 0.831 for the AUC and 0.552 for the Youden index. The optimal cutoff value for patients who were <50 years old was 11.5 ng/dL for post-CCT PAC and 8.4 ng/dL for post-SIT PAC. The suppression of post-CCT PAC turned to 18.2% for those of age 50 or older.

Conclusion: Compared with SIT, CCT had a higher diagnostic value when post-CCT PAC was used as the diagnostic criterion in Chinese people, while the selection of diagnostic thresholds depended on patient age.

KEYWORDS

primary aldosteronism, saline infusion test, captopril challenge test, age, Chinese

Introduction

Primary aldosteronism (PA), a common cause of secondary hypertension, occurs in 5%–10% of patients with hypertension, with higher ratios in those with resistant hypertension (1–3). Its pathogenesis is related to the increased secretion of autonomic aldosterone in one or both adrenal cortical globular zones, which causes water and sodium retention, leading to increased circulatory loading and blood pressure (4, 5). Recent research has shown that a higher incidence of cardiovascular events and more severe target organ damage are observed in PA compared with essential hypertension (EH) (3, 6–9). Therefore, early diagnosis and treatment of PA are of great significance.

According to the 2016 Endocrine Society guidelines, patients considered to have a possible PA diagnosis based on preliminary screening need to undergo confirmatory testing (10). There are four confirmatory tests for PA with diverse strengths and limitations: the fludrocortisone suppression test, the oral saline load test, the saline infusion test (SIT), and captopril challenge test (CCT). SIT and CCT are currently in wide clinical use due to their convenience and affordability (10, 11). However, some debates still remain among previous studies (12). There has not been a clear result comparing the accuracy of these confirmatory tests in diagnosing PA. There were several cutoff values in different guidelines (13–15). The interpretation of the results can be affected by factors such as the discrepant daily sodium intake in various countries (16). In addition, cutoff values are not fixed among various ethnic groups and ages (17–19). Leung et al. (20) pointed out that there were significant differences in the interpretation and verification of the results of the confirmatory tests, and there had been almost no effective reference standard to test at present, which made it difficult to distinguish.

The aim of our study is to compare the diagnostic efficiency between SIT and CCT and calculate the optimal cutoff value in different age groups among Chinese people to improve the diagnostic accuracy for PA.

Abbreviations: PA, primary aldosteronism; EH, essential hypertension; SIT, saline infusion test; CCT, captopril challenge test; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, dihydropyridine calcium channel blocker; OSA, obstructive sleep apnea; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LVEF, left ventricular ejection fraction; BMI, body mass index; eGFR, estimated glomerular filtration rate; Cr, creatinine; UA, uric acid; HbA1c, glycosylated hemoglobin; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BNP, type B natriuretic peptide; DRC, Direct renin concentration; PAC, plasma aldosterone concentration; ARR, Ratio of plasma aldosterone to renin concentration; ROC, receiver operator characteristic curve; AUC, calculating area under the curve; RAAS, renin–angiotensin–aldosterone system; PASO, Primary Aldosteronism Surgical Outcome.

Materials and methods

Study population

There were 2,546 patients diagnosed with hypertension who were admitted to the First Affiliated Hospital of Dalian Medical University in January 2019 and June 2021 and 346 patients aged 18–80 suspected with PA in the First Affiliated Hospital of Dalian Medical University were consecutive enrolled and performed SIT and CCT after drug eluting. Angiotensin-converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB), dihydropyridine calcium channel blocker (CCB), and β receptor blockers were stopped for at least 2 weeks, diuretics for at least 4 weeks, and aldosterone receptor antagonists for at least 6 weeks to eliminate the interference of drugs to the results. The antihypertensive drugs were chosen as α receptor blockers or non-dihydropyridine calcium channel blockers for blood pressure controlling. Sodium intake was not restricted. A total of 318 eligible patients were enrolled according to their symptoms, signs, and specific results of examination referring to the latest guideline (10).

Exclusion criteria were as follows: (1) other types of secondary hypertension [such as renal parenchymal hypertension, severe renal artery stenosis, Cushing's syndrome, pheochromocytoma, and severe obstructive sleep apnea (OSA)], white coat hypertension, pseudo hypertension, etc.; (2) severe liver or renal damage such as aspartate aminotransferase (AST) or alanine aminotransferase (ALT) more than three times of the upper limit of normal and estimated glomerular filtration rate (eGFR) <60 ml/min * 1.73 m^2; (3) heart failure with left ventricular ejection fraction (LVEF) <50%; (4) history of malignancy; (5) women taking contraceptives recently or pregnancy; and (6) those with mental and intellectual disorders and patients who refused to join the study.

Clinical characteristic

The characteristics of patients were collected including age, gender, body mass index (BMI), duration of hypertension, history of smoking, and drinking.

Biochemistry measurement

The laboratory examinations were collected from fasting serum for at least 8 h and urine for the first in the morning and throughout the day of patients. The fasting serum index included ALT, AST, creatinine (Cr), uric acid (UA), serum sodium and potassium, glycosylated hemoglobin (HbA1c), fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and type B natriuretic peptide (BNP). The urine index included 24 h urine sodium and potassium throughout the day. eGFR was calculated by CKD-EPI formula (21, 22). All laboratory examinations mentioned before were measured by automated biochemical instrument.

Screening and confirmatory test

Direct renin concentration (DRC) and plasma aldosterone concentration (PAC) in vertical and horizontal positions were measured by chemiluminescence (DiaSorin S.P.A, Saluggia, Italy). The diagnosis of PA relied on a combination with symptoms, signs, a positive ARR (≥3.7 ng/dL per mU/L), and one or more positive confirmatory tests following the guideline (10).

1. Screening test: patients were required to maintain a non-vertical (sitting or standing) position for at least 2 h after getting up in the morning. The horizontal DRC and PAC was collected after sitting for 5–15 min. The ratio of plasma aldosterone to renin concentration (ARR) was calculated by PAC/DRC, and the test would be considered positive when ARR ≥ 3.7 ng/dL per mU/L (10).
2. SIT: SIT was carried out at 8:00 a.m. with the intravenous infusion of 2 L of 0.9% sodium chloride solution for a 4-h test. DRC, PAC, and ARR before and after infusion were measured. The test is considered positive when post-SIT PAC was more than 10 ng/dL, whereas it was negative when post-SIT PAC was <5 ng/dL (10).
3. CCT: patients remained in a sitting position, and 50 mg captopril was administered orally. DRC, PAC, and ARR were measured before CCT and 1 and 2 h after taking the captopril. The standard included the PAC measurement value and PAC suppression at 2 h post-administration. The test was considered positive when post-CCT PAC was >11 ng/dl or the suppression of post-CCT PAC was <30% (10).

Statistical analysis

SPSS 24.0 software was used for statistical analysis. When comparing the differences between two groups, the data that conformed to the normal distribution and met the homogeneity of variance were analyzed by t-test; the data that conformed to the normal distribution but did not meet the homogeneity of variance were analyzed by the corrected t-test. The results above were described by mean ± standard deviation. The data that did not conform to the normal distribution were analyzed by Mann–Whitney U-test and were described by median and quartile. The counting data were analyzed by χ^2 test and were described by percentage. The comparison between SIT and CCT was carried by drawing the receiver operator characteristic curve (ROC) and calculating the area under the curve (AUC) to find the optimal cutoff value. Any p <0.05 was considered statistically significant.

Results

Clinical characteristic of patients

A total of 318 patients were consecutively enrolled, including 126 patients with PA and 192 patients with EH (Figure 1). The average age was 51.45 ± 10.48 years in the PA group and 45.84 ± 12.37 years in the EH group ($p < 0.05$). The patients in the PA group presented with longer hypertension duration and lower BMI than the EH group ($p < 0.05$). There was no difference between the two groups with respect to the proportion of smokers, alcohol history, or sex distribution ($p > 0.05$). Meanwhile, the concentration of serum potassium without supplementation before ARR screening was lower, and the concentration of serum sodium and 24-h urine potassium was higher in the PA group than in the EH group ($p < 0.05$). The PA group also had lower DRC and higher PAC compared with the EH group ($p < 0.05$). BNP and HDL-C was higher, and ALT, AST, TG and UA was lower in the PA group, although these laboratory test results were within normal ranges ($p < 0.05$) (Table 1).

Diagnostic efficacy of SIT and CCT by guideline

According to the diagnostic criteria in the guideline (10), 34 of 126 patients (26.7%) in the PA group had positive SIT results, and eight patients (6.3%) had negative results. The rest (66.7%) had

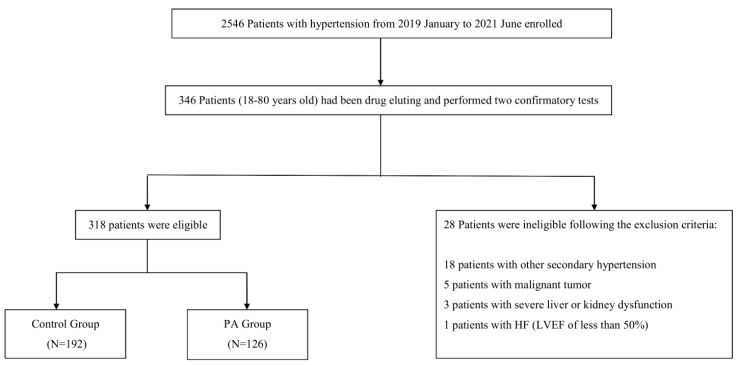


FIGURE 1
Flow chart of the study.

TABLE 1 Clinical characteristic of patients.

	PA group (n=126)	EH group (n=192)	*p*
Age (years)	51.45 ± 10.48	45.84 ± 12.37	<0.001*
Male (N,%)	55(43.7%)	95(49.5%)	0.309
BMI (kg/m^2)	26.28 ± 3.67	27.46 ± 4.06	0.009*
Duration of hypertension (years)	7.00(1.00, 10.25)	2.50(0.33, 7.00)	<0.001*
History of smoking (N, %)	25(19.8%)	43(22.4%)	0.587
History of drinking (N, %)	23(18.3%)	47(24.5%)	0.190
Laboratory examinations			
ALT (U/L)	17.00 (13.00, 25.25)	24.00 (15.25, 33.00)	<0.001*
AST (U/L)	16.00 (14.00, 20.25)	18.00 (16.00, 23.00)	0.001*
Cr (mmol/L)	63.56 ± 16.04	66.21 ± 16.45	0.157
eGFR (mL/min/1.73m^2)	102.07 ± 13.70	104.60 ± 14.26	0.117
BNP (ng/L)	34.08(18.34,56.94)	20.65 (10.97, 34.08)	<0.001*
Na^+ (mmol/L)	142.46 ± 2.58	141.33 ± 2.01	<0.001*
K^+ (mmol/L)	3.51 ± 0.46	3.71 ± 0.33	<0.001*
FPG (mmol/L)	4.73(4.42, 5.19)	4.71(4.33, 5.25)	0.784
HbA1c (%)	5.60(5.40, 5.80)	5.60(5.40, 5.90)	0.415
TC (mmol/L)	4.61 ± 0.86	4.76 ± 0.99	0.171
TG (mmol/L)	1.25(0.94,1.71)	1.50(1.02,2.19)	0.002*
HDL-C (mmol/L)	1.16 ± 0.28	1.08 ± 0.26	0.013*
LDL-C (mmol/L)	2.51 ± 0.63	2.66 ± 0.72	0.050
UA (μmol/L)	327.56 ± 82.31	368.80 ± 92.83	<0.001*
Horizontal DRC (mU/L)	1.21(0.50, 3.24)	4.32(1.83, 12.69)	<0.001*
Horizontal PAC (ng/dL)	17.0 ± 9.2	11.9 ± 5.4	<0.001*
Vertical DRC (mU/L)	4.03(1.69, 7.79)	12.50(4.92, 29.24)	<0.001*
Vertical PAC (ng/dL)	24.8 ± 12.9	19.5 ± 9.2	<0.001*
24 h urine sodium (mmol/24 h)	143.37 ± 68.65	141.29 ± 83.31	0.816
24 h urine potassium (mmol/24 h)	54.77 ± 23.58	42.05 ± 18.40	<0.001*

PA, primary aldosteronism; BMI=body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; eGFR, estimated glomerular filtration rate; BNP, type B natriuretic peptide; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin A1c; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; DRC, direct renin concentration; PAC, plasma aldosterone concentration.
*p <0.05.

indeterminate results (5–10 ng/dL). In the EH group, 44 of 192 patients (22.9%) were SIT negative and three (1.6%) were SIT positive. The remaining 75.5% were indeterminate. For CCT, the post-CCT PAC was positive in 102 of 126 patients (81.0%) in the PA group, whereas 137 of 192 patients (71.4%) in the EH group had negative post-CCT PAC. When using the suppression of post-CCT PAC as the criterion, 109 of 126 PA group patients (86.5%) were CCT positive, and 97 of 192 EH group patients (50.5%) were CCT negative (Table 2).

Diagnostic value of SIT and CCT

The optimal cutoff values were 10.7 ng/dL for post-CCT PAC, 6.8 ng/dL for post-SIT PAC, and 26.9% for post-CCT PAC suppression. The diagnostic value of post-CCT PAC was the highest, with an AUC of 0.831 [95% CI: (0.787, 0.875)] and Youden index of 0.552. For SIT, the AUC was 0.762 [95% CI: (0.708, 0.816)] and the Youden index was 0.425. There was a lowest diagnostic value of the suppression of post-CCT PAC, with an AUC of 0.684 [95% CI: (0.625, 0.743)] and Youden index of 0.385 (Table 3) (Figure 2).

Diagnostic value between SIT and CCT in different age groups

The patients were further divided into two groups according to age. The post-CCT PAC showed the greatest diagnostic value, with a higher AUC and Youden index regardless of age group (Figures 3A–C). As for the optimal cutoff values of each confirmatory test, it was increased to 11.5 ng/dL for post-CCT PAC and 8.4 ng/dL for post-SIT PAC among those who were <50 years old. The suppression of post-CCT PAC was 26.8%, which was similar to that mentioned above. For patients who were age 50 or older, the post-CCT PAC suppression was 18.2%, while the values of post-CCT PAC and post-SIT PAC did not change.

Discussion

It is necessary to identify, diagnose, and treat with PA in a timely manner to control blood pressure and reduce the risk of related complications. The consensus and guidelines for the diagnosis and treatment of PA have been continually updated for decades. Although the diagnostic process came to be gradually simplified, there was a wide overlap in ARR values between normokalemic patients with PA and those with EH, which need a caution if skipping the confirmatory test according to the research (23). The confirmatory test is also the key evidence for a clear diagnosis of PA and reduces unnecessary risks, such as invasive diagnosis and/or surgical treatment for patients when it is accurate

TABLE 2 The specific number of patients in each confirmatory test.

Confirmatory tests	PA(n=126)	EH(n=192)	Sensitivity/ specificity	Youden Index
Post-CCT PAC (+)	102	55	81.0%/71.4%	0.524
Post-CCT PAC (–)	24	137		
Post-SIT PAC (+)	34	3	27.0%/22.9%	–
Post-SIT PAC (–)	8	44		
Post-SIT PAC in gray gap	84	145		
Suppression of post-CCT PAC(+)	109	95	86.5%/50.5%	0.370
Suppression of post-CCT PAC(–)	17	97		

PA, primary aldosteronism; EH, essential hypertension; CCT, captopril challenge test; PAC, plasma aldosterone concentration; SIT, saline infusion test.

TABLE 3 Diagnostic value of each confirmatory test.

Confirmatory Tests	Cutoff value	AUC (95%CI)	Sensitivity	Specificity	Youden Index
Post-CCT PAC (ng/dL)	10.7	0.831 (0.787,0.875)	84.9%	70.3%	0.552
Post-SIT PAC (ng/dL)	6.8	0.762 (0.708,0.816)	72.2%	70.3%	0.425
Suppression of post-CCT PAC	26.9%	0.684 (0.625,0.743)	53.6%	84.9%	0.385

AUC, area under the curve; CCT, captopril challenge test; PAC, plasma aldosterone concentration; SIT, saline infusion test.

and reliable (10, 11). The present relevant guideline recommends four confirmatory tests (10). However, the sensitivity and specificity of each confirmatory test vary. It lacks sufficient evidence to recommend any single confirmatory test as the best one. Nowadays, confirmatory test selection is generally based on cost, patient compliance, local hospital conditions, etc. Considering multiple study factors, SIT and CCT are widely used and compared for PA diagnosis to select the appropriate method and identify their diagnostic values in Chinese people of different ages.

SIT and CCT had different mechanisms of action. SIT inhibited renin and aldosterone secretion via volume overload, while CCT suppressed aldosterone secretion by decreasing the angiotensinase activity and increasing the renin level. Thus, the diagnostic criteria of the two tests were inconsistent (24, 25). SIT had a high prevalence of gray zone in the PA and EH groups according to the criteria in the guideline, which were unable to clarify PA temporarily and required further confirmatory tests (10). Thus, the necessity for a definite diagnostic threshold in SIT was recognized, which was found to be controversial in previous studies (18, 26–28). In this study, the optimal cutoff value was 6.8 ng/dL for the post-SIT PAC. The post-SIT PAC was collected in various (sitting or horizontal) positions in different studies, and various detection methods, such as immunoassays or liquid chromatography coupled with tandem mass spectrometry, were used, which might explain the varied results (29, 30). The latest research recommended doubling the upper limit of salt intake in Chinese people (16, 31). A high salt intake could decrease aldosterone secretion through negative feedback regulation, leading to lower reactivity for SIT and aldosterone detection (32). The cutoff value in this study was below the one that the guidelines proposed, which might be attributed to higher salt intake according to the 24-h urine sodium test. For CCT, the oral dose of captopril, the difference in position, individual drug metabolisms when collecting the serum, and even the reference diagnostic criteria were inconsistent, leading to non-unified results (33, 34). In this study, the cutoff value was 10.7 ng/dL for post-CCT PAC and 26.9% for the suppression of post-CCT PAC, which was close to the guideline recommendation (10).

The comparison between the tests showed that all of them were feasible. The diagnostic efficacy of post-CCT PAC was relatively reliable, with a larger AUC and a higher Youden index, which was

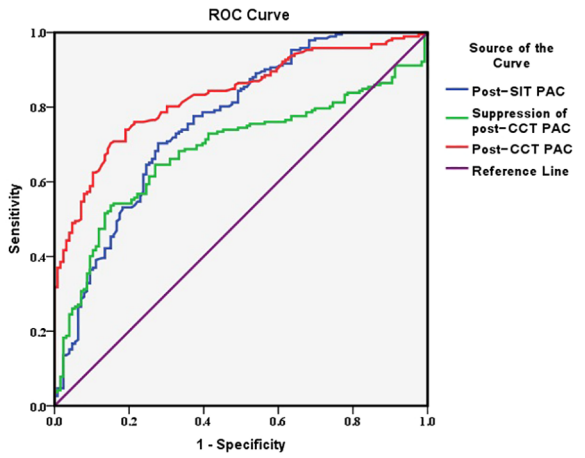


FIGURE 2
The ROC curve of each confirmatory test for PA among all ages.

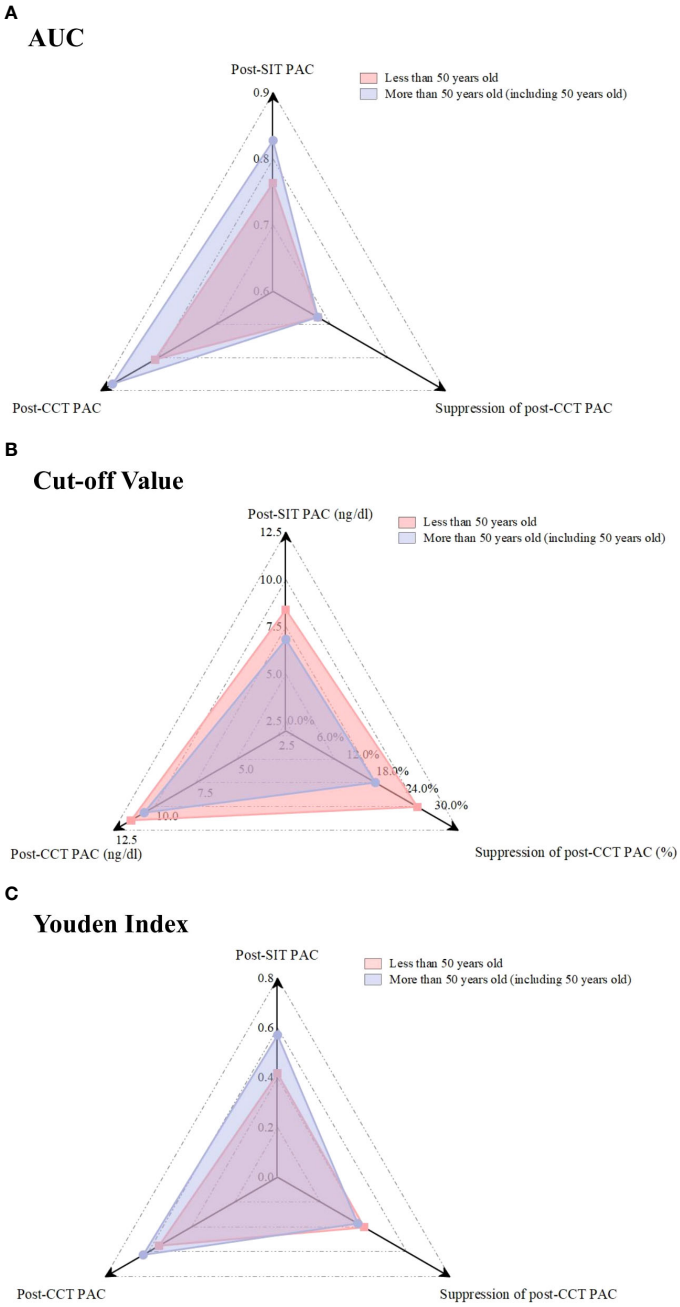


FIGURE 3
Comparison of the AUC, Cut-off value, and Youden Index for each confirmatory test in different age.

consistent with previous studies (18, 27, 29). The CCT was relatively simple and quick to operate. Additionally, it did not require infusion facilities and was more suitable for older patients with relatively high blood pressure (5, 35). Therefore, it might be more practicable when diagnosing with PA.

There was lack of a sufficient understanding of PA among senior citizens. One of the reasons was that there was a high incidence of cardiovascular disease with multiple drug treatment that could not withdraw these drugs among them, which influenced the screening test results and made performing the confirmatory test challenging (36). However, the resistant hypertension proportion and cardiovascular disease complications increased with age (37). Renin concentrations also decreased with age. Therefore, it is important to screen for PA among older populations. This study included a relatively wide range of patient ages, which might have impacted the results. Kuo et al. (38) showed that the average age at PA diagnosis was approximately 50 by reviewing previous studies. Therefore, the group was divided at age 50 to explore diagnostic accuracy and the optimal cutoff value. The optimal post-SIT PAC and post-CCT PAC cutoff values for patients who were less than 50 years old increased. The renin–angiotensin–aldosterone system (RAAS) activity decreased among older people,

leading to the hyposecretion of aldosterone (39). Therefore, the cutoff value was lower than that in younger ones. The diagnostic value was highest, had the largest AUC, and had a high Youden index when using the post-CCT PAC in each group, further proving the superiority of CCT.

This study had some limitations. First, this was an observational study, and some biases might exist. Second, the sample size was relatively small, and the patients were from a single center. Larger patient samples were required. Third, the study lacked details on patient management and follow-up. We collected the data on follow-up in PA group of which after the subtype differentiation and underwent medication treatment or surgery as shown in the Supplemental Table S1. The data of patients who underwent surgery and followed up were shown in the Supplementary Tables S2 and S3. We note that 11 patients reached biochemical complete success, and three patients reached clinical complete success in accordance with the Primary Aldosteronism Surgical Outcome (PASO) criteria (40). However, the rate of follow-up was relatively low. The incidence of PA in this study was nearly 5%, according to our statistics. In fact, there were about one-fifth of patients with elevated ARR but negative confirmatory test developed overt PA over time (41). We found that nearly one-third patients with a positive ARR but negative confirmatory tests in EH group. PA is a biochemical continuous process, and these patients may be in the early stages of the disease or in a subclinical state, requiring closer follow-up in the future to get a correct diagnosis at an earlier stage of the disease. In addition, the research showed that aldosterone levels were independently related to the degree of OSA and that PA and OSA interacted (42). To avoid the impact of severe OSA on the results, these patients with PA were excluded. Thus, the substantial proportion was even higher. Finally, some biochemical indices, such as HDL-C and UA, differed between the two groups, which previous studies also observed, and the mechanism requires further exploration (43, 44).

Conclusion

Compared with SIT, CCT had a higher diagnostic value when post-CCT PAC was used as the diagnostic criterion in Chinese people, while the selection of diagnostic thresholds depended on patient age.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

KS: Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – review & editing, Writing – original draft. MG: Writing – review & editing, Software, Methodology, Formal analysis, Data curation. YY: Writing – review & editing, Visualization, Software, Methodology. MY: Writing – review & editing, Project administration. YZ: Writing – review & editing, Resources. YJ: Writing – review & editing, Validation. WS: Writing – review & editing, Supervision.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

This research was supported by the First Affiliated Hospital of Dalian Medical University. The assistance of the staff is gratefully acknowledged.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1343704/full#supplementary-material

References

1. Xu Z, Yang J, Hu J, Song Y, He W, Luo T, et al. Primary aldosteronism in patients in China with recently detected hypertension. *J Am Coll Cardiol*. (2020) 75:1913–22. doi: 10.1016/j.jacc.2020.02.052

2. Mulatero P, Bertello C, Veglio F, Monticone S. Approach to the patient on antihypertensive therapy: screen for primary aldosteronism. *J Clin Endocrinol Metab*. (2022) 107:3175–81. doi: 10.1210/clinem/dgac460

3. Parasiliti-Caprino M, Lopez C, Prencipe N, Lucatello B, Settanni F, Giraudo G, et al. Prevalence of primary aldosteronism and association with cardiovascular complications in patients with resistant and refractory hypertension. *J Hypertens*. (2020) 38:1841–8. doi: 10.1097/HJH.0000000000002441

4. Vaidya A, Hundemer GL, Nanba K, Parksook WW, Brown JM. Primary aldosteronism: state-of-the-art review. *Am J Hypertens*. (2022) 35:967–88. doi: 10.1093/ajh/hpac079

5. Turcu AF, Yang J, Vaidya A. Primary aldosteronism - a multidimensional syndrome. *Nat Rev Endocrinol*. (2022) 18:665–82. doi: 10.1038/s41574-022-00730-2

6. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol*. (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4

7. Nguyen V, Tu TM, Mamauag MJB, Lai J, Saffari SE, Aw TC, et al. Primary aldosteronism more prevalent in patients with cardioembolic stroke and atrial fibrillation. *Front Endocrinol (Lausanne)*. (2022) 13:869980. doi: 10.3389/fendo.2022.869980

8. Yang Y, Zhu LM, Xu JZ, Tang XF, Gao PJ. Comparison of left ventricular structure and function in primary aldosteronism and essential hypertension by echocardiography. *Hypertens Res*. (2017) 40:243–50. doi: 10.1038/hr.2016.127

9. Ohno Y, Sone M, Inagaki N, Kawashima A, Takeda Y, Yoneda T, et al. Nadir aldosterone levels after confirmatory tests are correlated with left ventricular hypertrophy in primary aldosteronism. *Hypertension*. (2020) 75:1475–82. doi: 10.1161/HYPERTENSIONAHA.119.14601

10. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: Case detection, diagnosis, and treatment: An Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. (2016) 101:1889–916. doi: 10.1210/jc.2015-4061

11. Tezuka Y, Yamazaki Y, Nakamura Y, Sasano H, Satoh F. Recent development toward the next clinical practice of primary aldosteronism: A literature review. *Biomedicines*. (2021) 9:310. doi: 10.3390/biomedicines9030310

12. Funder JW, Carey RM. Primary aldosteronism: where are we now? *Where From Here? Hypertension*. (2022) 79:726–35. doi: 10.1161/HYPERTENSIONAHA.121.18761

13. Rossi GP, Bisogni V, Bacca AV, Belfiore A, Cesari M, Concistrè A, et al. The 2020 Italian Society of Arterial Hypertension (SIIA) practical guidelines for the management of primary aldosteronism. *Int J Cardiol Hypertens*. (2020) 5:100029. doi: 10.1016/j.ijchy.2020.100029

14. Naruse M, Katabami T, Shibata H, Sone M, Takahashi K, Tanabe A, et al. Japan Endocrine Society clinical practice guideline for the diagnosis and management of primary aldosteronism 2021. *Endocr J*. (2022) 69:327–59. doi: 10.1507/endocrj.EJ21-0508

15. Chinese Society of Endocrinology. Expert consensus on the diagnosis and treatment of primary aldosteronism (2020). *Chin J Endocrinol Metab*. (2020) 36:727–36. doi: 10.3760/cma.j.cn311282-20200615-00444

16. Powles J, Fahimi S, Micha R, Khatibzadeh S, Shi P, Ezzati M, et al. Global, regional and national sodium intakes in 1990 and 2010: a systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. *BMJ Open*. (2013) 3:e003733. doi: 10.1136/bmjopen-2013-003733

17. Kidoguchi S, Sugano N, Hayashi-Ishikawa N, Morisawa N, Tokudome G, Yokoo T. The characteristics of captopril challenge test-positive patients using various criteria. *J Renin Angiotensin Aldosterone Syst*. (2019) 20:1470320319870891. doi: 10.1177/1470320319870891

18. Meng X, Li Y, Wang X, Li J, Liu Y, Yu Y. Evaluation of the saline infusion test and the captopril challenge test in chinese patients with primary aldosteronism. *J Clin Endocrinol Metab*. (2018) 103:853–60. doi: 10.1210/jc.2017-01530

19. Lin C, Yang J, Fuller PJ, Jing H, Song Y, He W, et al. A combination of captopril challenge test after saline infusion test improves diagnostic accuracy for primary aldosteronism. *Clin Endocrinol (Oxf)*. (2020) 92:131–7. doi: 10.1111/cen.14134

20. Leung AA, Symonds CJ, Hundemer GL, Ronksley PE, Lorenzetti DL, Pasieka JL, et al. Performance of confirmatory tests for diagnosing primary aldosteronism: a systematic review and meta-analysis. *Hypertension*. (2022) 79:1835–44. doi: 10.1161/HYPERTENSIONAHA.122.19377

21. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006

22. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. (2012) 367:20–9. doi: 10.1056/NEJMoa1114248

23. Bioletto F, Lopez C, Bollati M, Arata S, Procopio M, Ponzetto F, et al. Predictive performance of aldosterone-to-renin ratio in the diagnosis of primary aldosteronism in patients with resistant hypertension. *Front Endocrinol (Lausanne)*. (2023) 14:1145186. doi: 10.3389/fendo.2023.1145186

24. Fukumoto T, Umakoshi H, Ogata M, Yokomoto-Umakoshi M, Matsuda Y, Motoya M, et al. Significance of discordant results between confirmatory tests in diagnosis of primary aldosteronism. *J Clin Endocrinol Metab*. (2021) 106:e866–74. doi: 10.1210/clinem/dgaa812

25. Wannachalee T, Lieberman L, Turcu AF. High prevalence of autonomous aldosterone production in hypertension: how to identify and treat it. *Curr Hypertens Rep*. (2022) 24:123–32. doi: 10.1007/s11906-022-01176-7

26. Li Y, Liu Y, Li J, Wang X, Yu Y. Sodium infusion test for diagnosis of primary aldosteronism in chinese population. *J Clin Endocrinol Metab*. (2016) 101:89–95. doi: 10.1210/jc.2015-2840

27. Song Y, Yang S, He W, Hu J, Cheng Q, Wang Y, et al. Confirmatory tests for the diagnosis of primary aldosteronism: A prospective diagnostic accuracy study. *Hypertension*. (2018) 71:118–24. doi: 10.1161/HYPERTENSIONAHA.117.10197

28. Fuss CT, Brohm K, Kurlbaum M, Hannemann A, Kendl S, Fassnacht M, et al. Confirmatory testing of primary aldosteronism with saline infusion test and LC-MS/MS. *Eur J Endocrinol*. (2021) 184:167–78. doi: 10.1530/EJE-20-0073

29. Fries CM, Bae YJ, Rayes N, Sandner B, Isermann B, Stumvoll M, et al. Prospective evaluation of aldosterone LC-MS/MS-specific cutoffs for the saline infusion test. *Eur J Endocrinol*. (2020) 183:191–201. doi: 10.1530/EJE-20-0030

30. Thuzar M, Young K, Ahmed AH, Ward G, Wolley M, Guo Z, et al. Diagnosis of primary aldosteronism by seated saline suppression test-variability between immunoassay and HPLC-MS/MS. *J Clin Endocrinol Metab*. (2020) 105:dgz150. doi: 10.1210/clinem/dgz150

31. Jiang K, He T, Ji Y, Zhu T, Jiang E. The perspective of hypertension and salt intake in Chinese population. *Front Public Health*. (2023) 11:1125608. doi: 10.3389/fpubh.2023.1125608

32. Gonsalez SR, Ferrão FM, Souza AM, Lowe J, Morcillo LDSL. Inappropriate activity of local renin-angiotensin-aldosterone system during high salt intake: impact on the cardio-renal axis. *J Bras Nefrol*. (2018) 40:170–8. doi: 10.1590/2175-8239-jbn-3661

33. Zhu KY, Zhang Y, Zhang WJ, Li HY, Feng WH, Zhu DL, et al. The captopril challenge test for diagnosing primary Aldosteronism in a Chinese population. *BMC Endocr Disord*. (2019) 19:65. doi: 10.1186/s12902-019-0390-3

34. Xiang Q, Chen T, Yu K, Li Y, Li Q, Tian H, et al. The value of different single or combined indexes of the captopril challenge test in the diagnosis of primary aldosteronism. *Front Endocrinol (Lausanne)*. (2021) 12:689618. doi: 10.3389/fendo.2021.689618

35. Wang Y, Cai J. Reliability analysis of a functional diagnostic test for primary hyperaldosteronism based on data analysis. *Comput Intell Neurosci*. (2022) 2022:6868941. doi: 10.1155/2022/6868941

36. Cesari M, Seccia TM, Maiolino G, Rossi GP. Primary aldosteronism in elderly, old, and very old patients. *J Hum Hypertens*. (2020) 34:807–13. doi: 10.1038/s41371-020-00395-2

37. Luo Q, Li NF, Yao XG, Zhang DL, Abulikemu SF, Chang GJ, et al. Potential effects of age on screening for primary aldosteronism. *J Hum Hypertens*. (2016) 30:53–61. doi: 10.1038/jhh.2015.21

38. Kuo CC, Balakrishnan P, Hsein YC, Wu VC, Chueh SC, Chen YM, et al. The value of losartan suppression test in the confirmatory diagnosis of primary aldosteronism in patients over 50 years old. *J Renin Angiotensin Aldosterone Syst*. (2015) 16:587–98. doi: 10.1177/1470320313498632

39. Nakama C, Kamide K, Kawai T, Hongyo K, Ito N, Onishi M, et al. The influence of aging on the diagnosis of primary aldosteronism. *Hypertens Res*. (2014) 37:1062–7. doi: 10.1038/hr.2014.129

40. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, et al. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol*. (2017) 5:689–99. doi: 10.1016/S2213-8587(17)30135-3

41. Buffolo F, Pecori A, Reincke M, Outland M, Veglio F, Schwarzlmüller P, et al. Long-term follow-up of patients with elevated aldosterone-to-renin ratio but negative confirmatory test: the progression of primary aldosteronism phenotypes. *Hypertension*. (2024) 81:340–7. doi: 10.1161/HYPERTENSIONAHA.123.21983

42. Wang Y, Li CX, Lin YN, Zhang LY, Li SQ, Zhang L, et al. The role of aldosterone in OSA and OSA-related hypertension. *Front Endocrinol (Lausanne)*. (2022) 12:801689. doi: 10.3389/fendo.2021.801689

43. Manosroi W, Phudphong P, Atthakomol P, Phimphilai M. The differences of serum lipid profiles between primary aldosteronism and essential hypertension: a meta-analysis and systematic review. *BMC Endocr Disord*. (2022) 22:217. doi: 10.1186/s12902-022-01135-y

44. Moon SJ, Jang HN, Kim JH, Moon MK. Lipid profiles in primary aldosteronism compared with essential hypertension: propensity-score matching study. *Endocrinol Metab (Seoul)*. (2021) 36:885–94. doi: 10.3803/EnM.2021.1012

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 26 March 2024
DOI 10.3389/fendo.2024.1370525

OPEN ACCESS

EDITED BY
Carmela Maniero,
Barts Health NHS Trust, United Kingdom

REVIEWED BY
Madson Almeida,
University of São Paulo, Brazil
Fabio Bioletto,
University of Turin, Italy

*CORRESPONDENCE
Zoran Erlic
zoran.erlic@usz.ch

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share last authorship

RECEIVED 14 January 2024
ACCEPTED 05 March 2024
PUBLISHED 26 March 2024

CITATION
Knuchel R, Erlic Z, Gruber S, Amar L, Larsen CK, Gimenez-Roqueplo A-P, Mulatero P, Tetti M, Pecori A, Pamporaki C, Langton K, Peitzsch M, Ceccato F, Prejbisz A, Januszewicz A, Adolf C, Remde H, Lenzini L, Dennedy M, Deinum J, Jefferson E, Blanchard A, Zennaro M-C, Eisenhofer G and Beuschlein F (2024) Association of adrenal steroids with metabolomic profiles in patients with primary and endocrine hypertension. *Front. Endocrinol.* 15:1370525. doi: 10.3389/fendo.2024.1370525

Association of adrenal steroids with metabolomic profiles in patients with primary and endocrine hypertension

Robin Knuchel[1†], Zoran Erlic[1*†], Sven Gruber[1], Laurence Amar[2,3,4], Casper K. Larsen[2], Anne-Paule Gimenez-Roqueplo[2,3], Paolo Mulatero[5], Martina Tetti[5], Alessio Pecori[5], Christina Pamporaki[6], Katharina Langton[6], Mirko Peitzsch[7], Filippo Ceccato[8], Aleksander Prejbisz[9], Andrzej Januszewicz[9], Christian Adolf[10], Hanna Remde[11], Livia Lenzini[12], Michael Dennedy[13], Jaap Deinum[14], Emily Jefferson[15], Anne Blanchard[16], Maria-Christina Zennaro[2,17], Graeme Eisenhofer[6‡] and Felix Beuschlein[1,10,18‡]

[1]Klinik für Endokrinologie, Diabetologie und Klinische Ernährung, UniversitätsSpital Zürich (USZ) und Universität Zürich (UZH), Zurich, Switzerland, [2]Université Paris Cité, Paris Cardiovascular Research Center (PARCC), L'Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France, [3]Département de Médecine Génomique des Tumeurs et des Cancers, Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France, [4]Centre de référence en maladies rares de la surrénale, Hôpital Européen Georges Pompidou, Paris, France, [5]Division of Internal Medicine and Hypertension Unit, Department of Medical Sciences, University of Torino, Torino, Italy, [6]Medical Clinic III, University Hospital Carl Gustav Carus, Technische Universität (TU) Dresden, Dresden, Germany, [7]Institute for Clinical Chemistry and Laboratory Medicine, University Hospital and Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, [8]Unita' Operativa Complessa (UOC) Endocrinologia, Dipartimento di Medicina DIMED, Azienda Ospedaliera-Università di Padova, Padua, Italy, [9]Department of Hypertension, National Institute of Cardiology, Warsaw, Poland, [10]Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Ludwig-Maximilians-Universität (LMU) München, Munich, Germany, [11]Department of Internal Medicine I, Division of Endocrinology and Diabetes, University Hospital, University of Würzburg, Würzburg, Germany, [12]Internal & Emergency Medicine Unit, Department of Medicine - DIMED, University of Padua, Padua, Italy, [13]The Discipline of Pharmacology and Therapeutics, School of Medicine, National University of Ireland, Galway, Ireland, [14]Department of Medicine, Section of Vascular Medicine, Radboud University Medical Center, Nijmegen, Netherlands, [15]Division of Population Health and Genomics, School of Medicine, University of Dundee, Dundee, United Kingdom, [16]Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Centre d'Investigations Cliniques, Paris, France, [17]Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Unité Hypertension artérielle, Paris, France, [18]The LOOP Zurich - Medical Research Center, Zurich, Switzerland

Introduction: Endocrine hypertension (EHT) due to pheochromocytoma/paraganglioma (PPGL), Cushing's syndrome (CS), or primary aldosteronism (PA) is linked to a variety of metabolic alterations and comorbidities. Accordingly, patients with EHT and primary hypertension (PHT) are characterized by distinct metabolic profiles. However, it remains unclear whether the metabolomic differences relate solely to the disease-defining hormonal parameters. Therefore, our objective was to study the association of disease defining hormonal excess and concomitant adrenal steroids with metabolomic alterations in patients with EHT.

Methods: Retrospective European multicenter study of 263 patients (mean age 49 years, 50% females; 58 PHT, 69 PPGL, 37 CS, 99 PA) in whom targeted metabolomic and adrenal steroid profiling was available. The association of 13 adrenal steroids with differences in 79 metabolites between PPGL, CS, PA and PHT was examined after correction for age, sex, BMI, and presence of diabetes mellitus.

Results: After adjustment for BMI and diabetes mellitus significant association between adrenal steroids and metabolites – 18 in PPGL, 15 in CS, and 23 in PA – were revealed. In PPGL, the majority of metabolite associations were linked to catecholamine excess, whereas in PA, only one metabolite was associated with aldosterone. In contrast, cortisone (16 metabolites), cortisol (6 metabolites), and DHEA (8 metabolites) had the highest number of associated metabolites in PA. In CS, 18-hydroxycortisol significantly influenced 5 metabolites, cortisol affected 4, and cortisone, 11-deoxycortisol, and DHEA each were linked to 3 metabolites.

Discussions: Our study indicates cortisol, cortisone, and catecholamine excess are significantly associated with metabolomic variances in EHT versus PHT patients. Notably, catecholamine excess is key to PPGL's metabolomic changes, whereas in PA, other non-defining adrenal steroids mainly account for metabolomic differences. In CS, cortisol, alongside other non-defining adrenal hormones, contributes to these differences, suggesting that metabolic disorders and cardiovascular morbidity in these conditions could also be affected by various adrenal steroids.

KEYWORDS

metabolomics, adrenal steroids, endocrine hypertension, primary hypertension, pheochromocytoma, primary aldosteronism, Cushing's syndrome

1 Introduction

Affecting more than 25% of the worldwide adult population, hypertension has become a global health challenge and is considered as one of the most important preventable risk factors for cardiovascular diseases (1, 2). Up to 90% of individuals with hypertension have no underlying disease and are therefore defined to have primary hypertension (PHT) (1, 2). However, among secondary and potentially curative causes, endocrine diseases such as pheochromocytoma and paraganglioma (PPGL), Cushing's syndrome (CS) and primary aldosteronism (PA) together are common but often missed or diagnosed late (3–5). In addition to causing arterial hypertension, these disorders have been associated with several metabolic alterations, possibly explaining the higher cardiovascular risk of affected patients in comparison to those with PHT (6–9). Specifically, CS is well known to cause a detrimental form of metabolic syndrome including impairment of glucose metabolism and dyslipidemia among many other clinical manifestations (9). Similarly, PA has a higher prevalence of metabolic syndrome compared to patients with PHT (10), as well as a higher prevalence of obesity and some lipid abnormalities (11). Similarly, hormonally active PPGL result in metabolic alterations including impaired glucose and lipid metabolism (12). Improvements in these metabolic alterations have been demonstrated in patients with PPGL and CS (9, 12), and to a lesser extent in PA (11, 13), following surgical intervention. This further highlights a causal relationship between the endocrine disorder and the described metabolic perturbations. However, not all pathogenic mechanisms underlying these alterations have yet been clarified (11, 12).

Targeted metabolomics (TM) offers a recent approach to assess metabolic alterations in patients with endocrine-related hypertension (EHT) (14–17). The approach enables measurements of dozens to hundreds of low-weight metabolites within one biological sample, thereby providing a means to delineate pathogenic mechanisms (18, 19) and enable prognostic and diagnostic characterization/stratification (14, 20, 21). From a study in patients with PPGL (15), it is known that several observed metabolite alterations have commonalities with those in patients with metabolic disorders (such as diabetes mellitus (DM) and obesity). These observations provide possible insights into the pathogenesis of metabolism that might contribute to the increased cardiovascular risk associated with PPGL.

In a recent study that compared patients with PHT and EHT (PPGL, CS, PA), we identified differences in biogenic amines/amino acids (AA), glycerophospholipids (GP), and acylcarnitines (AC), that offer promise for diagnostic discrimination of patients with PHT and EHT (14). It might seem obvious that the disease-defining hormonal excess - catecholamines for PPGL, cortisol for CS and aldosterone for PA, respectively - would be the main cause of the observed metabolomic alterations for each form of EHT. However, in other studies of patients with PPGL and PA, some unexpected hypersecretion of adrenal steroid have been described, which might also contribute to the metabolic disturbances in these endocrine disorders (22, 23). Therefore, the objective of this study was to further delineate whether the disease-defining hormonal excess impacts metabolic differences alongside the effects of other secreted adrenal steroids. The results could further deepen the knowledge of pathogenic mechanisms linking metabolic alterations with increased cardiovascular risk in patients with EHT.

2 Materials and methods

2.1 Subjects

Data for the present analysis were derived from a previously published cohort of 294 patients with arterial hypertension enrolled at eleven centers of the ENSAT-HT consortium (http://www.ensat-ht.eu) (14). Enrolled participants were diagnosed according to the current guidelines either with PPGL, CS, PA or with PHT. For patients with PHT, additional secondary causes of arterial hypertension, particularly renal disease, pharmacological causes, obstructive sleep apnea syndrome, and those with low-renin hypertension, were meticulously ruled out and excluded. Furthermore, patients with severe comorbidities (e.g. active malignancy, chronic kidney injury or heart diseases) or pregnancy were excluded. The current study involved a subgroup of 263 patients who underwent steroid profiling. In addition to retrieved clinical data, body mass index (BMI) and presence/absence of DM were gathered from patients' files. All participants provided written consent to participate in the study according to the protocol approved by the ethics committee of each center.

2.2 Laboratory testing

2.2.1 Targeted metabolomics

Plasma samples were analyzed using the LC-ESI-MS/MS and FIA-ESI-MS/MS measurements of 188 metabolites. Full details about the method, including assay performance characteristics, exclusion of metabolites and normalization of data are described previously (14). For the purpose of this study, we included the measured values of those metabolites, which were significantly differing between patients with endocrine hypertension (PPGL, CS, PA) and primary hypertension in the mentioned study (14). Accordingly, in total, 79 metabolites and 18 metabolic indices (MI, containing metabolite ratios and sums) were included in this study. Details of the included metabolites and MI for each subgroup analysis (see below) are provided in Supplementary Table 1.1.

2.2.2 Adrenal steroid profiling

Plasma steroid profiles included 15 different steroids (aldosterone, androstenedione, corticosterone, cortisol, cortisone, 11-deoxycorticosterone, 11-deoxycortisol, dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEAS), 17-hydroxyprogesterone, progesterone, testosterone, 21-deoxycortisol, 18-hydroxycortisol, 18-oxocortisol) that were analyzed using LC-MS/MS as described elsewhere (24).

2.2.3 Plasma metanephrines

Measurements of plasma normetanephrine, metanephrine, 3-methoxytyramine and 3-O-methyldopa were performed using an LC-MS/MS. Further details and information on the procedure have been described previously (25, 26).

2.2.4 Missing data estimation and data exclusion

We used a similar approach for exclusion and estimation for the adrenal steroids as we did for metabolite results previously (14, 15, 27, 28). In specific, we excluded those adrenal steroids with >10% of missing results (progesterone). The estimation of the values for those adrenal steroids with less than 10% of missing value was performed using the KNN approach (27, 28). In addition, for the purpose of this study we excluded the testosterone measurements, because of its strength relation to the sex. In total 13 out of 15 adrenal steroids were included in the further analyses.

2.3 Statistical analysis

Patients were categorized based on their diagnosis: PHT, PPGL, CS and PA. Baseline characteristics (age, sex) were compared across groups using the Pearson chi-squared test for sex and the Kruskal-Wallis test for age. Post-hoc analyses for comparisons between groups were performed using the Pearson chi-squared test for sex with a Bonferroni correction to account for multiple testing. The post-hoc analysis for age was conducted using Dunn-Bonferroni tests. For BMI comparisons, adjustments were made for age and sex using a one-way analysis of covariance (ANCOVA). When evaluating the presence of DM across groups, age and BMI were included as covariates. For these latter comparisons, BMI was logarithmically transformed due to non-normal distribution. A post-hoc Bonferroni correction for multiple testing was applied as well.

To assess differences in plasma steroid levels between the groups (PHT, PPGL, CS, and PA), a one-way ANCOVA was conducted with age and sex as covariates. Significance was determined using Bonferroni correction for multiple testing.

In a next step we focused on the 79 previously identified metabolomic differences and 18 different metabolic indices between the distinct clinical entities, namely PPGL, CS and PA, with PHT. In order to study the influence of the studied adrenal hormones, including also the leading (disease-identifying)

hormone, we built multiple linear regression models separately for each comparison (PPGL-PHT, CS-PHT and PA-PHT) on the respective patients. In specific, each identified metabolite from the different comparisons (Supplementary Table 1.1) was considered as a dependent variable, whilst the leading hormonal excess and other adrenal steroid hormones as independent variables. Since metanephrines are a good diagnostic tool for PPGL but do not reflect the biological activity of catecholamines, we did not include these values in the regression models. Therefore, we introduced for the subgroup analysis with patients with PPGL (PPGL-PHT) a categorical variable reflecting only the presence or absence of catecholamine excess (CE). Because of the different distribution among subgroups, we included age and sex as variables in the models. In addition, we performed the same analyses including in addition BMI and DM in the model, for the subgroup of patients where these data were available (Table 1). After building our multiple regression models, we only included the results of the independent variables when the F-value of the regression models was statistically significant (p-value ≤ 0.05) (Table 2).

Since not normally distributed, data values of targeted metabolomics and steroid profiles were normalized using the GLOG-Transformation in the MetaboAnalyst platform prior to the analyses (28, 29). Statistical Analysis and figure building were performed using SPSS Statistics v27.0 (IBM).

3 Results

3.1 Cohort description

A total of 263 subjects were included in the study. In Table 1, general patient characteristics are provided. In short, mean age of the patients was 49.3 years (13.4-77.9) varying between the subgroups with significantly older patients in the PPGL group compared to PA and PHT. The distribution of patients by sex differs between the groups, with a predominance of female patients in the CS group compared to all others. In addition, there was a significant difference in BMI between the groups with lower BMI values in the PPGL groups compared to PA and PHT in the subgroup analysis. At last, a significant higher occurrence of DM was found in individuals with CS and PPGL compared to PA and PHT.

As expected, aldosterone and 18oxo-cortisol levels were higher in patients with PA compared to other groups (CS, PPGL and PHT) (Supplementary Table 2). In addition, 11-deoxycortisol levels were also significantly higher in patients with PA than in PHT. However, this was also the case in patients with CS and PPGL. Furthermore, in patients with CS, cortisol levels were higher than in PA and PHT, but not significantly higher than in patients with PPGL. DHEA and DHEAS levels were lower in CS compared to all other patients.

3.2 Associations of plasma steroids on metabolomic features within distinct disease groups: Regression analyses results

Details of the regression models, such as p-values and standardized beta regression coefficients, are provided in the Supplementary Materials (Supplementary Tables 3.1, 3.2 for PPGL, Supplementary Tables 3.3, 3.4 for CS and Supplementary Tables 3.5, 3.6 for PA).

3.2.1 Pheochromocytoma and Paraganglioma

Regression models were constructed for 57 metabolites/MI (Supplementary Table 1.1) for the cohort encompassing PPGL-PHT patients (Table 2; Figure 1).

In both models, CE had the most associations with metabolite levels with 22 of 42 metabolites in the model without BMI and DM,

TABLE 1 General characteristics of the patients included in the study.

Diagnosis	PHT	PPGL	CS	PA	p-value
Patients, n	58	69	37	99	
Females, n (%)	19 (32.8)	38 (55.1)	34 (91.9)	42 (42.4)	<0.001[a]
Age (years)	44.8 (18.2-70.7)	53.8 (13.4-77.5)	51.2 (16.6-76.8)	48 (25.7-77.9)	<0.001[b]
Patients including BMI & DM, n	58	65	30	90	
Females, n (%)	19 (32.8)	36 (55.4)	27 (90.0)	39 (43.3)	<0.001[c]
Age (years)	44.8 (18.2-70.7)	53.7(13.4-77.5)	49.1 (16.6-73.3)	47.8 (25.7-77.9)	0.002 [d]
BMI (kg/m^2)	26.7 (19.6-40.6)	25.2 (16.0-34.3)	28.4 (20.9-39.5)	27.9 (18.0-41.0)	0.002 [e]
Diabetes mellitus (%)	0 (0.0)	16 (24.6)	8 (26.7)	4 (4.4)	<0.001[f]

Numeric values (age, BMI) are presented as mean with minimum and maximum. Categorical data (patients, females, diabetes mellitus) are shown as absolute numbers with percentage within the group.

[a]Analyses in the distribution of sex in subgroups revealed a significant disparity among CS-PPGL, CS-PHT and CS-PA (all p<0.001).

[b]Comparison of age in subgroups showed a significant difference between median age between PA-PPGL (p=0.013) and PHT-PPGL (p=0.001).

[c]Analyses in the distribution of sex in subgroups revealed a significant disparity among CS-PPGL (p=0.005), CS-PHT (p<0.001) and CS-PA (p<0.001).

[d]Comparison of age in subgroups showed a significant difference between median age between PA-PPGL (p=0.018) and PHT-PPGL (p=0.002).

[e]Comparison of BMI in subgroups showed a significant difference between PPGL-PA (p=0.001) and PPGL-CS (p=0.005) after correction for age and sex.

[f]Comparison of diabetes mellitus occurrence in subgroups showed a significant difference between CS-PA (p=0.04), CS-PHT (p=0.003), PPGL-PA (p<0.001) and PPGL-PHT (p<0.001) after correction for age and BMI.

PA, primary aldosteronism; CS, Cushing's syndrome; PPGL, pheochromocytoma and paraganglioma; PHT, primary hypertension; BMI, body mass index.

10.3389/fendo.2024.1370525

TABLE 2 List of studied metabolites and metabolic indices with the respective influencing adrenal steroids/catecholamines according to the subgroups.

	PPGL	CS	PA
Metabolite	Steroids & CE	Steroids	Steroids
Acylcarnitines			
C2	11DCS**		
C3-DC C4-OH	CE*, 11DCS*		
C7-DC			CST**, DHEAS**
C9		COL*, 11DCS*, 18OC*	18OHC*, 11DCS**, CST**, COL**, COS**, DHEA**
C10:1			11DCO*
C12:1			
C14:1	CE***, 11DCS***		
C14:2	COS*, 17OHP*		COS*, 11DCO***
C16			
C16:1	CE**, 11DCS**, 18OHC**		
C16:1-OH	11DCS*		
C18:1	CE**, 11DCS**, 18OHC**	11DCO**	Aldo*
C18:2	CE**, COL**, COS**, 11DCS*, 18OHC**	COL***, COS***	Aldo*, COS**
Amino Acids			
Arginine	CE**		
Aspartate	CE*, COL**, CST***	COL**	COL**, COS**, DHEA**, 17OHP**
Glutamate	COL**, 11DCO**, CST**	COL***, 18OHC***, DHEA***	Aldo*, CST*, COL***, COS***, DHEA**
Histidine	CE**, 21DC**		
Ornithine			CST**, COL**, COS**, DHEA**
Phenylalanine			Aldo*, 11DCS**, COS**, Ando**, DHEA**
Proline		18OHC**, 21DC***	
Serine			18OHC*, DHEA**, DHEAS**
Threonine			18OHC*, Ando**, DHEA**, DHEAS*
Biogenic Amines			
Spermidine	CE**, 17OHP**	11DCO*	Aldo**, 11DCO***
alpha-AAA	COL**, CST**, 18OC**		
Glycerophospholipids			
lysoPC a C14:0		COS**, 18OHC**, 17OHP**	
lysoPC a C16:0			COL*, COS*
lysoPC a C16:1		Ando**	
lysoPC a C17:0		Aldo*, 11DCS*	
lysoPC a C18:0	CST***		
lysoPC a C18:2	CE***, DHEAS***		
lysoPC C20:4	11DCS*		COS**
lysoPC a C24:0	CE**, COS**		

(Continued)

10.3389/fendo.2024.1370525

TABLE 2 Continued

	PPGL	CS	PA
Glycerophospholipids			
PC aa C32:1			
PC aa C32:2	CE*, DHEAS*		
PC aa C34:2			COL*, COS**, Ando**
PC aa C34:4	CE*, COS*, 21DC*		
PC aa C36:2	CE**, CST**		
PC aa C36:4	11DCS*		
PC aa C38:4	CST**, 11DCS*		
PC aa C38:6	CE*, 11DCO*, 18OC***, 17OHP**		
PC aa C40:1			COL*, COS*
PC aa C40:6	CE**, 11DCO*, 17OHP**		
PC aa C42:0			COL*, COS**, 11DCO*, DHEAS**
PC aa C42:1		11DCO**, 11DCS*, 18OHC**, 18OC**, DHEA**, 21DC***	COL*, COS**, 11DCO*, DHEAS**
PC aa C42:4			Aldo*
PC aa C42:5	CE***		
PC ae C32:1			
PC ae C32:2			11DCS*
PC ae C34:2	CE**, 18OHC**, 18OC**, DHEAS**		
PC ae C34:3	CE**, 11DCS*, 18OHC**, DHEAS***	COS***, Aldo**, 11DCO*, 11DCS*, 18OHC*, 18OC*	
PC ae C36:1		CST***, 11DCS**	
PC ae C36:3	COS*, 18OHC*, CE*		
PC ae C38:1			DHEA***
PC ae C40:3			11DCS**, COL*, COS*
PC ae C40:5			
PC ae C42:0	CE**, Ando*, 11DCO*	Aldo**, 11DCO**, 18OHC**, 18OC**, DHEA*, 17OHP**	18OHC*
PC ae C42:1			COL**, COS**
PC ae C42:2		11DCO*, 11DCS**	COL*, COS**, 11DCO*
PC ae C42:3			18OHC*, 18OC**, COL*, COS**, 11DCO*
PC ae C42:5			COL*, COS**, 11DCO*
PC ae C44:3			
PC ae C44:4		11DCO*, 11DCS*	
PC ae C44:5			COS***
PC ae C44:6			COL**, COS**, 11DCO*, 21DC*
Sphingolipids			
SM C16:1		CST***	
SM C18:0	CE**		
SM C18:1	CE**		Ando*
SM C20:2		COL***	

(Continued)

TABLE 2 Continued

	PPGL	CS	PA
Sphingolipids			
SM C24:1	CE**, 11DCO*		
SM(OH) C16:1		11DCS*	
Monosaccharides			
H1	CE*	Ando**, DHEA**	
Metabolic indices			
C2/C0			
Fischer Ratio	18OHC*		Aldo***, Ando***
CPT-I Ratio			Aldo**, COS**
Citrulline/Arginine	CE*		
Citrulline/Ornithine		COL**, COS**, 21DC**	CST*, COL**, COS**
Met-SO/Methionine	CE**		
Ornithine/Arginine	CE**, COS**	11DCO**	CST***, COL**, COS**, DHEA**
Putrescine/Ornithine			COL*, COS*
Spermidine/ Putrescine	CE*, 17OHP**	18OHC*, DHEA*	Aldo*
Tyrosine/ Phenylalanine	CE*, 11DCS***, DHEA*	DHEA*	
Total DMA/Arginine	CE**, COS**, DHEA**, 18OHC*		Aldo*
AAA			11DCS*, Ando*, DHEA*
BCAA			11DCS**, Ando**, DHEA**
Essential AA			11-DCS*, COS***, Ando**, DHEA**
Non-essential AA	21DC**		
Glucogenic AA		Ando*	
Total AA	21DC***		

Significant associated metabolites with steroids according to the subgroups (PPGL 127 patients without BMI/DM, 123 including BMI/DM and 57 metabolites/metabolic indices, CS 95 patients without BMI/DM, 88 including BMI/DM and 47 metabolites/metabolic indices, PA 157 patients without BMI/DM, 148 including BMI/DM and 57 metabolites/metabolic indices).
Highlighted fields represent not included metabolites in the subgroup analysis (dark grey) and metabolites where the regression model itself was not significant (lighter grey). Empty fields (white) represent missing predictor (adrenal steroid or catecholamine excess) in an otherwise significant regression model.
* Significantly associated metabolites in linear regression models only without BMI and DM in the model.
** Significantly associated metabolites in linear regression models with and without BMI and DM in the model.
*** Significantly associated metabolites in linear regression models only with BMI and DM in the model.
Abbreviations metabolites and metabolic indices: a, acyl; AA, amino acids; AAA, aromatic amino acids; aa, diacyl; ae, acyl-alkyl; BCAA, branched chain amino acids; CPT-I ratio, carnitine palmitoyltransferase I ratio; Cx:y shows the lipid chain composition where "x" is the number of carbons and "y" of double bonds. DMA, dimethylarginine; H1, sum of Hexoses (including Glucose); lysoPC, lysophosphatidylcholine; Met-SO, methionine sulfoxide; PC, phosphatidylcholine; SM, sphingomyelin.
Abbreviations steroids and clinical data: Aldo, Aldosterone; Ando, Androstenedione; BMI, body mass index; CE, catecholamine excess; COL, cortisol; COS, cortisone; CST, corticosterone; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DM, diabetes mellitus; CS, Cushing's syndrome; PA, primary hyperaldosteronism; PHT, primary hypertension; PPGL, pheochromocytoma/paraganglioma; 11DCO, 11-deoxycortisol; 11DCS, 11-deoxycorticosterone; 17OHP, 17-hydroxyprogesterone; 18OC, 18-oxocortisol; 18OHC, 18-hydroxycortisol; 21DC, 21-deoxycortisol.

and 18 of 42 metabolites in the model including BMI and DM. The association was positive for all ACs, sphingolipids (SP), spermidine, and H1, while it was predominantly negative for AA and GP. Among all metabolites, GPs were the predominant one (10 out of 19 metabolites in the model without BMI and DM and 8 out of 19 metabolites in the model including BMI and DM). In both models, 11-deoxycorticosterone showed the most significant positive relationship with ACs (6 out of 12 metabolites in the model without BMI and DM and 4 out of 12 metabolites in the model including BMI and DM). Cortisol and corticosterone shared their association on AA with CE (2 out of 4 metabolites in both models) following aldosterone, DHEA, and androstenedione, which did not show any significant association.

3.2.2 Cushing's syndrome

Regression models were constructed for 47 metabolites/MI (Supplementary Table 1.1) for the cohort containing CS-PHT patients. (Table 2; Figure 2).

Cortisol had a negative association with C9 and citrulline/ornithine and a positive association with aspartate. Cortisone was

10.3389/fendo.2024.1370525

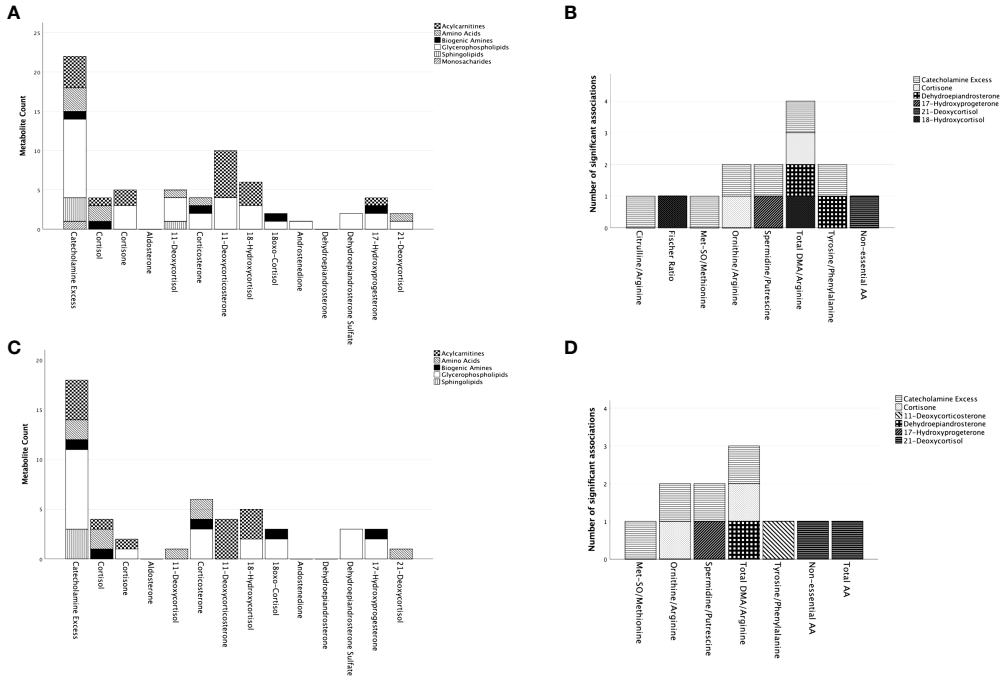


FIGURE 1
Summary of significant associations of adrenal steroids on the included metabolites and metabolic indices in patients with paraganglioma/pheochromocytoma. Represented are metabolites and metabolites indices for which the model in the multiple regression analyses resulted significant (Supplementary Tables 3.1, 3.2). In **(A)** (with adjustment for age/sex) and **(C)** (with adjustment for age/sex/BMI/DM) on the x-axis are represented the adrenal steroids with the total number of metabolites being significantly predicted for each steroid on the y-axis. The metabolites are represented by groups (acylcarnitines, amino acids, biogenic amines, glycerophospholipids, sphingolipids) defined by distinct pattering as specified in the figure legend. To note is that one metabolite might have significant associations with multiple adrenal steroids. **(B)** (with adjustment for age/sex) and **(D)** (with adjustment for age/sex/BMI/DM) represent the metabolite indices on the x-axis and the total number of adrenal steroid(s) significantly associated with the metabolic indices on the y-axis. Each adrenal steroid is represented by a different pattern as specified in the figure legend. AA, amino acids; DMA, dimethylarginine; Met-SO, sulfoxidized methionine.

only related to one metabolite, lysoPC acyl (a) C14:0. However, when BMI and DM were included in the linear regression models, the number of associations increased for cortisol (4 out of 37 metabolites) and cortisone (3 out of 37 metabolites). Specifically, C18:2 showed a significant relationship with both cortisol and cortisone. Additionally, glutamate and sphingomyelin (SM) C20:2 had positive relationships with cortisol, while PC acyl-alkyl (ae) C34:3 had a negative relationship with cortisone. Without including BMI and DM, 11-deoxycortisone (8 out of 37) and 11-deoxycortisol (7 out of 37) had the most significant associations with the metabolites. However, the number of their associations was less pronounced after the inclusion of BMI and DM in the model (11-deoxycortisone 2 out of 37, 11-deoxycortisol total 3 out of 37), whereas cortisol, 18-hydroxycortisol (5 out of 37), and DHEA (3 out of 37) had more associations with the metabolites. Moreover, monosaccharides showed associations with androstenedione and DHEA in both models (Supplementary Figure 1), while AA had associations with only cortisol (1 out of 6) and 18-hydroxycortisol (1 out of 6) without considering BMI and DM. After including BMI and DM, associations for AA were observed with cortisol (2 out of 6), 21-deoxycortisol (1 out of 6), 18-hydroxycortisol (2 out of 6), and DHEA (1 out of 6). Additionally, upon including BMI and DM, AC had associations with cortisol, cortisone, and 11-deoxycortisol (all 1 out of 10). GP mainly showed associations with 11-deoxycorticosterone (6 out of 13), then 11-deoxycortisol (5 out of 13), 18-hydroxycortisol (4 out of 13), and 18-oxocortisol (3 out of 13) before adding BMI and DM to the regression models. Ornithine, phenylalanine, and alanine did not have predictive associations with any of the studied adrenal steroids.

3.2.3 Primary aldosteronism

Multiple linear regression analyses were performed for each of the 57 metabolites/MI (Supplementary Table 1.1) studied in the cohort consisting of patients with PA and PHT (Table 2; Figure 3).

Among the 57 metabolites/MI, only nine were associated with aldosterone levels. Among these, C18:2, spermidine, and phosphatidylcholine (PC) diacyl (aa) C42:4, as well as CPT-I ratio, spermidine/putrescine, and total DMA/arginine, were positively associated with aldosterone. However, after adding BMI and DM to the model, these associations with aldosterone were no longer significant, except for spermidine, CPT-I ratio, and the Fisher-ratio (not resulted significant in the former model).

In contrast, a higher number of metabolites (14 in total) were predicted by cortisol, including a negative association with short-chain AC, two positively associated AA, as well as 11 GP. Similarly, 17 metabolites were predicted by cortisone, containing two negatively and one positively associated AC, three negatively associated AA, as well as 11 negatively associated GP. Interestingly, cortisone and cortisol had

10.3389/fendo.2024.1370525

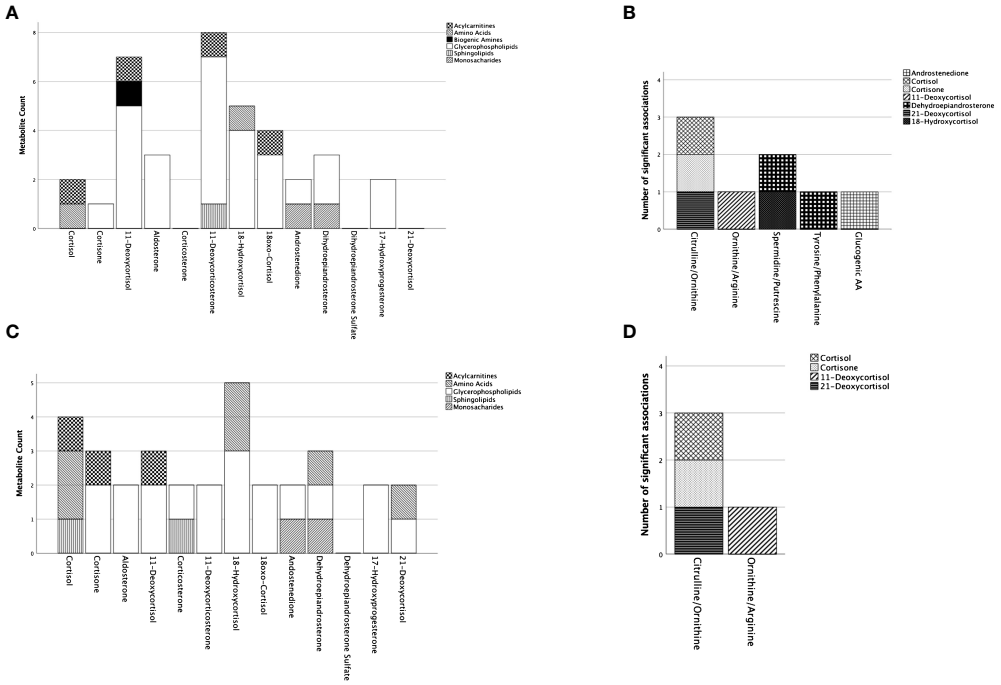


FIGURE 2
Summary of significant associations of adrenal steroids on the included metabolites and metabolic indices in patients with Cushing's syndrome. Represented are metabolites and metabolites indices for which the model in the multiple regression analyses resulted significant (Supplementary Tables 3.3, 3.4). In **(A)** (with adjustment for age/sex) and **(C)** (with adjustment for age/sex/BMI/DM) on the x-axis are represented the adrenal steroids with the total number of metabolites being significantly predicted for each steroid on the y-axis. The metabolites are represented by groups (acylcarnitines, amino acids, biogenic amines, glycerophospholipids, sphingolipids) defined by distinct pattering as specified in the figure legend. To note is that one metabolite might have significant associations with multiple adrenal steroids. **(B)** (with adjustment for age/sex) and **(D)** (with adjustment for age/sex/BMI/DM) represent the metabolite indices on the x-axis and the total number of adrenal steroid(s) significantly associated with the metabolic indices on the y-axis. Each adrenal steroid is represented by a different pattern as specified in the figure legend. AA, amino acids.

the most associations with GP (11 out of 27) and DHEA on AA (6 out of 7). After including BMI and diabetes in the model, the significance of glucocorticoids persisted. However, cortisol had fewer associations with GP (2 out of 27), whereas cortisone remained associated with almost the same number of GP (10 out of 27), AA (4 out of 7), and AC (2 out of 9). Additionally, the significance of the association between DHEA and AA did not change. The other parameters (18-hydroxycortisol, 18oxo-cortisol, 11-deoxycortisone, corticosterone, 11-deoxycortisol, androstenedione, DHEAS, 17-hydroxyprogesterone) showed only a few associations.

4 Discussion

From earlier studies (23), it has been appreciated that hormonal alterations in patients with PPGL, CS and PA are not only characterized by excessive secretion of the disease-defining adrenal hormones but also by concurrent hormonal alterations that vary significantly between the disorders. In this study, we further identified associations between these hormonal changes and the metabolomic differences previously reported (14), at times exceeding the associations seen with the disease-defining hormonal excess.

Considering the whole cohort of hypertensive patients, we identified a high number of associations between distinct adrenal steroids on the acylcarnitines and glycerophospholipids. From the literature, it is known that long-chain acylcarnitines are associated with cardiac morbidities such as arrhythmic disorders (6), dilated cardiomyopathy as well as heart failure (30). In addition, long-chain acylcarnitines have been associated with insulin resistance and DM type 2 (31). It is of interest that all these clinical features are more prominent in patients with endocrine hypertension in comparison to those with PHT (6–9). Interestingly, in addition to the disease defining catecholamine excess in patients with PPGL, and cortisol excess in patients with CS, we found that cortisone, 11-deoxycortisol and 11-deoxycorticosterone exhibited a significant association with long-chain acylcarnitines.

Another crucial aspect to consider across all three EHT entities is the increased catabolic state and loss of skeletal muscle (32–34), leading to increased protein turnover. This is reflected in higher levels of aspartate and glutamate within the metabolomic profile of patients affected by EHT. We also observed an association between cortisol and both amino acids in all EHT subgroups, in concomitance with the association of aspartate with catecholamine excess in PPGL and glutamate with aldosterone in the PA patients. Surprisingly, DHEA was associated with both metabolites in PA and with glutamate in CS subgroup analysis. Notably, considering the potential role of aspartate and glutamate in the pathogenesis of glucose homeostasis disorders (35), it is intriguing to observe that lower levels of DHEA, which tend to be

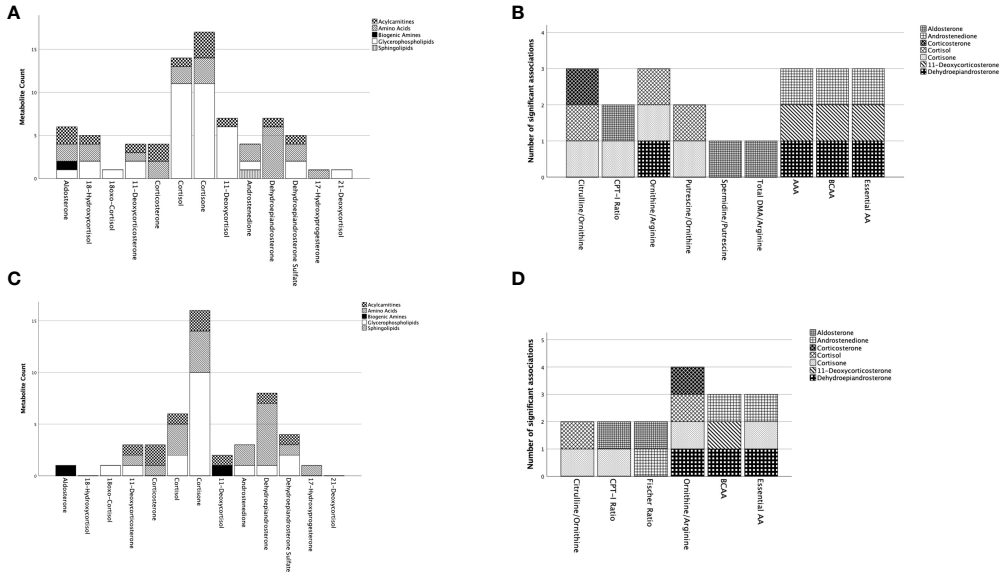


FIGURE 3
Summary of significant associations of adrenal steroids on the included metabolites and metabolic indices in patients with primary aldosteronism. Represented are metabolites and metabolites indices for which the model in the multiple regression analyses resulted significant (Supplementary Tables 3.5, 3.6). In **(A)** (with adjustment for age/sex) and **(C)** (with adjustment for age/sex/BMI/DM) on the x-axis are represented the adrenal steroids with the total number of metabolites being significantly predicted for each steroid on the y-axis. The metabolites are represented by groups (acylcarnitines, amino acids, biogenic amines, glycerophospholipids, sphingolipids) defined by distinct pattering as specified in the figure legend. To note is that one metabolite might have significant associations with multiple adrenal steroids. **(B)** (with adjustment for age/sex) and **(D)** (with adjustment for age/sex/BMI/DM) represent the metabolite indices on the x-axis and the total number of adrenal steroid(s) significantly associated with the metabolic indices on the y-axis. Each adrenal steroid is represented by a different pattern as specified in the figure legend. AA, amino acids; AAA, aromatic amino acids; BCAA, branched chain amino acids; CPT-I ratio, carnitine palmitoyltransferase I ratio; DMA, dimethylarginine.

present in patients with CS and PA compared to patients with PHT, were associated with higher levels of these two amino acids.

In addition, considering the measured metabolic indices as surrogate markers of specific metabolic pathways, we identified a large number of associations with urea cycle activity (represented by citrulline/ornithine, ornithine/arginine), activity of protein arginine methyl transferases (represented by total DMA/arginine), and CPT-I activity. Similarly to the above-described metabolites, available data reveal the importance of arginase and protein arginine methyl transferases in the pathogenesis of hypertension, obesity, insulin resistance and diabetes mellitus (36–41).

In patients with PPGL the most significant association on the observed metabolomic differences compared to patients with PHT was related the catecholamine excess itself. Specifically, metabolomic markers associated with higher cardiovascular risk and dysglycemia (long-chain acylcarnitines, glycerophospholipids, arginine) as well as metabolomic indices, including PRMT (total DMA/Arginine) and arginase (ornithine/arginine) activity (6, 30, 31, 36–43) were mainly associated with the extent of catecholamine excess. Nevertheless, adrenal steroids, such as 11-deoxycortisone, and after adjustment for BMI and DM, corticosterone, 18-hydroxycortisol, and cortisol, also showed associations with these metabolic markers. Interestingly, 11-deoxycortisone had most of the positive associations with long-chain acylcarnitines followed by 18-hydroxycortisol, adding some additional impact on the increased cardiovascular risk observed in the patients with PPGL (7). However, a CE-independent metabolomic difference regarded the alpha-aminoadipic acid (AAA). AAA is a product of the lysine breakdown pathway, and one study hypothesized that AAA might be part of the carbonyl stress pathway seen in diabetes (44). Furthermore, higher levels of AAA have been linked with diabetes mellitus and disturbed glucose metabolism (45), which are more often seen in patients with PPGL (12).

In patients with CS, it was evident that the metabolomic traits, specifically GP (e.g., PC aa C42:1, PC ae C42:2, PC ae C44:4), were mainly associated with 11-deoxycorticosterone, 18-hydroxycortisol (positively), and 11-deoxycortisol (negatively), rather than cortisol itself before adjusting for DM and BMI. However, this changed after adjustment for these features, where, in addition to 11-deoxcycortisol, cortisol was mainly associated with the metabolomic profile. It is therefore tempting to speculate that, in addition to the well-known impact of cortisol on insulin resistance (9), mineralocorticoids may contribute to the respective metabolomic pattern (GP), related to insulin resistance (46, 47). The significant association with the low arginine level, potentially due to decreased urea cycle activity and heightened arginase activity, also involves 21-deoxycortisol and 11-deoxycortisol. This relationship further relates to the metabolic syndrome phenotype and increased cardiovascular risk (34, 41, 46). One particularly intriguing discovery is the notable association between glucose levels (indicated by monosaccharides) and DHEA, which is significantly reduced in patients with CS within our cohort. Lower DHEA is primarily observed in patients with adrenal-based, and thus ACTH-independent CS, which are mainly represented in our cohort (data has not been presented). This relationship is noteworthy, as a higher prevalence of diabetes

mellitus is observed among patients with ACTH-dependent CS, which corresponds to elevated DHEAS levels when compared to those with ACTH-independent CS (47).

Intriguingly, in patients with PA, the analysis did not suggest aldosterone as the hormone with most associations with the metabolomic differences, but rather cortisone followed by cortisol and DHEA. Specifically, a strong association was observed between cortisone (negative correlation) and cortisol (positive correlation) with several GP (e.g., lyso PC a C16:0, PC aa C34:2, PC aa C42:1, PC ae C42:1, PC ae C44:6) before accounting for BMI and DM in patients with PA. Recent studies have shown a significant association between several GP and SP and insulin resistance/metabolic syndrome (46, 48, 49), which are phenotypic features observed in patients with PA (10, 11). Additionally, lyso PCs increase oxidative stress and regional vascular inflammation, and higher circulating levels of lyso PCs are associated with early coronary atherosclerosis leading to a higher cardiovascular risk (50, 51), which has been observed in patients with PA compared to PHT (3). Therefore, the metabolome traits distinguishing PA from PHT and associated with glucose metabolism alteration and cardiovascular morbidity are mainly associated with the concomitantly secreted adrenal steroids, rather than aldosterone itself. In line with the metabolomic traits, the MI in patients with PA, such as citrulline/ornithine and ornithine/arginine, which are associated with increased cardiovascular risk and phenotypic features of metabolic syndrome, were mainly associated with cortisol, cortisone, corticosterone, and DHEA in our regression analysis. Based on these observations, it can be speculated that the poorer cardiovascular outcomes in patients with PA treated pharmacologically by inhibiting aldosterone activity compared to surgery (52) might be related to the unopposed activity of the other adrenal steroids.

Overall, these findings provide indirect evidence that various adrenal hormones are linked to alterations in specific metabolic pathways that may also influence cardiovascular and metabolic risk.

The current study has several strengths, including a multicentric approach, a well-characterized patient cohort with arterial hypertension, and comprehensive endocrine assessments for diagnosis. The study protocol included standardized blood sample collection, minimizing the impact of external factors, and all measurements were performed on blood specimens obtained at the same time point.

There are, however, some limitations to our study: While the diagnosis of endocrine hypertension was made using standard laboratory screening tests in line with the guidelines at the study's inception, no subsequent data were available to verify the initial diagnosis. Particularly concerning the diagnosis of PA, recent findings indicate potential over-diagnosis based on current cut-offs, dependent on the assay used (53, 54). Consequently, we cannot rule out potential misclassification of some cases in our cohort. Moreover, due to the study's design, the modest patient count, and its retrospective nature, there might be an overestimation in our results, suggesting associations rather than definitive causations. The statistical approach, utilizing linear regression, encompassed multiple predictors. This increases the likelihood of collinearity or confounding, especially compared to models with fewer parameters. Unfortunately, data on substance abuse (nicotine, alcohol) weren't accessible, and only a limited array of clinical data was on hand. The sample sizes for certain subgroup disorders, notably in the CS group, were relatively minimal. Therefore, our study's findings should serve as a foundational reference for subsequent research in a prospective environment, emphasizing the accumulation of broader clinical information from a more extensive population. Data post-treatment would further validate the nature of the relationship. Additionally, any speculative interpretation of the observed links requires extensive background validation and mechanistic studies to ascertain causality. Addressing this point, it's important to recognize that, although distinct metabolic patterns have been observed, certain associated adrenal steroid concentrations didn't showcase significant variance between the groups. This highlights a possible context-specific exposure yet to be identified.

In conclusion, our study suggests a significant impact of cortisol, cortisone, and catecholamine excess on the metabolomic differences observed in patients with EHT compared to PHT. Surprisingly, apart from patients with PPGL, where the catecholamine excess played a major role in the metabolomic changes, the majority of metabolomic differences observed in patients with PA were associated with non-disease defining hormonal excess represented by other adrenal steroids from the studied panel. In CS, cortisol was also not the leading adrenal steroid but was associated with metabolomic differences along with other non-disease defining adrenal hormones. These findings suggest that the metabolic disorders and increased cardiovascular morbidity in these patients may be influenced by other adrenal steroids as well.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Kantonale Ethikkomission Zürich. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

RK: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. ZE: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SG: Data curation,

Investigation, Resources, Writing – review & editing. LA: Data curation, Investigation, Resources, Writing – review & editing. CL: Data curation, Investigation, Resources, Writing – review & editing. A-PG-R: Data curation, Investigation, Resources, Writing – review & editing. PM: Data curation, Investigation, Resources, Writing – review & editing. MT: Data curation, Investigation, Resources, Writing – review & editing. APe: Data curation, Investigation, Resources, Writing – review & editing. CP: Data curation, Investigation, Resources, Writing – review & editing. KL: Data curation, Investigation, Resources, Writing – review & editing. MP: Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Writing – review & editing. FC: Data curation, Investigation, Resources, Writing – review & editing. APr: Data curation, Investigation, Resources, Writing – review & editing. AJ: Data curation, Investigation, Resources, Writing – review & editing. CA: Data curation, Investigation, Resources, Writing – review & editing. HR: Data curation, Investigation, Resources, Writing – review & editing. LL: Data curation, Investigation, Resources, Writing – review & editing. MD: Data curation, Investigation, Resources, Writing – review & editing. JD: Data curation, Investigation, Resources, Writing – review & editing. EJ: Data curation, Investigation, Resources, Writing – review & editing. AB: Data curation, Investigation, Resources, Writing – review & editing. M-CZ: Data curation, Investigation, Project administration, Resources, Supervision, Writing – review & editing. GE: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. FB: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 633983 (ENSAT-HT to all authors, except MD), by the Clinical Research Priority Program of the University of Zurich for the CRPP HYRENE (to FB), the Deutsche Forschungsgemeinschaft project number 314061271 (CRC/Transregio 205/1 "The Adrenal: Central relay of health and disease" to GE, MP, CP, FB) and the Philhuman Stiftung Vaduz, Lichtenstein (to ZE).

Acknowledgments

We thank the COST Action CA20122 Harmonization and ENSAT for supportive networking.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1370525/full#supplementary-material

References

1. Elliott WJ. Systemic hypertension. *Curr Problems Cardiol.* (2007) 32:201–59. doi: 10.1016/j.cpcardiol.2007.01.002

2. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet.* (2005) 365:217–23. doi: 10.1016/S0140-6736(05)70151-3

3. Rossi GP. Primary aldosteronism: JACC state-of-the-art review. *J Am Coll Cardiol.* (2019) 74:2799–811. doi: 10.1016/j.jacc.2019.09.057

4. Fleseriu M, Auchus R, Bancos I, Ben-Shlomo A, Bertherat J, Biermasz NR, et al. Consensus on diagnosis and management of Cushing's disease: a guideline update. *Lancet Diabetes Endocrinol.* (2021) 9:847–75. doi: 10.1016/S2213-8587(21)00235-7

5. Lenders JWM, Kerstens MN, Amar L, Prejbisz A, Robledo M, Taieb D, et al. Genetics, diagnosis, management and future directions of research of phaeochromocytoma and paraganglioma: a position statement and consensus of the Working Group on Endocrine Hypertension of the European Society of Hypertension. *J Hypertens.* (2020) 38:1443–56. doi: 10.1097/HJH.0000000000002438

6. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol.* (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4

7. Stolk RF, Bakx C, Mulder J, Timmers HJ, Lenders JW. Is the excess cardiovascular morbidity in pheochromocytoma related to blood pressure or to catecholamines? *J Clin Endocrinol Metab.* (2013) 98:1100–6. doi: 10.1210/jc.2012-3669

8. Stowasser M, Gordon RD. Primary aldosteronism: changing definitions and new concepts of physiology and pathophysiology both inside and outside the kidney. *Physiol Rev.* (2016) 96:1327–84. doi: 10.1152/physrev.00026.2015

9. Braun LT, Vogel F, Reincke M. Long-term morbidity and mortality in patients with Cushing's syndrome. *J Neuroendocrinol.* (2022) 34(8):e13113. doi: 10.1111/jne.13113

10. Fallo F, Veglio F, Bertello C, Sonino N, Della Mea P, Ermani M, et al. Prevalence and characteristics of the metabolic syndrome in primary aldosteronism. *J Clin Endocrinol Metab.* (2006) 91:454–9. doi: 10.1210/jc.2005-1733

11. Bothou C, Beuschlein F, Spyroglou A. Links between aldosterone excess and metabolic complications: A comprehensive review. *Diabetes Metab.* (2020) 46:1–7. doi: 10.1016/j.diabet.2019.02.003

12. Erlic Z, Beuschlein F. Metabolic alterations in patients with pheochromocytoma. *Exp Clin Endocrinol Diabetes*. (2019) 127:129–36. doi: 10.1055/a-0649-0960

13. Kwak MK, Lee JY, Kim BJ, Lee SH, Koh JM. Effects of primary aldosteronism and different therapeutic modalities on glucose metabolism. *J Clin Med*. (2019) 8 (12):2194. doi: 10.3390/jcm8122194

14. Erlic Z, Reel P, Reel S, Amar L, Pecori A, Larsen CK, et al. Targeted metabolomics as a tool in discriminating endocrine from primary hypertension. *J Clin Endocrinol Metab*. (2021) 106:e1111–28. doi: 10.1210/clinem/dgaa954

15. Erlic Z, Kurlbaum M, Deutschbein T, Nolting S, Prejbisz A, Timmers H, et al. Metabolic impact of pheochromocytoma/paraganglioma: targeted metabolomics in patients before and after tumor removal. *Eur J Endocrinol*. (2019) 181:647–57. doi: 10.1530/EJE-19-0589

16. Lana A, Alexander K, Castagna A, D'Alessandro A, Morandini F, Pizzolo F, et al. Urinary metabolic signature of primary aldosteronism: gender and subtype-specific alterations. *Proteomics Clin Appl*. (2019) 13:e1800049. doi: 10.1002/prca.201800049

17. Di Dalmazi G, Quinkler M, Deutschbein T, Prehn C, Rayes N, Kroiss M, et al. Cortisol-related metabolic alterations assessed by mass spectrometry assay in patients with Cushing's syndrome. *Eur J Endocrinol*. (2017) 177:227–37. doi: 10.1530/EJE-17-0109

18. Newgard CB. Metabolomics and metabolic diseases: where do we stand? *Cell Metab*. (2017) 25:43–56. doi: 10.1016/j.cmet.2016.09.018

19. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. *Nat Rev Mol Cell Biol*. (2016) 17:451–9. doi: 10.1038/nrm.2016.25

20. Ussher JR, Elmariah S, Gerszten RE, Dyck JRB. The emerging role of metabolomics in the diagnosis and prognosis of cardiovascular disease. *J Am Coll Cardiol*. (2016) 68:2850–70. doi: 10.1016/j.jacc.2016.09.972

21. Zordoky BN, Sung MM, Ezekowitz J, Mandal R, Han B, Bjorndahl TC, et al. Metabolomic fingerprint of heart failure with preserved ejection fraction. *PloS One*. (2015) 10:e0124844. doi: 10.1371/journal.pone.0124844

22. Arlt W, Lang K, Sitch AJ, Dietz AS, Rhayem Y, Bancos I, et al. Steroid metabolome analysis reveals prevalent glucocorticoid excess in primary aldosteronism. *JCI Insight*. (2017) 2(8):e93136. doi: 10.1172/jci.insight.93136

23. Constantinescu G, Langton K, Conrad C, Amar L, Assie G, Gimenez-Roqueplo AP, et al. Glucocorticoid excess in patients with pheochromocytoma compared with paraganglioma and other forms of hypertension. *J Clin Endocrinol Metab*. (2020) 105: e3374–83. doi: 10.1210/clinem/dgaa423

24. Peitzsch M, Dekkers T, Haase M, Sweep FC, Quack I, Antoch G, et al. An LC-MS/MS method for steroid profiling during adrenal venous sampling for investigation of primary aldosteronism. *J Steroid Biochem Mol Biol*. (2015) 145:75–84. doi: 10.1016/j.jsbmb.2014.10.006

25. Peitzsch M, Prejbisz A, Kroiss M, Beuschlein F, Arlt W, Januszewicz A, et al. Analysis of plasma 3-methoxytyramine, normetanephrine and metanephrine by ultraperformance liquid chromatography-tandem mass spectrometry: utility for diagnosis of dopamine-producing metastatic phaeochromocytoma. *Ann Clin Biochem*. (2013) 50:147–55. doi: 10.1258/acb.2012.012112

26. Peitzsch M, Adaway JE, Eisenhofer G. Interference from 3-O-methyldopa with ultra-high performance LC-MS/MS measurements of plasma metanephrines: chromatographic separation remains important. *Clin Chem*. (2015) 61:993–6. doi: 10.1373/clinchem.2015.239962

27. Troyanskaya O, Cantor M, Sherlock G, Brown P, Hastie T, Tibshirani R, et al. Missing value estimation methods for DNA microarrays. *Bioinformatics*. (2001) 17:520–5. doi: 10.1093/bioinformatics/17.6.520

28. Xia J, Wishart DS. Using metaboAnalyst 3.0 for comprehensive metabolomics data analysis. *Curr Protoc Bioinf*. (2016) 55:14.0.1–.0.91. doi: 10.1002/cpbi.11

29. Durbin BP, Hardin JS, Hawkins DM, Rocke DM. A variance-stabilizing transformation for gene-expression microarray data. *Bioinformatics*. (2002) 18 Suppl 1:S105–10. doi: 10.1093/bioinformatics/18.suppl_1.S105

30. Aitken-Buck HM, Krause J, van Hout I, Davis PJ, Bunton RW, Parry DJ, et al. Long-chain acylcarnitine 18:1 acutely increases human atrial myocardial contractility and arrhythmia susceptibility. *Am J Physiol Heart Circ Physiol*. (2021) 321:H162–h74. doi: 10.1152/ajpheart.00184.2021

31. Schooneman MG, Vaz FM, Houten SM, Soeters MR. Acylcarnitines: reflecting or inflicting insulin resistance? *Diabetes*. (2013) 62:1–8. doi: 10.2337/db12-0466

32. Kwak MK, Lee SE, Cho YY, Suh S, Kim BJ, Song KH, et al. The differential effect of excess aldosterone on skeletal muscle mass by sex. *Front Endocrinol (Lausanne)*. (2019) 10:195. doi: 10.3389/fendo.2019.00195

33. Brillon DJ, Zheng B, Campbell RG, Matthews DE. Effect of cortisol on energy expenditure and amino acid metabolism in humans. *Am J Physiol*. (1995) 268:E501–13. doi: 10.1152/ajpendo.1995.268.3.E501

34. Lee SH, Kwak MK, Ahn SH, Kim H, Cho YY, Suh S, et al. Change of skeletal muscle mass in patients with pheochromocytoma. *J Bone Miner Metab*. (2019) 37:694–702. doi: 10.1007/s00774-018-0959-3

35. Wishart DS. Metabolomics for investigating physiological and pathophysiological processes. *Physiol Rev*. (2019) 99:1819–75. doi: 10.1152/physrev.00035.2018

36. Ren Y, Li Z, Li W, Fan X, Han F, Huang Y, et al. Arginase: biological and therapeutic implications in diabetes mellitus and its complications. *Oxid Med Cell Longevity*. (2022) 2022:2419412. doi: 10.1155/2022/2419412

37. Förstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, et al. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. *Hypertension*. (1994) 23:1121–31. doi: 10.1161/01.HYP.23.6.1121

38. Hogg N, Kalyanaraman B, Joseph J, Struck A, Parthasarathy S. Inhibition of low-density lipoprotein oxidation by nitric oxide. Potential role in atherogenesis. *FEBS Lett*. (1993) 334:170–4. doi: 10.1016/0014-5793(93)81706-6

39. Hermann M, Flammer A, Lüscher TF. Nitric oxide in hypertension. *J Clin Hypertens (Greenwich)*. (2006) 8:17–29. doi: 10.1111/j.1524-6175.2006.06032.x

40. Couto ESA, Wu CY, Citadin CT, Clemons GA, Possoit HE, Grames MS, et al. Protein arginine methyltransferases in cardiovascular and neuronal function. *Mol Neurobiol*. (2020) 57:1716–32. doi: 10.1007/s12035-019-01850-z

41. Lee W, Lee HJ, Jang HB, Kim HJ, Ban HJ, Kim KY, et al. Asymmetric dimethylarginine (ADMA) is identified as a potential biomarker of insulin resistance in skeletal muscle. *Sci Rep*. (2018) 8:2133. doi: 10.1038/s41598-018-20549-0

42. Wu G, Meininger CJ. Nitric oxide and vascular insulin resistance. *Biofactors*. (2009) 35:21–7. doi: 10.1002/biof.3

43. Hu S, Han M, Rezaei A, Li D, Wu G, Ma X. L-arginine modulates glucose and lipid metabolism in obesity and diabetes. *Curr Protein Pept Sci*. (2017) 18:599–608. doi: 10.2174/1389203717666160627074017

44. Sell DR, Strauch CM, Shen W, Monnier VM. 2-aminoadipic acid is a marker of protein carbonyl oxidation in the aging human skin: effects of diabetes, renal failure and sepsis. *Biochem J*. (2007) 404:269–77. doi: 10.1042/BJ20061645

45. Wang TJ, Ngo D, Psychogios N, Dejam A, Larson MG, Vasan RS, et al. 2-Aminoadipic acid is a biomarker for diabetes risk. *J Clin Invest*. (2013) 123:4309–17. doi: 10.1172/JCI64801

46. Chang W, Hatch GM, Wang Y, Yu F, Wang M. The relationship between phospholipids and insulin resistance: From clinical to experimental studies. *J Cell Mol Med*. (2019) 23:702–10. doi: 10.1111/jcmm.13984

47. Brookes JC, Galigniana MD, Harker AH, Stoneham AM, Vinson GP. System among the corticosteroids: specificity and molecular dynamics. *J R Soc Interf*. (2012) 9:43–53. doi: 10.1098/rsif.2011.0183

48. Floegel A, Stefan N, Yu Z, Mühlenbruch K, Drogan D, Joost H-G, et al. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. *Diabetes*. (2013) 62:639. doi: 10.2337/db12-0495

49. Then C, Wahl S, Kirchhofer A, Grallert H, Krug S, Kastenmüller G, et al. Plasma metabolomics reveal alterations of sphingo- and glycerophospholipid levels in non-diabetic carriers of the transcription factor 7-like 2 polymorphism rs7903146. *PloS One*. (2013) 8:e78430. doi: 10.1371/journal.pone.0078430

50. Cole LK, Vance JE, Vance DE. Phosphatidylcholine biosynthesis and lipoprotein metabolism. *Biochim Biophys Acta*. (2012) 1821:754–61. doi: 10.1016/j.bbalip.2011.09.009

51. Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, et al. Local production of lipoprotein-associated phospholipase A and lysophosphatidylcholine in the coronary circulation. *Circulation*. (2007) 115:2715–21. doi: 10.1161/CIRCULATIONAHA.106.671420

52. Jing Y, Liao K, Li R, Yang S, Song Y, He W, et al. Cardiovascular events and all-cause mortality in surgically or medically treated primary aldosteronism: A Meta-analysis. *J Renin Angiotensin Aldosterone Syst*. (2021) 22:14703203211003781. doi: 10.1177/14703203211003781

53. Eisenhofer G, Kurlbaum M, Peitzsch M, Constantinescu G, Remde H, Schulze M, et al. The saline infusion test for primary aldosteronism: implications of immunoassay inaccuracy. *J Clin Endocrinol Metab*. (2022) 107:e2027–e36. doi: 10.1210/clinem/dgab924

54. Brown JM, Auchus RJ, Honzel B, Luther JM, Yozamp N, Vaidya A. Recalibrating interpretations of aldosterone assays across the physiologic range: immunoassay and liquid chromatography-tandem mass spectrometry measurements under multiple controlled conditions. *J Endocr Soc*. (2022) 6:bvac049. doi: 10.1210/jendso/bvac049

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 30 April 2024
DOI 10.3389/fendo.2024.1369582

OPEN ACCESS

EDITED BY
Henrik Falhammar,
Karolinska Institutet (KI), Sweden

REVIEWED BY
Susmeeta Tewari Sharma,
MedStar Washington Hospital Center, United States
Maniselvan Kuppusamy,
University of Virginia, United States
Juilee Rege,
University of Michigan, United States

*CORRESPONDENCE
Weiqing Wang
wqingw61@163.com

†These authors have contributed equally to this work

RECEIVED 12 January 2024
ACCEPTED 15 April 2024
PUBLISHED 30 April 2024

CITATION
Jiang Y, Zhou L, Zhang C, Su T, Jiang L, Zhou W, Zhong X, Wu L and Wang W (2024) The influence of cortisol co-secretion on clinical characteristics and postoperative outcomes in unilateral primary aldosteronism. *Front. Endocrinol.* 15:1369582. doi: 10.3389/fendo.2024.1369582

The influence of cortisol co-secretion on clinical characteristics and postoperative outcomes in unilateral primary aldosteronism

Yiran Jiang[1†], Lihua Zhou[1†], Cui Zhang[1†], Tingwei Su[1], Lei Jiang[1], Weiwei Zhou[1], Xu Zhong[1], Luming Wu[1] and Weiqing Wang[1,2*]

[1]Shanghai Key Laboratory for Endocrine Tumors, Shanghai Clinical Centre for Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China, [2]Laboratory for Endocrine and Metabolic diseases of Institute of Health Science, Shanghai Jiaotong University School of Medicine and Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Context: The prevalence of unilateral primary aldosteronism (UPA) with cortisol co-secretion varies geographically.

Objective: To investigate the prevalence and clinical characteristics of UPA with cortisol co-secretion in a Chinese population.

Design: Retrospective cohort study.

Methods: We recruited 580 patients with UPA who underwent cosyntropin stimulation test (CST) after the 1-mg dexamethasone suppression test (DST) and retrospectively analyzed the clinical characteristics and postoperative outcomes of UPA with and without cortisol co-secretion.

Results: UPA with cortisol co-secretion (1 mg DST>1.8 ug/dL) was identified in 65 of 580 (11.2%) patients. These patients were characterized by older age, longer duration of hypertension, higher concentration of plasma aldosterone and midnight cortisol, lower adrenocorticotropic hormone (ACTH) and dehydroepiandrosterone sulfate (DHEAS), larger tumor diameter, and more history of diabetes mellitus. Cortisol and aldosterone levels were higher and DHEAS level was lower in UPA with cortisol co-secretion at 0–120 min after CST. Among 342 UPA patients with *KCNJ5* gene sequencing and follow-up results, the complete clinical success rate was lower in UPA with cortisol co-secretion (33.3% vs. 56.4%, P<0.05); the complete biochemical success rate and *KCNJ5* mutation did not differ between the two groups. Age, tumor size, and ACTH were independent predictors of UPA with cortisol co-secretion. Sex, BMI, duration of hypertension, *KCNJ5* mutation, and cortisol co-secretion were independent predictors for complete clinical success in UPA after surgery.

Conclusions: UPA with cortisol co-secretion is not uncommon in China, but the clinical features were distinctly different from those without co-secretion. Cortisol co-secretion is an independent risk factor for incomplete clinical success after surgery in UPA.

KEYWORDS

primary aldosteronism, cortisol, complete clinical success, cosyntropin stimulation test, KCNJ5

Introduction

Primary aldosterone (PA), first reported in 1955, is a known cause for hypertension (1). PA is the most common form of secondary hypertension, accounting for approximately 5–15% of all hypertension cases (2). PA is divided into unilateral PA (UPA) and bilateral PA (BPA), with aldosterone-producing adenoma (APA) and bilateral adrenal hyperplasia (BAH) being the most common forms of PA. APA is mainly treated with unilateral adrenalectomy, while BAH is treated with mineralocorticoid receptor antagonist (MRA) (3). PA is associated with a greater risk of cardiovascular, cerebrovascular, renal, and metabolic disease than essential hypertension (4–8). In recent years, the reported prevalence of PA combined with cortisol co-secretion is about 10–30% in different regions (9–13). Elevated serum cortisol further increases the risk of cardiovascular disease, glucose tolerance/diabetes, and osteoporosis associated with elevated serum aldosterone (9, 14–17). Several studies have investigated certain clinical characteristics of PA combined with cortisol co-secretion. Owing to regional and demographic differences, there is no consensus on the clinical features of PA combined with cortisol co-secretion, but there seems to be an agreement on the association of larger tumor size with PA (11, 14, 18).

KCNJ5 is the most common gene mutation in PA, and the results of a recent study in China showed that *KCNJ5* was mutated in 70.7% of APA (19). The mutation status of *KCNJ5* in PA with and without cortisol co-secretion is controversial. A study showed that APA with cortisol co-secretion had a significantly lower rate of *KCNJ5* mutations than those without (10). However, a study from Japan showed that there was no difference in *KCNJ5* mutations between APA with cortisol co-secretion and those without (20). Adrenocorticotropic hormone (ACTH) regulates both aldosterone and cortisol secretion and can further promote aldosterone secretion in combination with ACTH receptor (MC2R) (21, 22).

The cosyntropin stimulation test (CST) was first reported in 1978 (23). Currently, it is mostly used to differentiate APA or UPA from bilateral PA (24, 25). The use of CST to differentiate between the subtypes of primary hyperaldosteronism has been proposed but not fully validated and is hence currently not used as standard practice in many countries including USA.

This study was designed to further explore the clinical characteristics and postoperative outcomes of UPA with cortisol co-secretion in China and examine, for the first time, its response to CST.

Materials and methods

Patients

We screened 960 consecutive patients with UPA who had showed lateralization on adrenal venous sampling (AVS) and underwent an adrenalectomy. Finally, we included 580 UPA patients who underwent a CST (50 IU) after 1-mg dexamethasone suppression test (DST). Of these, 373 had data of Sanger sequencing for *KCNJ5*, and 342 cases had follow-up data for at least 6 months after surgery. All patients were referred to Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine, from December 2010 to February 2022 (Figure 1). This study was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. All patients provided informed consent for participation.

Diagnostic criteria for PA and subtype identification

PA was diagnosed according to the 2008 and 2016 endocrine society clinical practice guidelines (26, 27). The aldosterone-to-renin ratio (ARR) was used as a screening indicator for PA. Patients with ARR>30[(ng/dl)/(ng/ml/h)] were further subjected to a saline infusion test (SIT). Patients were made to lie down for at least 2 h and then, 2 L 0.9% saline was slowly infused via the peripheral vein over 4 h. PA was diagnosed if the plasma aldosterone concentration (PAC) was >10 ng/dL after infusion.

Adrenal computed tomography (CT) and AVS were used to differentiate between UPA and BPA. Cannulation was considered successful if the ratio of cortisol (adrenal vein)/cortisol (peripheral vein) was >3 without cosyntropin stimulation. Cortisol-corrected aldosterone ratio (A/C) served to correct adrenal venous

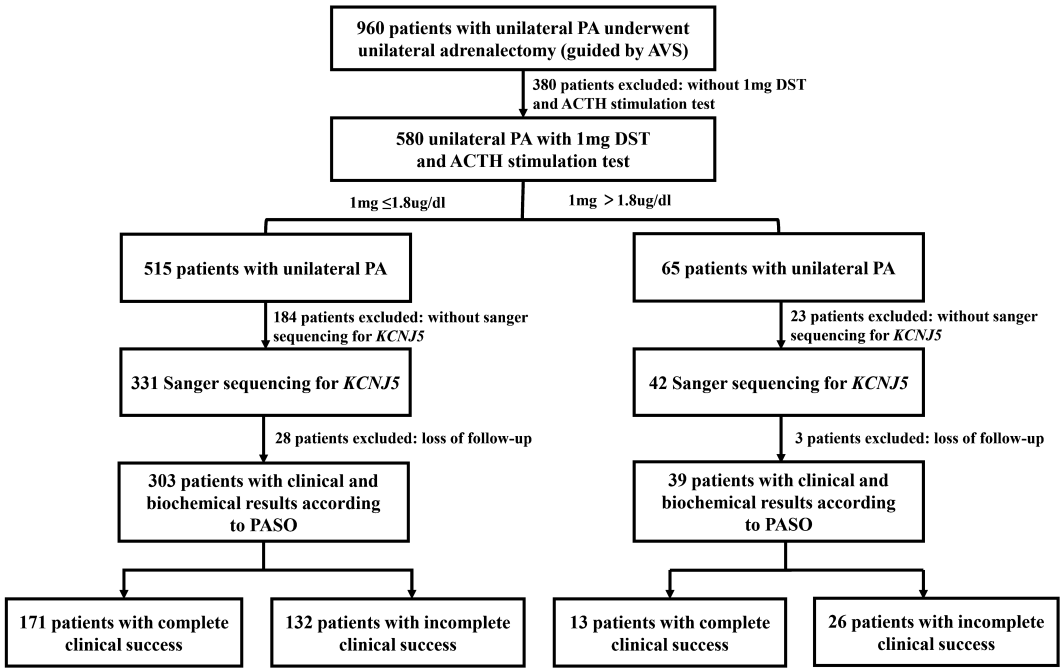


FIGURE 1
Flow chart.

aldosterone concentration for differing degrees of dilution of adrenal versus peripheral venous blood. Patients with $(A/C)_{\text{adrenal vein}}/(A/C)_{\text{contralateral adrenal vein}}>2$ were considered to have dominant secretion (26).

The primary aldosteronism surgical outcome (PASO) criteria (28) were used to assess outcomes after adrenalectomy for UPA. Complete clinical success in patients was defined normalized blood pressure and non-use of any antihypertensive medicine. Patients without hypokalemia (if present preoperatively) and normalized ARR were classified as having complete biochemical success.

Diagnostic criteria for PA with cortisol co-secretion

The diagnosis of PA with cortisol co-secretion is based on the 2016 European Guidelines for Unexpected Adrenal Tumors (29): (1) a confirmed PA diagnosis; (2) post-dexamethasone serum cortisol levels >1.8 ug/dL; (3) absence of typical overt Cushing's Syndrome (CS) features such as striae, skin atrophy, facial plethora, and central obesity; and (4) presence of an adrenal mass confirmed via CT before surgery, and a pathological diagnosis of the adrenal mass as an adrenal adenoma after surgery.

ACTH stimulation test under 1-mg DST

Patients received 1 mg dexamethasone orally at midnight. The following morning at 0800 h, 4 mL (50 IU) of cosyntropin (produced by Shanghai No.1 biochemical&pharmaceutical corporation) was injected slowly via a peripheral vein within 1 min, and then peripheral venous blood samples were collected every 30 min for 2 h for cortisol, aldosterone, and dehydroepiandrosterone sulfate (DHEAS) measurements.

Laboratory measurements

All tests were carried out in a College of American Pathologists (CAP)-accredited laboratory (CAP No. 7217913). Serum aldosterone and plasma renin activity were measured by radioimmunoassay (RIA) following the manufacturer's instructions (Beckman Coulter). The intra-assay and inter assay coefficients of variation were 9.3 and 9.5% for aldosterone and 10.1 and 10.2% for renin activity, respectively. The respective reference values were 3.81–31.33 ng/dL and 0.1–5.5 ng/mL/h. Serum cortisol and serum ACTH were measured by immunoluminescence and radio immunoassay (RIA) following the manufacturer's instructions (Beckman Coulter). The intra-assay and inter assay coefficients of variation were 6.7 and 7.9% for cortisol and 6.1 and 5.3% for ACTH, respectively. The respective reference values were 6.7–22.6 ug/dL and 12–78 pg/mL, respectively.

Molecular analysis

Genetic testing of adrenal tumors was performed in specimens from UPA patients who underwent unilateral adrenalectomy. The genomic DNA was prepared using the QIAGEN DNeasy tissue kit (Qiagen, Hilden, Germany). Polymerase chain reaction was performed

in a Dual 96-well GeneAmp PCR system 9700 (Applied Biosystems Courtaboeuf, France) using 20 ng of template DNA from each frozen sample per reaction. The products were sequenced on a 3730xl DNA analyzer (Applied Biosystems). All of the sequences were analyzed using sequencing analysis software version 5.2 (Applied Biosystems). The tumor samples were screened for mutations in the hotspot area of *KCNJ5*. The specific primer sequences are listed as follows:

KCNJ5-F: GGTGACCTGGACCATGTTGGCG

KCNJ5-R: CTTGGCAGGTCATGCCTGTGGC

Statistical analysis

Patients were categorized separately based on whether their serum cortisol level was >1.8 μg/dL after the 1-mg DST and whether they had complete clinical success after surgery. SPSS software ((version 26.0; IBM Corporation, Armonk, NY, USA) was used for statistical analyses. Normally distributed data are presented as means ± standard deviation (SD) and non-normally distributed data are expressed as medians (interquartile range: 25^{th}–75^{th} percentile). Categorical variables are presented as frequencies or percentages. The *t*-test and chi-square test were used for comparisons between two groups for continuous and categorical variables, respectively. The diagnostic value of PAC after ACTH stimulation for cortisol co-secretion in UPA was assessed based on receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC). Multivariable regression analysis (method: LR) was performed to investigate the factors influencing serum cortisol >1.8 ug/dL after 1-mg DST and complete clinical success after surgery. The ROC curves and line plots were plotted using MedCalc (15.2) and GraphPad (8.0), respectively. A *P* value of <0.05 was considered to indicate statistically significant differences.

Results

Baseline characteristics and demographics

Our study included a total of 580 patients diagnosed with UPA. Based on the serum cortisol level after 1-mg DST, there were 65 (11.2%) UPA cases with 1-mg DST>1.8 ug/dL and 515 (88.8%) UPA cases without 1-mg DST>1.8 ug/dL. The baseline characteristics between the two groups are presented in Table 1. Patients in the UPA with 1-mg DST>1.8 ug/dL group were older (52.8 ± 8.7 vs. 46.7 ± 11.2 years, P<0.001), had longer duration of hypertension (10.0 [4.5–15.5] vs. 6.0 [2.0–10.0] years, P<0.05) and a higher prevalence of diabetes mellitus (18.5% vs. 9.1%, P<0.05) than the UPA without 1-mg DST>1.8 ug/dL group. The UPA with 1-mg DST>1.8 ug/dL group had higher PAC (55.0 [34.6–90.6] vs. 43.4 [30.0–69.0] ng/dL, P<0.05); midnight cortisol (3.2 [2.3–4.9] vs. 2.1 [1.4–3.6] ug/dL, P<0.001); and serum cortisol after 1-mg DST (2.2 [2.0–3.3] vs. 1.0 [0.8–1.2] ug/dL, P<0.001). While, ACTH (27.2 ± 12.3 vs. 35.2 ± 22.7 pg/mL, P<0.05) and DHEAS 128.2 [74.6–190.4] vs. 182.6 [124.7–258.1] ug/dL, P<0.001] were lower in the UPA with 1-mg DST>1.8 ug/dL. However, there was no difference between the two groups with respect to systolic blood pressure (SBP), diastolic blood pressure (DBP), serum sodium, serum potassium, plasma renin activity (PRA), ARR, serum cortisol (0800 h and 1600 h) and 24-h urinary free cortisol (24h-UFC). The maximum diameter of the adrenal tumor was larger in UPA with 1-mg DST>1.8 ug/dL (1.7 [1.3–2.2] vs. 1.4 [1.1–1.7] cm, P<0.001).

TABLE 1 Baseline characteristics of UPA with different serum cortisol levels after the 1-mg DST.

Characteristics	UPA without 1-mg DST>1.8	UPA with 1-mg DST>1.8	*P*
Case number, *N* (%)	515 (88.8%)	65 (11.2%)	
Age (year)	46.7 ± 11.2	52.8 ± 8.7	<0.001
Male, *N* (%)	282 (54.8%)	32 (49.2%)	0.399
BMI (kg/m^2)	24.5 ± 3.7	24.0 ± 2.9	0.194
Duration of hypertension (year)	6.0 (2.0-10.0)	10.0 (4.5-15.5)	0.002
History of diabetes mellitus, *N* (%)	47 (9.1%)	12 (18.5%)	0.019
SBP (mmHg)	172.6 ± 22.6	174.2 ± 21.8	0.585
DBP (mmHg)	105.7 ± 15.8	104.7 ± 13.4	0.627
PAC (ng/dL)	43.4 (30.0-69.0)	55.0 (34.6-90.6)	0.016
PRA (ng/mL/h)	0.24 (0.08-0.59)	0.21 (0.10-0.48)	0.561
ARR [(ng/ml)/(ng/ml/h)]	192.3 (74.2-605.5)	241.7 (84.6-906.2)	0.213
Serum cortisol 0800 h (ug/dL)	11.5 (8.7-14.3)	11.6 (9.2-15.0)	0.490
Serum cortisol 1600 h (ug/dL)	5.5 (4.3-7.3)	6.0 (4.5-8.0)	0.192
Serum cortisol midnight (ug/dL)	2.1 (1.4-3.6)	3.2 (2.3-4.9)	<0.001
1mg DST (ug/dL)	1.0 (0.8-1.2)	2.2 (2.0-3.3)	<0.001
ACTH (pg/mL)	35.2 ± 22.7	27.2 ± 12.3	0.006
24h-UFC (ug/24 h)	75.3 (57.0-96.6)	77.7 (56.9-94.7)	0.933
DHEAS (ug/dL)	182.6 (124.7-258.1)	128.2 (74.6-190.4)	<0.001
Serum sodium (mmol/L)	143.3 ± 2.8	143.0 ± 3.3	0.479
Serum potassium (mmol/L)	3.0 ± 0.4	3.0 ± 0.4	0.774
Tumor size (cm)	1.4 (1.1-1.7)	1.7 (1.3-2.2)	<0.001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, aldosterone renin ratio; 1-mg DST, 1-mg dexamethasone suppression test; ACTH, adrenal corticotropic hormone; 24h-UFC, 24-h urinary free cortisol; DHEAS, dehydroepiandrosterone sulfate.

Response of UPA to CST at different serum cortisol levels

The patient's AVS parameters are shown in Supplementary Table S1. There was no difference in aldosterone, cortisol, and A/C between the dominant and nondominant adrenal veins in the two groups. There was no difference in selection index (SI) on the

10.3389/fendo.2024.1369582

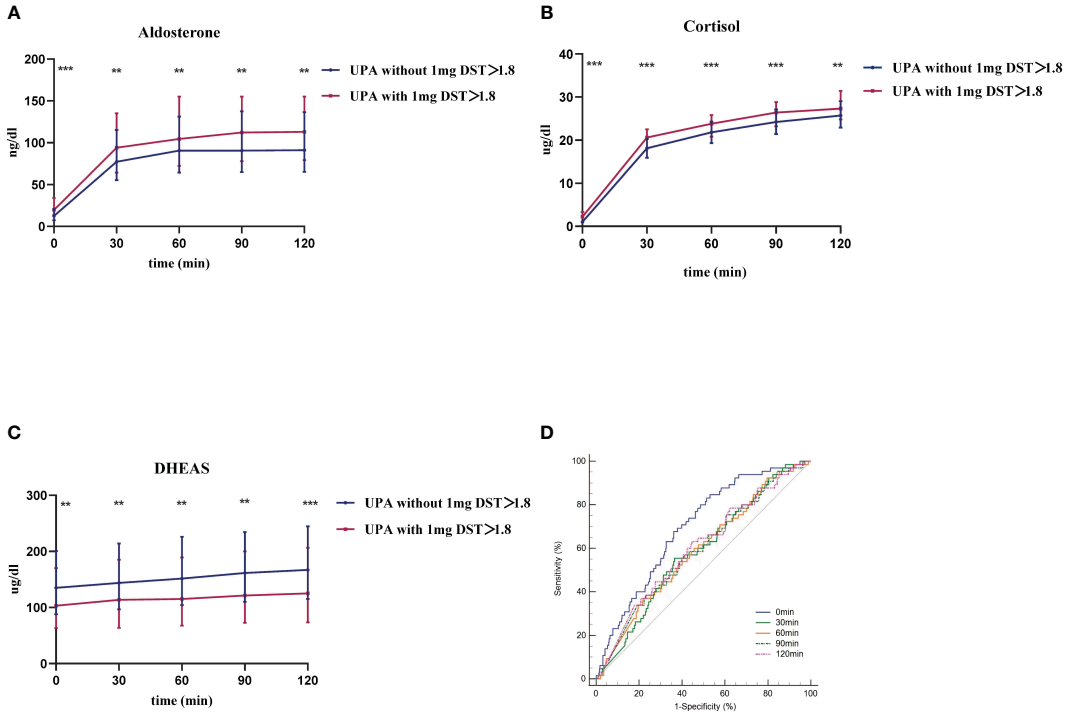


FIGURE 2
The response of UPA to cosyntropin stimulation test at different cortisol levels. **(A–C)** respective represent aldosterone, cortisol, and DHEAS of both groups after cosyntropin stimulation test under the influence of 1-mg dexamethasone suppression test. Data are shown as median with quartile range. **(D)** represents the ROC curve of aldosterone level at 0 min, 30 min, 60 min, 90 min, and 120 min after cosyntropin injection in the differential diagnosis between UPA with 1-mg DST>1.8 and those without. **P<0.05. ***P<0.001.

dominant side, but it was lower on the nondominant side. Furthermore, there was no difference in lateralization index (LI) and contralateral suppression index (CSI) in the two groups.

The changes in aldosterone, cortisol, and DHEAS levels after CST between the two groups are shown in Figure 2. Post cosyntropin administration, aldosterone, and cortisol were higher and DHEAS was lower in the UPA with 1-mg DST>1.8 ug/dL group (Figures 2A, B) than the UPA without 1-mg DST>1.8 group (Figure 2C) at each time point (P<0.05).

We first evaluated the diagnostic value of CST in distinguishing UPA with cortisol co-secretion and those without. Figure 2D shows the ROC curve of PAC at each point after cosyntropin injection for the differential diagnosis of UPA with or without cortisol co-secretion. The AUCs (95% confidence interval [CI]) of aldosterone at 0 min, 30 min, 60 min, 90 min, and 120 min were 0.696 (0.657–0.733), 0.587 (0.546–0.628), 0.591 (0.550–0.632), 0.594 (0.553–0.634), and 0.603 (0.562–0.643), respectively. Among these curves, aldosterone at 0 min was the best marker for the diagnosis of UPA with cortisol co-secretion. The optimal cut-off was 11.99 ng/dL, which displayed a sensitivity of 83.08% and specificity of 48.54%.

Comparison of preoperative and postoperative parameters for UPA

We analyzed the postoperative parameters in 342 UPA with *KCNJ5* gene sequencing (Table 2), and a comparison of their baseline characteristics are presented in Supplementary Table S2. After surgery, SBP, DBP, PAC, and ARR were significantly decreased and PRA was elevated in both groups (P<0.001). Postoperative ACTH increased significantly in both groups (P<0.001), and DHEAS did not change significantly, but DHEAS remained lower in the UPA with 1-mg DST>1.8 ug/dL group. After surgery, serum sodium was significantly decreased and serum potassium was obviously increased in both groups. Complete clinical success rate was higher in the group of UPA without 1-mg DST>1.8 ug/dL after surgery (56.4% vs. 33.3%, P<0.05), but there was no difference in complete biochemical success between the two groups. The total mutation rate of *KCNJ5* was 71.6% (245/342), but there was no significant difference between the two groups (71.3% vs. 74.4%, P>0.05).

Factors influencing UPA with 1-mg DST>1.8 ug/dL

We performed a binary logistic regression analysis of the factors associated with 1-mg DST>1.8 ug/dL (Table 3). Univariate regression analysis revealed that age (1.075 [1.036–1.115], P<0.001); duration of hypertension (1.069 [1.025–1.115], P<0.05); tumor size (3.921 [2.180–7.054], P<0.001); ACTH (0.971 [0.945–0.998], P<0.05); and DHEAS (0.995 [0.991–0.999], P<0.05) were significantly associated with UPA with 1-mg DST>1.8 ug/dL. Further multiple regression analysis revealed that age (1.094

TABLE 2 Comparison of post-operative parameters between the two groups.

	UPA without 1-mg DST>1.8 (N=303)			UPA with 1-mg DST>1.8 (N=39)			
	Baseline	Follow-up	P^1	Baseline	Follow-up	P^1	P^2
SBP (mmHg)	171.5 ± 22.7	130.0 ± 15.0	<0.001	170.0 ± 20.4	136.0 ± 16.7	<0.001	0.022
DBP (mmHg)	105.5 ± 14.6	82.4 ± 9.8	<0.001	104.7 ± 13.7	83.0 ± 10.3	<0.001	0.726
PAC (ng/dL)	42.1 (29.8-68.3)	8.9 (5.3-12.6)	<0.001	54.5 (33.5-95.6)	9.6 (5.3-12.2)	<0.001	0.913
PRA (mg/dL/h)	0.3 (0.1-0.6)	1.8 (0.9-3.0)	<0.001	0.2 (0.1-0.5)	1.8 (1.0-2.9)	<0.001	0.975
ARR [(ng/dL)/(mg/dL/h)]	173.5 (68.8-521.7)	5.7 (3.2-10.5)	<0.001	207.4 (82.1-705.9)	5.2 (3.8-11.2)	<0.001	0.917
Serum cortisol 0800 h (ug/dL)	11.3 (8.7-14.2)	11.7 (9.4-13.9)	0.313	12.7 (9.8-16.0)	11.8 (9.5-14.6)	0.617	0.645
Serum cortisol 1600 h (ug/dL)	5.5 (4.3-7.2)	6.3 (5.0-8.0)	0.005	6.4 (4.5-8.5)	6.6 (4.8-8.3)	0.880	0.956
Serum cortisol midnight (ug/dL)	2.1 (1.4-3.5)	2.2 (1.3-3.7)	0.572	3.1 (2.3-4.9)	2.3 (1.6-5.6)	0.099	0.251
ACTH (pg/mL)	33.7 ± 17.9	52.0 ± 29.3	<0.001	27.6 ± 11.4	44.5 ± 19.4	<0.001	0.184
DHEAS (ug/dL)	179.6 (126.5-256.4)	178.2 (114.4-243.9)	0.314	137.0 (77.6-207.4)	121.5 (78.9-186.8)	0.617	0.040
Serum sodium (mmol/L)	143.3 ± 3.0	140.5 ± 2.1	<0.001	143.0 ± 3.3	140.0 ± 1.9	<0.001	0.318
Serum potassium (mmol/L)	3.0 ± 0.4	4.2 ± 0.4	<0.001	3.0 ± 0.3	4.2 ± 0.4	<0.001	0.536
Complete clinical success, *N* (%)		171/303 (56.4%)			13/39 (33.3%)		0.006
Complete biochemistry success, *N* (%)		296/303 (97.7%)			38/39 (97.4%)		1.000
KCNJ5, *N* (%)		216/303 (71.3%)			29/39 (74.4%)		0.689

[1]Comparison between baseline and follow up data within each group[2]. Comparison of follow-up information between the two groups. SBP, systolic blood pressure; DBP, diastolic blood pressure; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, aldosterone renin ratio; ACTH, adrenal corticotropic hormone; DHEAS, dehydroepiandrosterone sulfate.

[1.049–1.141], P<0.001) and tumor size (4.508 [2.370–8.576], P<0.001) were independent risk factors for 1-mg DST>1.8 ug/dL, and ACTH (0.967 [0.938–0.997], P<0.05) was a protective factor for it. Our further analysis of age, tumor sizes, and duration of hypertension revealed that the UPA with 1-mg DST>1.8 ug/dL group was characterized by the following clinical features (Figure 3): age>50 years (66.7%), duration of hypertension>10 years (56.4%), and maximum tumor diameter>1.5 cm (64.1%).

Factors influencing complete clinical success of UPA after surgery

We analyzed the factors associated with complete clinical success after surgery in UPA (Table 4). Univariate logistic regression analysis revealed that age (1.060 [1.037–1.083], P<0.001); male sex (3.591 [2.287–5.639], P<0.001); BMI (1.249 [1.160–1.344], P<0.001); duration of hypertension (1.120 [1.079–1.163], P<0.001); renin activity (1.712 [1.141–2.569], P<0.05); 1-mg DST>1.8 ug/dL (2.591 [1.282–5.235], P<0.05); and *KCNJ5* mutation (0.342 [0.209–0.558], P<0.001) were significantly association with complete clinical success. Tumor size was not associated with complete clinical success (P>0.05). Multiple regression analysis showed that male sex, higher BMI, longer duration of hypertension, cortisol co-secretion (1-mg DST>1.8 μg/dL), and absence of *KCNJ5* mutation were independent predictors for complete clinical success.

Discussion

An epidemiological survey showed that the prevalence of PA in patients with newly diagnosed hypertension in China was at least 4% (30). According to European guidelines (29), we chose 1.8 as the cortisol cut-off point after 1-mg DST and divided the patients into two groups. In this study, the prevalence of UPA with cortisol co-secretion was 11.20%, which is similar to reports from Greece (31), but lower than that in Japan (12, 18) and Germany (17). In a study with 82 PA subjects in Taiwan, the prevalence of APA with 1-mg DST>1.8 ug/dL was 26.8% (10). Differences in prevalence may be related to ethnicity and geography. Our study suggests that while the prevalence of UPA with cortisol co-secretion is not low in China, this particular subtype of PA requires further research attention.

Our study found that UPA patients with cortisol co-secretion were older, had a longer duration of hypertension, and a higher prevalence of diabetes; these characteristics were similar to some of the previous findings (9, 11). In our study, UPA with cortisol co-secretion had a higher PAC, while PRA and ARR did not show significant differences with UPA without cortisol co-secretion. A study by Tsai et al. (9) also suggested that PA with cortisol co-secretion had higher aldosterone concentrations, while the results from O'Toole (13) and Peng (10) did not support this view. Taken together, whether PA with cortisol co-secretion is accompanied by higher aldosterone levels remains a controversial issue. Serum DHEAS concentration was lower in the UPA with cortisol co-secretion group, which was associated with high serum cortisol concentrations suppressing ACTH in our group. Our study found

TABLE 3 Factors associated with 1-mg DST>1.8 ug/dL in 342 UPA.

	Univariate		Multivariate	
	Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)	*P*
Age (year)	1.075 (1.036-1.115)	<0.001	1.094 (1.049-1.141)	<0.001
Male (yes)	0.998 (0.511-1.948)	0.995		
BMI (kg/m^2)	0.958 (0.870-1.055)	0.381		
Duration of hypertension (year)	1.069 (1.025-1.115)	0.002		
PAC (ng/dL)	1.000 (1.000-1.001)	0.155		
PRA (mg/dL/h)	1.092 (0.646-1.846)	0.742		
Serum cortisol 0800 h (ug/dL)	1.062 (0.982-1.148)	0.131		
Serum cortisol 1600 h (ug/dL)	1.099 (0.984-1.228)	0.094		
Serum cortisol midnight (ug/dL)	1.056 (0.979-1.138)	0.158		
ACTH (pg/mL)	0.971 (0.945-0.998)	0.036	0.967 (0.938-0.997)	0.032
Serum potassium (mmol/L)	0.682 (0.285-1.632)	0.390		
Serum sodium (mmol/L)	0.961 (0.855-1.079)	0.499		
DHEAS (ug/dL)	0.995 (0.991-0.999)	0.020		
Tumor size (cm)	3.921 (2.180-7.054)	<0.001	4.508 (2.370-8.576)	<0.001
KCNJ5 mutation, yes	1.463 (0.688-3.110)	0.323		

BMI, body mass index; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ACTH, adrenal corticotropic hormone; DHEAS, dehydroepiandrosterone sulfate.

that UPA with cortisol co-secretion had larger adrenal tumors than those without cortisol co-secretion in China. Some studies have come to a similar conclusion. Tang et al. (14) found that the tumor sizes of APA with cortisol co-secretion were 6 mm larger than those of pure APA (24.50 ± 11.34 vs. 18.92 ± 7.98 mm, P<0.05). This was also consistent with Yasuda et al's study (11), in that PA patients with cortisol co-secretion had a larger tumor than those without cortisol co-secretion (2.7 ± 0.1 vs. 1.4 ± 0.1 cm, P<0.05).

Aldosterone is produced by the adrenal zona glomerulosa, which is regulated by ACTH, serum potassium, and angiotensin II (32). We found that after cosyntropin was administered per peripheral IV, aldosterone and cortisol rose more significantly in UPA with cortisol co-secretion, while DHEAS levels were lower in this group. This suggests that ACTH promotes the production of both aldosterone and cortisol from the adrenocortical globus and fasciculus, and that this effect was more pronounced in patients with UPA with cortisol co-secretion. A study by St. Jean et al. (33) suggested that ACTH only promotes the production of aldosterone in PA without cortisol co-secretion, while it promotes both aldosterone and cortisol secretion in PA with cortisol co-secretion. We speculated that PA patients with cortisol co-secretion have a higher number of ACTH receptors or a higher sensitivity of ACTH receptors than those with PA without cortisol co-secretion, which is why the former can produce more aldosterone and cortisol. In addition, we believe this is the first study to discuss whether CST can be used to distinguish between UPA with and without cortisol co-secretion. Our results showed that CST was not an effective differentiating tool in this regard. Although our study did not find an association between *KCNJ5* and cortisol co-secretion, some studies have found an association. Inoue et al. (34) found that *KCNJ5*-muted-APA had lower aldosterone concentration than *KCNJ5*-wild-APA after dexamethasone, suggesting that *KCNJ5*-muted-APA is more responsive to endogenous ACTH and that the ACTH pathway may be more sensitive and easily activated. The cause of PA with cortisol co-secretion is controversial. Our results revealed that age, tumor size, and ACTH were significantly correlated with PA with cortisol co-secretion. This is also similar to the study of Peng et al. (10), who concluded that tumor size was positively correlated with PA with cortisol co-secretion.

In our study, UPA patients with cortisol co-secretion had a lower complete clinical success rate than those without cortisol co-

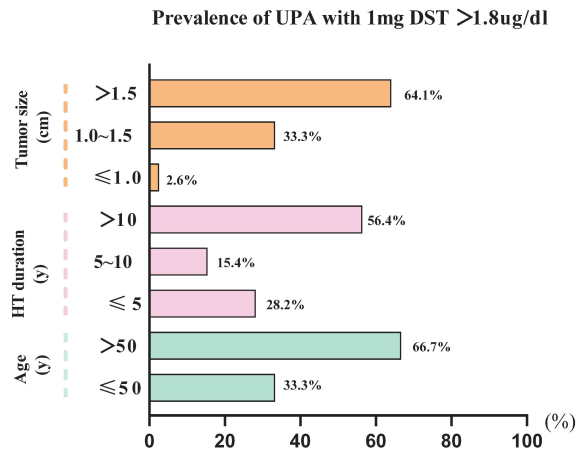


FIGURE 3
The characteristics of UPA with 1-mg DST>1.8 ug/dL. Data are expressed as a percentage.

TABLE 4 Factors associated with complete clinical success in 342 UPA.

	Univariate		Multivariate	
	Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)	*P*
Age (year)	1.060 (1.037-1.083)	<0.001		
Male, yes	3.591 (2.287-5.639)	<0.001	2.496 (1.434-4.355)	0.001
BMI (kg/m^2)	1.249 (1.160-1.344)	<0.001	1.169 (1.076-1.271)	<0.001
Duration of hypertension (year)	1.120 (1.079-1.163)	<0.001	1.116 (1.070-1.164)	<0.001
PAC (ng/dL)	1.000 (1.000-1.001)	0.601		
PRA (mg/dL/h)	1.712 (1.141-2.569)	0.009		
Serum cortisol 0800 h (ug/dL)	1.036 (0.982-1.092)	0.195		
ACTH (pg/mL)	1.003 (0.991-1.015)	0.666		
1-mg DST>1.8, yes	2.591 (1.282-5.235)	0.008	2.461 (1.094-5.535)	0.029
Tumor size (cm)	0.854 (0.564-1.294)	0.457		
KCNJ5 mutation, yes	0.342 (0.209-0.558)	<0.001	0.516 (0.294-0.906)	0.021
DHEAS (ug/dL)	1.000 (0.998-1.002)	0.977		
Serum potassium (mmol/L)	1.066 (0.630-1.803)	0.811		
Serum sodium (mmol/L)	0.992 (0.924-1.065)	0.992		

BMI, body mass index; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ACTH, adrenal corticotropic hormone; 1mg DST, 1-mg dexamethasone suppression test; DHEAS, dehydroepiandrosterone sulfate.

secretion after surgery. The results were similar to the findings of a Taiwanese study (10), although its findings were not significant (62.5% vs. 38.5%, P>0.05). This study reveals that the duration of hypertension and cortisol co-secretion were independent risk factors for complete clinical remission in UPA after surgery, while *KCNJ5* mutation was a protective factor. This finding is consistent with the findings of Peng et al. (10) However, Peng et al. also revealed that the *KCNJ5* mutation was negatively associated with cortisol co-secretion (OR=0.23, 95%CI: 0.06–0.83, P=0.024). This could be because their sample size was smaller than ours. In addition, the total mutation rate of *KCNJ5* (71.6%) in this study was consistent with previous findings from our center (19), and UPA with and without cortisol co-secretion had similar *KCNJ5* mutation rates, which is consistent with a study from Japan (20).

Limitations

First, we did not discuss the characteristics of immunohisto chemistry (CYP11B1 and CYP11B2) in UPA with cortisol co-secretion. Second, some relevant genetic mutations such as *ATP1A1*, *ATP2B3*, *CTNNB1*, *CACNA1D*, and *PRKACA* were not studied. Third, our study was limited to the clinical features and prognosis of UPA with cortisol co-secretion, and did not explore the underlying mechanisms. Last, the subjects in our study did not undergo the 1-mg DST and AST after surgery. Therefore, more studies are needed to explore and summarize the characteristics and mechanisms of PA with cortisol co-secretion.

Conclusions

In China, there is a high prevalence of UPA with cortisol co-secretion, and their clinical features are distinctly different from those without cortisol co-secretion. UPA patients with cortisol co-secretion are more responsive to ACTH than those without. Cortisol co-secretion is associated with a decreased chance of complete clinical success in UPA after surgery. The 1-mg DST should be routinely performed in the daily practice of PA to detect cortisol co-secretion early.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

YJ: Writing – original draft, Writing – review & editing. LZ: Data curation, Formal analysis, Funding acquisition, Methodology, Software, Writing – original draft, Writing – review & editing. CZ: Methodology, Project administration, Resources, Software, Writing – review & editing. TS: Conceptualization, Data curation, Formal analysis, Writing – review & editing. LJ: Data curation, Formal analysis, Investigation, Writing – review & editing. WZ: Resources, Software, Writing – review & editing. XZ: Resources, Software, Supervision, Writing – review & editing. LW: Formal analysis, Resources, Software, Writing – review & editing. WW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Key Research and Development Program of China (2021YFC2501600, 2021YFC2501603); the Shanghai Shenkang Hospital Development Center (SHDC 2020CR2002A, SHDC2020CR6015); Natural Science Foundation of Shanghai (22ZR1439100); and National Natural Science Foundation of China (82170797).

Acknowledgments

We are grateful to Prof. Guang Ning, MD, for his critical revision and input on the study design.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1369582/full#supplementary-material

References

1. Conn JW. Primary aldosteronism. *J Lab Clin Med*. (1955) 45:661–4.

2. Mulatero P, Stowasser M, Loh KC, Fardella CE, Gordon RD, Mosso L. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. *J Clin Endocrinol Metab*. (2004) 89:1045–50. doi: 10.1210/jc.2003-031337

3. Reincke M, Bancos I, Mulatero P, Scholl UI, Stowasser M, Williams TA. Diagnosis and treatment of primary aldosteronism. *Lancet Diabetes Endocrinol*. (2021) 9:876–92. doi: 10.1016/S2213-8587(21)00210-2

4. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol*. (2005) 45:1243–8. doi: 10.1016/j.jacc.2005.01.015

5. Rossi G, Boscaro M, Ronconi V, Funder JW. Aldosterone as a cardiovascular risk factor. *Trends Endocrinol Metab*. (2005) 16:104–7. doi: 10.1016/j.tem.2005.02.010

6. Born-Frontsberg E, Reincke M, Rump LC, Hahner S, Diederich S, Lorenz R, et al. Cardiovascular and cerebrovascular comorbidities of hypokalemic and normokalemic primary aldosteronism: results of the German Conn's Registry. *J Clin Endocrinol Metab*. (2009) 94:1125–30. doi: 10.1210/jc.2008-2116

7. Quinkler M, Born-Frontsberg E, Fourkiotis VG. Comorbidities in primary aldosteronism. *Horm Metab Res*. (2010) 42:429–34. doi: 10.1055/s-0029-1243257

8. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol*. (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4

9. Tsai CH, Liao CW, Wu XM, Chen ZW, Pan CT, Chang YY, et al. Autonomous cortisol secretion is associated with worse arterial stiffness and vascular fibrosis in primary aldosteronism: a cross-sectional study with follow-up data. *Eur J Endocrinol*. (2022) 187:197–208. doi: 10.1530/EJE-21-1157

10. Peng KY, Liao HW, Chan CK, Lin WC, Yang SY, Tsai Y-C, et al. Presence of subclinical hypercortisolism in clinical aldosterone-producing adenomas predicts lower clinical success. *Hypertension*. (2020) 76:1537–44. doi: 10.1161/HYPERTENSIONAHA.120.15328

11. Yasuda S, Hikima Y, Kabeya Y, lida S, Oikawa Y, Isshiki M, et al. Clinical characterization of patients with primary aldosteronism plus subclinical Cushing's syndrome. *BMC Endocr Disord*. (2020) 20:9. doi: 10.1186/s12902-020-0490-0

12. Nakajima Y, Yamada M, Taguchi R, Satoh T, Hashimoto K, Ozawa A, et al. Cardiovascular complications of patients with aldosteronism associated with autonomous cortisol secretion. *J Clin Endocrinol Metab*. (2011) 96:2512—2518. doi: 10.1210/jc.2010-2743

13. O'Toole SM, Sze WC, Chung TT, Akker SA, Druce MR, Waterhouse M, et al. Low-grade cortisol cosecretion has limited impact on ACTH-stimulated AVS parameters in primary aldosteronism. *J Clin Endocrinol Metab*. (2020) 105(10). doi: 10.1210/clinem/dgaa519

14. Tang L, Li X, Wang B, Ma X, Li H, Gao Y, et al. Clinical characteristics of aldosterone- and cortisol-coproducing adrenal adenoma in primary aldosteronism. *Int J Endocrinol*. (2018) 2018:4920841. doi: 10.1155/2018/4920841

15. Arlt W, Lang K, Sitch AJ, Dietz AS, Rhayem Y, Bancos I, et al. Steroid metabolome analysis reveals prevalent glucocorticoid excess in primary aldosteronism. *JCI Insight*. (2017) 2(8):e93136. doi: 10.1172/jci.insight.93136

16. Adolf C, Köhler A, Franke A, Lang K, Riester A, Löw A, et al. Cortisol excess in patients with primary aldosteronism impacts left ventricular hypertrophy. *J Clin Endocrinol Metab*. (2018) 103:4543–52. doi: 10.1210/jc.2018-00617

17. Gerards J, Heinrich DA, Adolf C, Meisinger C, Rathmann W, Sturm L, et al. Impaired glucose metabolism in primary aldosteronism is associated with cortisol cosecretion. *J Clin Endocrinol Metab*. (2019) 104:3192–202. doi: 10.1210/jc.2019-00299

18. Hiraishi K, Yoshimoto T, Tsuchiya K, Minami I, Doi M, Izumiyama H, et al. Clinicopathological features of primary aldosteronism associated with subclinical Cushing's syndrome. *Endocr J*. (2011) 58:543–51. doi: 10.1507/endocrj.K10E-402

19. Zhang C, Wu L, Jiang L, Su T, Zhou W, Zhong X, et al. KCNJ5 mutation contributes to complete clinical success in aldosterone-producing adenoma: a study from a Single Center. *Endocr Pract*. (2021) 27:736–42. doi: 10.1016/j.eprac.2021.01.007

20. Kometani M, Yoneda T, Demura M, Aono D, Gondoh Y, Karashima S, et al. Genetic and epigenetic analyses of aldosterone-producing adenoma with hypercortisolemia. *Steroids*. (2019) 151:108470. doi: 10.1016/j.steroids.2019.108470

21. Funder JW. The potential of ACTH in the genesis of primary aldosteronism. *Front Endocrinol (Lausanne)*. (2016) 7:40. doi: 10.3389/fendo.2016.00040

22. Young M, Funder JW. Eplerenone, but not steroid withdrawal, reverses cardiac fibrosis in deoxycorticosterone/salt-treated rats. *Endocrinology*. (2004) 145:3153–7. doi: 10.1210/en.2004-0005

23. Kem DC, Weinberger MH, Higgins JR, Kramer NJ, Gomez-Sanchez C, Holland OB. Plasma aldosterone response to ACTH in primary aldosteronism and in patients with low renin hypertension. *J Clin Endocrinol Metab*. (1978) 46:552–60. doi: 10.1210/jcem-46-4-552

24. Sonoyama T, Sone M, Miyashita K, Tamura N, Yamahara K, Park K, et al. Significance of adrenocorticotropin stimulation test in the diagnosis of an aldosterone-producing adenoma. *J Clin Endocrinol Metab*. (2011) 96:2771–8. doi: 10.1210/jc.2011-0573

25. Jiang Y, Zhang C, Wang W, Su T, Zhou W, Jiang L, et al. Diagnostic value of ACTH stimulation test in determining the subtypes of primary aldosteronism. *J Clin Endocrinol Metab*. (2015) 100:1837–44. doi: 10.1210/jc.2014-3551

26. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasse M, et al. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. (2008) 93:3266–81. doi: 10.1210/jc.2008-0104

27. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: case detection, diagnosis, and treatment: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. (2016) 101:1889–916. doi: 10.1210/jc.2015-4061

28. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, et al. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol*. (2017) 5:689–99. doi: 10.1016/S2213-8587(17)30135-3

29. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, et al. Management of adrenal incidentalomas: European society of endocrinology clinical practice guideline in collaboration with the European network for the study of adrenal tumors. *Eur J Endocrinol*. (2016) 175:G1–G34. doi: 10.1530/EJE-16-0467

30. Xu Z, Yang J, Hu J, Song Y, He W, Luo T. Primary aldosteronism in patients in China with recently detected hypertension. *J Am Coll Cardiol*. (2020) 75:1913–22. doi: 10.1016/j.jacc.2020.02.052

31. Piaditis GP, Kaltsas GA, Androulakis II, Gouli A, Makras P, Papadogias D, et al. High prevalence of autonomous cortisol and aldosterone secretion from adrenal adenomas. *Clin Endocrinol (Oxf)*. (2009) 71:772–8. doi: 10.1111/j.1365-2265.2009.03551.x

32. Young MJ, Funder JW. The renin-angiotensin-aldosterone system in experimental mineralocorticoid-salt-induced cardiac fibrosis. *Am J Physiol*. (1996) 271:E883–8. doi: 10.1152/ajpendo.1996.271.5.E883

33. St-Jean M, Bourdeau I, Martin M, Lacroix A. Aldosterone is aberrantly regulated by various stimuli in a high proportion of patients with primary aldosteronism. *J Clin Endocrinol Metab*. (2021) 106:e45–60. doi: 10.1210/clinem/dgaa703

34. Inoue K, Yamazaki Y, Kitamoto T, Hirose R, Saito J, Omura M, et al. Aldosterone suppression by dexamethasone in patients with KCNJ5-mutated aldosterone-producing adenoma. *J Clin Endocrinol Metab*. (2018) 103:3477–85. doi: 10.1210/jc.2018-00738

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 20 June 2024
DOI 10.3389/fendo.2024.1416287

OPEN ACCESS

EDITED BY
Carl D. Malchoff,
University of Connecticut Health Center, United States

REVIEWED BY
Francesca Pizzolo,
University of Verona, Italy
Juilee Rege,
University of Michigan, United States

*CORRESPONDENCE
Wei Song
songwei8124@163.com
Deyong Yang
yangdeyong@dmu.edu.cn
Xinqing Zhu
252555508@qq.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 12 April 2024
ACCEPTED 06 June 2024
PUBLISHED 20 June 2024

CITATION
Xiang H, Zhang T, Song W, Yang D and Zhu X (2024) Adrenalectomy for primary aldosteronism and its related surgical characteristics.
Front. Endocrinol. 15:1416287.
doi: 10.3389/fendo.2024.1416287

Adrenalectomy for primary aldosteronism and its related surgical characteristics

Hao Xiang[1†], Tingting Zhang[2†], Wei Song[3*], Deyong Yang[1,4*] and Xinqing Zhu[1*]

[1]Department of Urology, First Affiliated Hospital of Dalian Medical University, Dalian, China,
[2]Department of Neurology, First Affiliated Hospital of Dalian Medical University, Dalian, China,
[3]Department of Hypertension, First Affiliated Hospital of Dalian Medical University, Dalian, China,
[4]Department of Surgery, Healinghands Clinic, Dalian, Liaoning, China

Primary aldosteronism (PA) is a common cause of secondary hypertension. Adrenalectomy is an effective treatment for unilateral PA, particularly aldosterone-producing adenoma (APA), resulting in improvements in biochemical parameters and blood pressure in the vast majority of patients. The article provides a comprehensive overview of PA, focusing on the outcomes of adrenalectomy for PA and the factors that may suggest prognostic implications. Analysis of the outcome of different PA patients undergoing adrenalectomy in terms of preoperative factors, vascular and adipose conditions, type of pathology, and somatic variants. In addition, it is recommended to use the histopathology of primary aldosteronism (HISTALDO) consensus to classify the patient's pathological type, with classical and nonclassical pathological types showing a different prognosis and possibly being associated with an unresected contralateral adrenal gland. The primary aldosteronism surgical outcome (PASO) consensus sets uniform standards for postoperative outcomes in unilateral PA, but its setting of thresholds remains controversial. Partial adrenalectomy shows similar surgical results and fewer postoperative complications than total adrenalectomy, but there is a risk of missing the true source of abnormal aldosterone secretion. Steroid profiling and functional imaging techniques offer alternative options to adrenal vein sampling (AVS) for unilateral and bilateral judgments in patients with PA. A combination of factors is needed to predict the prognosis of PA patients undergoing adrenalectomy in order to manage patient expectations of the outcome of the procedure and to closely monitor blood pressure and biochemical parameters in patients who suggest a poorer prognosis.

KEYWORDS

adrenalectomy, primary hyperaldosteronism, hypertension, prognosis, CYP11B2

1 Introduction

Primary aldosteronism (PA) was first described by Jerome W. Conn in 1954 and is characterized by hypokalemia and excessive production of aldosterone independent of the renin-angiotensin system (1). PA is the most common endocrine form of secondary hypertension. It has a prevalence of 5% to 15% in the general hypertensive population and up to 20% in those with severe or refractory hypertension (2, 3). Of these, approximately 27% have aldosterone-producing adenoma (APA) and 64% have bilateral adrenal hyperplasia (BAH) (4). The APA recommends unilateral adrenalectomy, while patients with BAH are treated with mineralocorticoid receptor antagonists.

2 Pathogenesis

Aldosterone is synthesized from cholesterol in the zona glomerulosa of the adrenal cortex by a variety of enzymes, the key step in the synthesis being aldosterone synthase (CYP11B2). PA mainly increases the gene transcription of CYP11B2 through various pathogenesis, and ultimately leads to increased aldosterone synthesis and cell proliferation. Based on the effect of gene mutate, somatic mutations are broadly categorized into three types: Ion Channels, Ion Transporters, and Cell Signaling Systems, which are discussed separately.

2.1 Ion channels (KCNJ5, CACNA1D, CACNA1H, CLCN2, SLC30A1)

KCNJ5 encodes a potassium inwardly rectifying channel (GIRK4), and mutations in KCNJ5 lead to channel selectivity changes that increase intracellular sodium influx, leading to cell depolarization (5). CACNA1D codes calcium voltage-gated channel subunit alpha1 D, CACNA1H codes calcium voltage-gated channel subunit alpha1 H, mutations in these genes lead to enhanced function of calcium channels and increased intracellular calcium concentration (6, 7). CLCN2 encodes chloride voltage-gated channel 2. The mutation resulted in enhanced chloride channel function and increased chloride ion permeability and depolarization (8). Rege, et al. (9) recently identified somatic mutations in the SLC30A1 gene in APAs. The SLC30A1 gene encodes the zinc transporter protein ZnT1. Mutations in SLC30A1 can potentially cause alterations in the cell membrane potential, which may impact the activity of voltage-gated calcium channels and consequently affect the influx of calcium ions and the regulation of intracellular calcium levels.

2.2 Ion transporters (ATP1A1, ATP2B3)

ATP1A1 encodes ATPase Na^+/K^+ transporting subunit alpha 1. The ATP1A1 mutate found in APAs leads to impaired potassium ion affinity and ATPase activity, leading to membrane depolarization. ATP2B encodes the ATPase plasma membrane Ca^{2+} transporting 3, which is responsible for pumping calcium ions inside the cell to the outside. Mutations in ATP2B3 affect the binding and transport of calcium ions, leading to the accumulation of calcium ions within the cell (10). Mutations in genes encoding ion channels or transporters ultimately result in an increase in intracellular Ca^{2+} concentration, activating a phosphorylation cascade that leads to increased aldosterone synthesis (11).

2.3 Cell signaling systems (GNAS, GNAQ, GNA11, PRKACA, CTNNB1, CADM1)

GNAS, GNAQ, and GNA1 encode G protein alpha subunits, and mutations in them can lead to abnormal activation of G protein signaling (12). PRKACA encodes protein kinase cAMP-activated catalytic subunit alpha. PRKACA mutates found in APAs lead to persistent activation of the cAMP/PKA signaling pathway, resulting in dysregulation of cell proliferation (13). Somatic mutate of the *CTNNB1* gene, which encodes a β-catenin, have been identified in APA, and the affected WNT/β-catenin signaling pathway is essential for the regulation of proliferation, differentiation and tumorigenesis in the adrenal cortex (14). However, the potential mechanism by which mutations in the *CTNNB1* gene lead to aldosterone overproduction is unclear.

In addition, somatic mutation of CADM1 was recently discovered in APAs, which is a synaptic cell adhesion molecule mainly expressed in the nervous system. The mutation of CADM1 leads to significant upregulation of CYP11B2 expression. This upregulation is associated with inhibition of intercellular communication, particularly by inhibiting communication at the gap junction (GJ) (15).

3 Diagnosis

The prevalence of hypertension combined with atrial fibrillation or diabetes mellitus was reported to be significantly higher in patients with PA than in those with essential hypertension (EHT) (16). Patients with PA also had a higher incidence of stroke than patients with EHT (12.9% v. s. 3.4%; 95% CI 2.0 to 8.6) (17). In addition, PA can lead to an increased risk of renal dysfunction and metabolic syndrome (18). Because even without considering the effect on blood pressure, aldosterone itself promotes cardiac and vascular fibrosis and tissue damage, leading to an increased incidence of cardiovascular and cerebrovascular events (11). The higher prevalence of diabetes in PA patients is mainly associated with subclinical hypercortisolism (SH) (19). Adequate treatment of PA can significantly reduce morbidity and mortality by reducing increased aldosterone and relieving renin suppression and hypertension (2). Early diagnosis and appropriate treatment of PA are therefore essential to reduce the increased risk associated with the disease.

The diagnosis of PA involves three stages: screening tests, case confirmation and classification of PA subtypes (20). Screening tests:

Plasma aldosterone renin ratio (ARR), derived from measurement of plasma aldosterone concentrations (PACs) and plasma renin activity (PRA) or direct renin concentration (DRC), is the currently recommended screening method. In recent years, as ARR has been used in an increasing number of hypertensive patients, the detection of PA has increased significantly, especially in patients without hypokalemia (16). Confirmatory testing: The test was based on the assumption that aldosterone production would decrease if renin production were completely inhibited or if angiotensin II production was blocked. Common confirmatory tests include the fludrocortisone suppression test (FST), the saline infusion test (SIT), the oral salt loading test (SLT) and the captopril challenge test (CCT). FST has been used less frequently due to the need for hospitalization. SIT and SLT are the most commonly used in China and CCT is preferred for patients at risk of volume overload (16). Classification of PA subtypes: In most cases, PA is caused by either APA or BAH. The differential diagnosis between the two subtypes is important because the treatment of the two varies considerably. Masoni first introduced AVS in 1957 (21) and it has now become the gold standard for differentiating between unilateral and bilateral forms of PA. Conventional AVS collects blood samples from both central adrenal veins, identifying the laterality by comparison of steroid secretion, classifying PA subtypes, and usually guide for total adrenalectomy. In a multicenter study including 761 patients, unilateral PA patients diagnosed by AVS and subsequently treated by surgery have a higher rate of postoperative complete biochemical success than the CT group (22). In a study involving 19 centers and 1,625 patients, AVS-guided adrenalectomy patients had higher rates of hypertension cure than non-AVS-guided patients. The super-selective adrenal venous sampling, also known as segmental AVS (S-AVS), has been proposed providing a new basis for partial adrenalectomy. In contrast to central AVS (C-AVS), in addition to distinguishing between unilateral and bilateral diseases, S-AVS can assess intra-adrenal hormone distribution, pinpoint the site of aldosterone hypersecretion and make it possible for patients with PA treated by partial adrenalectomy (23).

However, as an invasive operation, AVS is technically difficult and needs to be performed in an experienced medical center. The overall complication rate for AVS is approximately 2.5%, the most common complication being groin hematoma and, in severe cases, adrenal hemorrhage and adrenal vein dissection (24).

4 Surgical treatment

Laparoscopic adrenalectomy is now a safe and effective standard surgical treatment option. Compared to traditional open surgery, laparoscopic adrenalectomy has shown significant advantages in terms of patient recovery and perioperative complications (25). Higashihara, et al. (26) first described laparoscopic transperitoneal adrenalectomy (LTA) in 1992 (Figure 1). Gagner, et al. (27) first introduced the lateral position in 1994 and since then LTA has been widely used. Mercan, et al. (28) first described retroperitoneoscopic adrenalectomy (RP) in 1995, but it was not routinely performed until the mid-2000s when Walz published modified techniques (29). In addition, Walz, et al. (30) first proposed partial adrenalectomy using a retroperitoneoscopic technique in 1996. The retroperitoneal approach has the advantage of not interfering the abdominal organs, avoiding intra-abdominal complications (e.g., postoperative intestinal obstruction, adhesions) and a shorter operative time, but the narrow space for the retroperitoneal approach makes it unsuitable for patients with large tumor diameters or poor periadrenal fat conditions (31). At the same

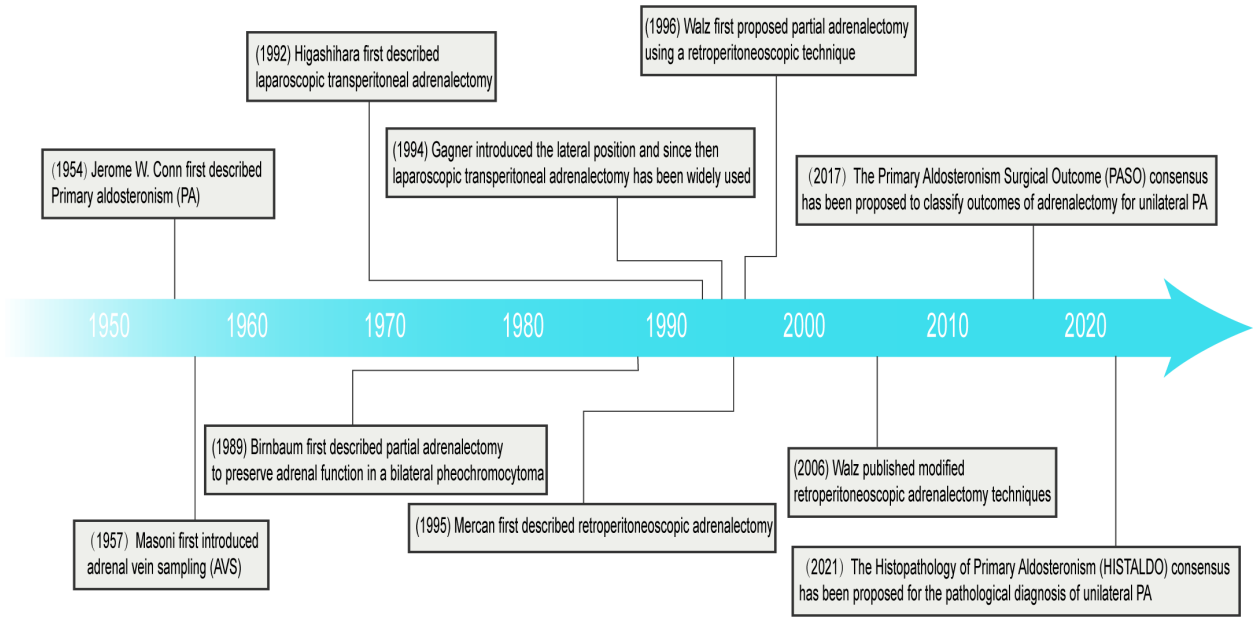


FIGURE 1
Milestones in primary aldosteronism and adrenalectomy.

time, all processes can be carried out with the assistance of a robot. Morino, et al. (32) compared the feasibility and safety of robot-assisted adrenalectomy (n = 10) with laparoscopic adrenalectomy (n = 10), and robot-assisted adrenalectomy did not show a significant advantage; instead, the robotic group had a longer operative time ($p < 0.001$), a higher perioperative complication rate (20% vs 0%), and a higher operative cost ($3,467 vs $2,737; $p < 0.01$). Therefore, further research is needed to fully define the role of robotic-assisted adrenalectomy in adrenalectomy (33).

5 Assessment of surgical outcomes

There is considerable variation between studies in the outcome of adrenalectomy in patients with PA, mainly due to differences in the definition of clinical success, resulting in considerable heterogeneity in prognosis (2). Williams, et al. (34) presented the Primary Aldosteronism Surgical Outcome (PASO) in 2017 to establish a standardized set of international consensuses for clinical and biochemical outcomes in unilateral PA adrenalectomy. Consensus assesses clinical outcomes based on blood pressure and use of antihypertensive medication, and biochemical outcomes based on blood potassium, ARR, and aldosterone concentrations. Clinical and biochemical outcomes were categorized as complete, partial and absent success (Table 1). They evaluated 705 patients with unilateral PA undergoing adrenalectomy at 12 centers from 1994 to 2015 using the PASO consensus. 259 (37%) of the 705 patients had complete clinical success and 334 (47%) had partial clinical success, i.e., over 80% of patients had improved blood pressure control. In addition, 656 of 699 patients (94%) had complete biochemical success (34).

TABLE 1 The Primary Aldosteronism Surgical Outcome (PASO).

Outcome	Clinical	Biochemical
Complete success	Normal blood pressure, no need for antihypertensive medication	Correction of hypokalemia (if present preoperatively) and normalization of ARR; in patients with high postoperative ARR, suppression of aldosterone secretion should be performed in the confirmatory test
Partial success	Same blood pressure as before surgery with less antihypertensive medication; or lower blood pressure with the same or less medication	Correction of hypokalemia (if present preoperatively); patients with elevated ARR who have more than 50% reduction in baseline plasma aldosterone concentration compared to preoperative or who have abnormal but improved postoperative confirmatory test results
Absent success	No change or increase in blood pressure, no change or increase in use of antihypertensive medication	Persistent hypokalemia (if preoperative) or persistent elevated ARR or both, failure of postoperative confirmatory tests to suppress aldosterone secretion

An initial postoperative outcome assessment of at least blood pressure and serum potassium concentration should be performed within the first 3 months to adjust antihypertensive medication and correct hyperkalemia/hyperkalemia if necessary. However, final results should be assessed at 6-12 months and reassessed annually after that.

Sawyer, et al. (35) conducted follow-up evaluations on 47 Australian PA patients who underwent unilateral adrenalectomy using the PASO criteria. The results showed that among the 40 patients who achieved clinical outcomes, 35.0% (14/40) had complete clinical success, and 47.5% (19/40) had partial clinical success. Among the 30 patients who achieved biochemical outcomes, 83.8% (31/37) had complete biochemical success. A total of 93.6% (44/47) of patients benefited from adrenalectomy. Similarly, Anceschi, et al. (36) assessed 90 PA patients who underwent unilateral adrenalectomy using the PASO criteria. Sixty-one patients underwent minimally-invasive total adrenalectomy, with 54% (33/61) achieving complete clinical success and 23% (14/61) achieving partial clinical success. Additionally, 81.9% (50/61) achieved complete biochemical success.

6 Factors affecting prognosis

Although the majority of patients with unilateral PA treated with adrenalectomy have significantly improved clinical and biochemical outcomes, some patients still have persistent postoperative hypertension or abnormal biochemical parameters. It is therefore hoped that an analysis of the likely prognosis of patients in terms of preoperative factors, vascular and adipose conditions, postoperative pathology and somatic cell variation will help manage patient expectations of postoperative outcomes and identify patients who require close follow-up or ongoing monitoring of blood pressure and biochemical parameters (3).

6.1 Preoperative factors

Predicting patient prognosis through preoperative factors also facilitates the selection of an appropriate treatment strategy for patients, particularly in patients at high risk for surgery and in patients with imaging of adrenal nodules for whom conclusive evidence of lateralized aldosterone excess is not available (37). In a retrospective study of 96 patients undergoing laparoscopic adrenalectomy for unilateral PA, Bokuda, et al. (38) concluded that BMI ($p = 0.0473$) and contralateral ratio ($p = 0.0199$) were significantly associated with normal postoperative blood pressure and no need for antihypertensive medication by multivariate logistic regression (Table 2).

Williams, et al. (34) used logistic regression analysis to identify preoperative factors associated with clinical and biochemical outcomes following the establishment of the PASO consensus, suggesting that younger and female patients were more likely to have complete clinical success or clinical improvement (complete + partial clinical success), while preoperative antihypertensive medication use and left ventricular hypertrophy were negatively associated with complete clinical success. Similarly, Picado, et al. (39) used the PASO consensus to assess long-term outcomes in 37 patients with PA who underwent adrenalectomy and to identify preoperative predictors associated with persistent postoperative

TABLE 2 Preoperative factors affecting prognosis.

First author (year)	Design	Patients (n)	Characteristics	Outcome measure	Statistical analyses	Results
Williams (2017) (34)	Retrospective	unilateral PA Clinical data (n = 706) Biochemical data (n = 699)	Age, Sex, BMI Antihypertensive medication, Systolic blood pressure	PASO (complete plus partial clinical success)	Adjusted logistic regression analyses	Younger age (p = 0.004), female sex (p = 0.002), lower BMI (p = 0.001), higher systolic blood pressure (p = 0.005) and antihypertensive medication (p = 0.003) at baseline were determinants of clinical benefit.
Burrello (2020) (3)	Retrospective	unilateral PA (n = 380)	Duration of hypertension, Sex, BMI, AntiHT medication, Target organ damage, Largest nodule at imaging	PASO (complete clinical success)	Unadjusted univariate and adjusted multivariate logistic regression analyses	Duration of hypertension (p < 0.001), Sex (p < 0.001), BMI (p < 0.001), AntiHT medication (p < 0.001), Target organ damage (p < 0.001), and largest nodule at imaging (p = 0.048) were confirmed as predictors.
Bokuda (2017) (38)	Retrospective	unilateral PA (n = 96)	Age, Sex, BMI Antihypertensive medication, UA, CR	12-month follow-up Cured: normotensive without drugs Not cured: not normotensive	Multivariate logistic regression analyses	Higher BMI (p = 0.0473) significantly correlated with not cured, while lower CR (p = 0.0199) significantly correlated with cured.
Rossi (2008) (37)	Prospectively	APA (n = 50)	BMI, Systolic BP, M/L, Known duration of HT	Cured Markedly Improved Mildly Improved	Backward stepwise multivariable logistic regression analysis	M/L (p = 0.038; OR: 0.5992, 95%CI: 0.3695 to 0.9718); Known duration of HT (p = 0.033; OR: 0.9812, 95%CI: 0.9642 to 0.9985).
Picado (2021) (39)	Retrospective	PA (n = 37)	Age, sex, race, ethnicity, preoperative aldosterone and renin level, tumor size, BMI, duration of hypertension, the number of blood pressure medications	PASO (absent clinical success)	Multivariate logistic regression analyses	BMI (p = 0.04; OR: 1.13, 95%CI: 1.01 to 1.29); duration of hypertension (p < 0.05; OR: 1.11, 95%CI: 1.03 to 1.25); the number of blood pressure medications (p < 0.05; OR: 2.30, 95%CI: 1.07 to 4.93) were associated with absent clinical success.

AntiHT medication, antihypertensive medication; APA, aldosterone-producing adenoma; BMI, body mass index; BP, blood pressure; CR, contralateral ratio; HT, hypertension; M/L, media: lumen ratio; PA, primary aldosteronism; PASO, primary aldosteronism surgical outcome; UA, uric acid.

hypertension. The results showed complete biochemical success in all patients, while clinical outcomes were complete success 15 (41%), partial success 14 (38%) and absent success 8 (21%). Multivariate logistic regression analysis showed that BMI (p = 0.04), duration of hypertension (p < 0.05) and the number of antihypertensive drugs used (p < 0.05) were significantly associated with absent clinical success.

Burrello, et al. (3) developed a 25-point scoring system using preoperative factors to predict clinical outcomes after unilateral PA. Data from 380 patients undergoing adrenalectomy for unilateral PA were first analyzed by unadjusted and adjusted logistic regression to select variables associated with clinical complete success, followed by the training and testing of linear discriminant analysis models to establish scores based on data from these 380 patients. A total of six variables were screened for the study: "duration of hypertension," "sex," "body mass index (BMI)," "antihypertensive medication," "target organ damage" and "largest nodule at imaging." Of these, duration of hypertension (negative correlation) was the strongest predictor of clinical complete success, followed by anti-hypertensive medication (negative correlation) and largest nodule at imaging (positive correlation). Each variable is assigned a different score, with higher total scores suggesting a better prognosis. Using a score of 16 as a cut-off value results in an accuracy of 79.2%, with sensitivities and specificities of 71.3% and 84.4% respectively.

6.2 Variations in the anatomy of the adrenal veins

Management of the central adrenal vein is a key step in adrenalectomy and can lead to hemorrhage if not handled correctly (25). In addition, there may be variants of the adrenal vasculature, so a thorough knowledge of adrenal vein anatomy by the operator is required to avoid medically induced injury. The most common

anatomy of the adrenal veins is that the left adrenal vein receives inferior phrenic and drains into left renal vein, while the right adrenal vein drains directly into the inferior vena cava (Figure 2).

Cesmebasi, et al. (40) argue that the variations in adrenal venous drainage cannot be described independently, but rather the overall appearance of the adrenal veins and their accompanying renal veins are described in a unified manner. For example, the anatomical variation of the left adrenal vein is described as "Adrenal vein joins renal alone, renal vein receives independent inferior phrenic vein," and "Double adrenal veins, one receives inferior phrenic vein, renal vein receives adrenal vein and inferior phrenic vein common trunk and an accessory adrenal vein (Figure 3)." Given the proximity of the right adrenal vein to the inferior vena cava and the variability of the right adrenal vein, it is recommended that special attention be paid to venous dissection during right adrenalectomy or AVS operations. Scholten, et al. (41) suggested that adrenal vein anatomical variation could be described in terms of both number and location. Of the 546 laparoscopic adrenalectomies collected, 70 (13%) had variations in adrenal vein anatomy. 63 had variation in the number of veins, including "no identifiable central adrenal vein," "one central adrenal vein with additional prominent small veins," and "multiple adrenal veins with or without small veins." Seven cases were locational variations related to the hepatic vein, the inferior vena cava or the inferior phrenic vein. For instance, "the right adrenal vein joins the right hepatic vein." Patients with variant venous anatomy had larger tumors (5.1 vs 3.3 cm; $p < 0.01$) and a higher proportion of pheochromocytomas (24 (35%) vs 100 (21%); $p = 0.02$) compared to patients with normal venous anatomy. The mean operative time was longer in patients with venous variants ($p < 0.05$) and the estimated blood loss (EBL) was also higher ($p = 0.01$). It was also found that more venous variants occurred on the right side than on the left (42 (17%) vs 28 (9%); $p = 0.02$), so the risk of medically induced injury during surgery was greater on the right side. Sun, et al. (42) reached a similar conclusion. In a retrospective analysis of 303 adrenalectomies, 62 cases (20%) had adrenal vein variation. Multiple logistic regression analysis showed that tumor size and pheochromocytoma were independent factors associated with variant veins. Multiple linear regression modeling of bleeding showed an increase of approximately 42.5% in patients with variant veins compared to normal veins ($p = 0.009$). In addition, patients with adrenal vein variants had more blood loss ($p < 0.001$), longer operative time ($p < 0.001$), longer postoperative hospital stay ($p = 0.004$) and higher operative costs ($p = 0.014$) compared to normal anatomy. Unfortunately, there is a lack of studies comparing variant adrenal venous anatomy with normal venous anatomy for long-term outcome after adrenalectomy.

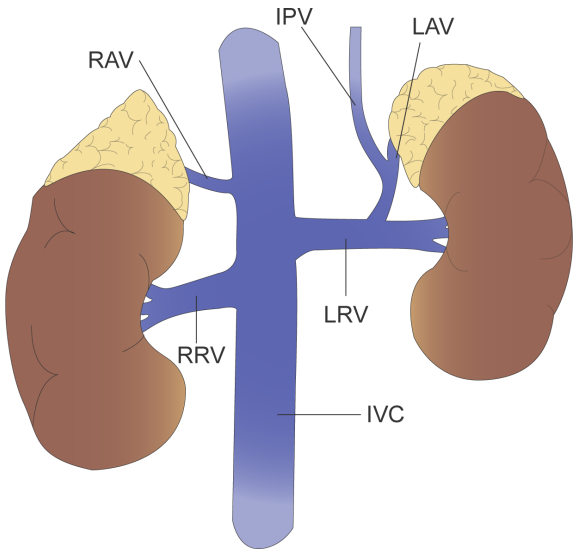


FIGURE 2
The left adrenal vein (LAV) receives inferior phrenic vein (IPV) and drains into left renal vein (LRV), while the right adrenal vein (RAV) drains directly into the inferior vena cava (IVC).

It is important to note that in AVS procedures, although very rare, the use of unsuitable catheters and catheterization techniques may result in serious complications such as adrenal vein dissection due to inadequate knowledge of adrenal vein anatomy (43). In addition, even if the central vein is successfully cannulated, variant venous drainage of an APA may lead to misinterpretation of the results and even a "double-down" AVS result (bilateral adrenal suppression) (44). A thorough understanding of the normal anatomy of the adrenal vein and its many variants is therefore essential to avoid complications and medically induced injuries during procedures such as AVS and adrenalectomy.

6.3 Periadrenal adipose tissue

The adrenal glands are located in the retroperitoneal space above the kidneys and are surrounded by periadrenal adipose tissue. The increased amount of periadrenal adipose tissue increases the operational difficulty of laparoscopic surgery and the difficulty of dissecting anatomical landmarks such as the inferior vena cava and adrenal veins, prolonging the operative time and increasing the incidence of postoperative complications. Although BMI is the most commonly used anthropometric measure to assess obesity, it does not always accurately reflect the extent of visceral fat in patients (45). Lindeman, et al. (29) introduced the concept of the posterior adiposity index (PAI), which is the sum of the distance from the skin to the Gerota fascia (S-GF) and the perirenal fat distance (PNF), i.e., the distance from the skin to the renal parenchyma. In a multifactorial regression analysis of predictors of operative time in 56 patients undergoing retroperitoneoscopic adrenalectomy, PAI (PAI ≥ 9; $p = 0.02$) predicted increased operative time and morbidly obese patients significantly increased the challenge of retroperitoneoscopic surgery (Table 3). Pearlstein, et al. (46) explored the predictors of operative time for retroperitoneoscopic adrenalectomy over BMI, with periadrenal fat volume being an independent predictor of increased operative time in both univariate and multivariate analyses (both $p < 0.01$). However, PAI was a significant predictor of operative time in the univariate analysis ($p < 0.01$) but not statistically significant in the multivariate analysis ($p = 0.81$). They concluded that BMI per se did not affect operative time when controlling for variables such as periadrenal fat volume and left-right side of surgery. In contrast to Pearlstein's

10.3389/fendo.2024.1416287

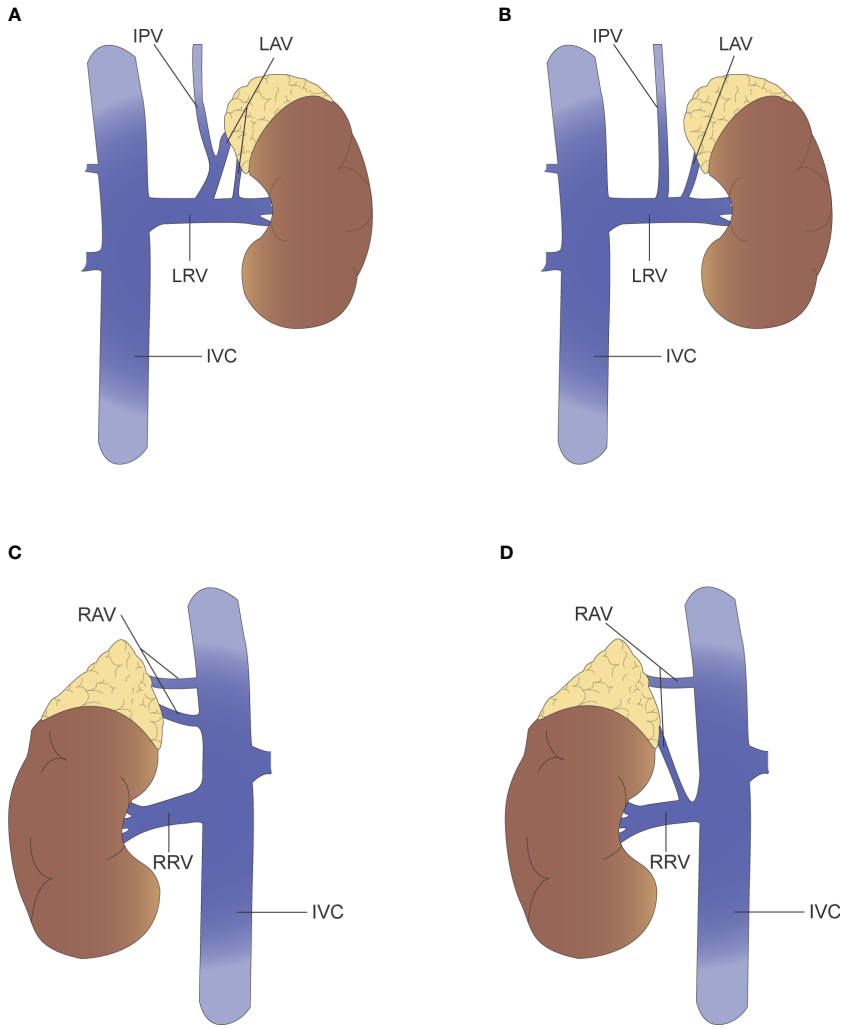


FIGURE 3
(A) Left renal vein receives adrenal vein and inferior phrenic vein common trunk and an accessory adrenal vein. **(B)** Left adrenal vein joins renal vein alone, renal vein receives independent inferior phrenic vein. **(C)** Two right adrenal vein and all drain into the IVC. **(D)** Two right adrenal vein, where one drains into the IVC and the other into the renal vein.

report that periadrenal volume including adrenal lesions was an independent predictor of prolonged operative time for retroperitoneoscopic adrenalectomy, Rah, et al. (47) directly measured and analyzed the volume of periadrenal fat excluding the adrenal mass. Multiple regression analysis showed that both PAI (p = 0.027) and periadrenal fat volume (p = 0.024) were predictors of longer operative time, while BMI was not statistically significant (p = 0.239). However, after grouping based on the learning curve, periadrenal fat volume was an independent predictor of prolonged operative time only before the learning curve (p = 0.009). After the learning curve, the difficulties posed by periadrenal fat would be overcome (p = 0.054). It is important to emphasize that although adipose tissue may extend the duration of surgery, it is not significantly associated with estimated blood loss (EBL) and does not apparently influence a negative surgical outcome (29).

Er, et al. (48) examined the relationship between visceral adipose tissue and postoperative clinical outcomes in patients with PA. One hundred patients with APA who underwent adrenalectomy and 41 control patients with primary hypertension were included in the study. The visceral fat area (VFA) of each patient was measured by CT and showed that patients with PA had a significantly smaller VFA than patients with essential hypertension (p = 0.021). Logistic regression analysis showed that a smaller VAF ($p < 0.001$) and shorter duration of hypertension (p = 0.011) predicted complete clinical success after adrenalectomy. The reason for this may be that patients with a larger VAF are associated with obesity-related hypertension and do not fully normalize their blood pressure after undergoing adrenalectomy.

6.4 Type of pathology

Hematoxylin-eosin (HE) staining, routinely performed in the pathology laboratory, provides only morphological information, but it is not suitable for functional histopathological analysis and

TABLE 3 Assessment of the periadrenal adipose tissue.

First author (year)	Design	Patients (n)	Characteristics	Outcome measure	Statistical analyses	Results
Lindeman (2019) (29)	Retrospective	LA (n = 57) RP (n = 56)	PAI Lesion size Side	Operative time Estimated blood loss	Multivariable linear regression analyses	Increasing PAI (p = 0.02), larger lesions (p = 0.01) and right site (p = 0.03) were predictive of longer operative time in RP; Nothing was significantly associated with estimated blood loss.
Pearlstein (2020) (46)	Retrospective	RP (n = 83)	Periadrenal fat volume Side Order of operation	Operative time	Multivariable linear random effects model	Periadrenal volume (p < 0.01), side (p < 0.01) and order of operation (p = 0.02) retained significance.
Rah (2021) (47)	Retrospective	RP (n = 284)	Depth of descended adrenal tumor location to kidney PAI Periadrenal fat volume Sex, Side, Surgeon, Diagnosis	Operative time	Multivariate logistic regression	Depth of descended adrenal tumor location to kidney (p = 0.002), PAI (p = 0.027), large periadrenal fat volume (p = 0.024), male (p = 0.012), right site (p = 0.031), surgeon A (p = 0.002) and pheochromocytoma (p = 0.003) were predictive of longer operative time.
Er (2020) (48)	Retrospective	APA (n = 100) EH (n = 41)	VFA Duration of hypertension	PASO	Logistic regression analysis	APA patients had smaller VFA (p = 0.021) than EH patents; smaller Log VFA (p < 0.001) and shorter duration of hypertension to PA diagnosis (p = 0.011) could independently predict the cure of hypertension.

APA, aldosterone-producing adenoma; BMI, body mass index; EH, essential hypertension; LA, laparoscopic transabdominal adrenalectomy; PAI, posterior adiposity index; PASO, primary aldosteronism surgical outcome; RP, retroperitoneoscopic adrenalectomy; VFA, visceral fat area.

cannot determine the source of excess aldosterone (14). Immunohistochemical staining for CYP11B2, a key enzyme involved in aldosterone synthesis present in the zona glomerulosa, is important for the diagnosis of potential sources of excess aldosterone production and pathology in PA. The proposal of the international consensus on Histopathology of Primary Aldosteronism (HISTALDO), based on CYP11B2 immunohistochemical staining for classification and diagnosis, contributed to the standardized nomenclature of resected PA pathology and the consistency of histopathological diagnosis of unilateral PA (49). Consensus groups lesions into five categories: Aldosterone-producing adenoma (APA), Aldosterone-producing nodule (APN), Aldosterone-producing micronodule (APM) (previously known as aldosterone-producing cell clusters), Multiple aldosterone-producing nodules or multiple aldosterone-producing micronodules (MAPN or MAPM) (previously known as nodular hyperplasia or micronodular hyperplasia) and Aldosterone-producing diffuse hyperplasia (APDH). Of these, APA and APN are classified as classical unilateral primary aldosteronism and the others as nonclassical unilateral primary aldosteronism.

Williams, et al. (49) compared the classical group (n = 24) with the nonclassical group (n = 12). At baseline in the nonclassical group, hypertension lasted longer (p = 0.01), pathological nodules were smaller (p = 0.019), the lateralization index was lower (p = 0.048), and serum potassium concentrations were higher (p = 0.031); however, during postoperative follow-up, the nonclassical group showed lower serum potassium concentrations (p = 0.006) and a higher ARR (p = 0.006); according to the PASO criteria, although there were no statistical differences in clinical outcomes between the two groups, the biochemical results were worse in the nonclassical group than in the classical group (p = 0.009). Nanba, et al. (50) divided 32 patients undergoing unilateral adrenalectomy for PA into an APA group (n = 22) and a non-APA group (n = 10). The preoperative APA group had lower serum potassium concentrations (p < 0.05), a higher prevalence of hypokalaemia (p < 0.01) and a higher ARR (p < 0.01) than the non-APA group. Similarly, Meyer, et al. (51) divided 60 patients with unilateral PA who underwent adrenalectomy into classical group (n = 45) and nonclassical group (n = 15). Classical group exhibited higher plasma aldosterone concentrations (p = 0.008) and ARR (p = 0.002) at baseline level. In addition, the classical group had a significantly higher proportion of complete biochemical success (97.6% vs 66.7%, p = 0.004). These results suggest that PA patients in the classical group had more severe preoperative biochemical indicators, but had a better prognosis after undergoing adrenalectomy than PA patients in the nonclassical group.

In addition to CYP11B2 immunohistochemical staining, chemokine receptors CXCR4 immunohistochemical staining was also characteristically expressed. Heinze, et al. (52) found that CXCR4 showed strong staining in the subcapsular region of normal adrenal glands, as well as strong staining in APA and a significant positive correlation with CYP11B2 (p < 0.01), but was almost negative in non-functioning adenomas.

6.5 Somatic variants

Somatic variants of the *KCNJ5*, *CACNA1D*, *CACNA1H*, *CLCN2*, *ATP1A1*, *ATP2B3* and *CTNNB1* genes were found in unilateral PA (53). APA is the predominant lesion type in unilateral PA, with an overall somatic variant detection rate of approximately 50–58.4% (54–56). However, recent researchers have used immunohistochemistry to guide lesion selection, resulting in detectable somatic variation of up to 90% in APA (57). The dominant somatic variant in APA is a mutation in the *KCNJ5* gene, with an incidence of approximately 40% in studies from Western countries and an even higher incidence in

studies from Asian countries, which can reach approximately 70%, but the *CACNA1D* variant is more common in African Americans, accounting for 42% (58).

Vilela, et al. (54) conducted a retrospective study of *KCNJ5* somatic variants associated with clinical outcomes in unilateral PA adrenalectomy, where *KCNJ5* somatic variants were identified in 33 (43.4%) of 76 patients who had their genes sequenced. When patients were divided into *KCNJ5* variant and wild-type groups for comparison, the proportion of complete clinical success was significantly higher in the variant group than in the wild-type group (57.6% vs 16.2%; p = 0.0001). Multiple logistic regression also showed that the *KCNJ5* somatic mutation was an independent predictor of complete clinical success (RR 6.418, 95% CI 1.83 to 22.93; p = 0.004). Interestingly, a higher proportion of patients in the *KCNJ5* variant group were female (p = 0.004) and the size of the tumor was larger (p = 0.001) compared to the wild-type group, which is consistent with the preoperative factors suggestive of a good prognosis for adrenalectomy described above (3, 34). Similar results were obtained by Kitamoto, et al. (59), where *KCNJ5* somatic variants were present in 106 (74.6%) of 142 patients with APA. 136 (95.8%) patients achieved complete endocrinological remission after adrenalectomy, with 81 (59.6%) patients cured of hypertension and 55 (40.4%) improved. When compared between the two groups, the proportion of *KCNJ5* somatic variants was significantly higher in the cured group (85.2% vs 60%; p = 0.002). Stepwise regression analysis also demonstrated that *KCNJ5* somatic variants, duration of hypertension and number of antihypertensive medications used were independently associated with postoperative hypertensive remission. Also, patients with the *KCNJ5* somatic variants were younger, had larger tumors, had a more severe PA phenotype and showed more aggressive disease progression than patients with the wild-type *KCNJ5* gene.

7 Discussion

7.1 Preoperative prognostic indicators that may influence surgical decisions

It has been suggested that persistent postoperative hypertension may be due to the diagnosis of highly asymmetric bilateral PA as unilateral PA during preoperative classification by AVS (51). In other words, the poor prognosis of unilateral adrenalectomy may have a strong association with the contralateral adrenal gland that was not removed. In a single-center prospective cohort study by Meyer, et al. (51), the biochemical outcomes were significantly better in the classical pathology type group than in the nonclassical group, while the ratio of absolute aldosterone concentration in the contralateral adrenal vein to the peripheral vein was significantly higher in the nonclassical group compared to the classical group (p = 0.004), with weaker contralateral suppression in this type of patient, asymmetric bilateral disease may have been present preoperatively. In another study, 43 patients with a biochemical outcome of absent + partial success compared with 52 patients with complete success, patients in the absent + partial success group exhibited a lower lateralization index and a higher contralateral ratio (60). Suggesting weaker contralateral adrenal suppression and abnormal postoperative aldosterone secretion on the contralateral side contributed to the inability to achieve a biochemical outcome of complete success. It has also been suggested that primary hypertension due to obesity, old age or long-term vascular damage and remodeling is likely to prevent patients from returning to normal blood pressure after adrenalectomy (39, 48). However, compared to the inability of mineralocorticoid antagonists to completely inhibit the systemic effects of aldosterone (atrial fibrillation, cardiac fibrosis), surgical treatment has a high biochemical success rate, allowing for the normalization of plasma aldosterone concentrations and providing better long-term benefits (61).

7.2 Histological/genetic features that may influence long-term prognosis

From a pathogenetic point of view, the prognosis of nonclassical PA differs from that of classical PA, probably because of a different genetic profile (53). The *KCNJ5* gene variant is the most common in APA (59, 62), while the *CACNA1D* gene variant is more common in APM (63, 64), with different somatic variants leading to different degrees of symptoms and prognosis. Moreover, the proportion of somatic variants and pathological types varies widely by region and ethnicity, especially in Asia compared with Western countries, where a higher proportion of APA patients have *KCNJ5* gene variants and a lower proportion of nonclassical PA (49, 58, 65).

7.3 Application of PASO consensus

Previous studies on unilateral PA have lacked uniform criteria for postoperative assessment, making the results highly heterogeneous and difficult to compare between studies. The advent of the PASO consensus has provided norms for the clinical and biochemical assessment of unilateral PA patients after adrenalectomy, but there are still some shortcomings in the postoperative assessment of adrenalectomy. Vorselaars, et al. (66) conducted a multicenter retrospective study to classify the postoperative outcomes of 380 patients from 16 treatment centers using the PASO consensus. However, 11% and 47% (16% of the total cohort) of patients classified as partial and absent clinical success, respectively, were considered to be misclassified or debatable. The main reasons for the debatable grouping of results were the PASO consensus's use of high thresholds to determine relevant changes in systolic blood pressure (SBP) and the use of percentages rather than absolute values to determine changes in the defined daily dose. The PASO consensus defines a change in SBP of more than 20 mmHg to be considered a change in blood pressure. However, a systematic review by Ettehad, et al. (67) demonstrated that a 10mmHg reduction in SBP in hypertensive patients reduced the risk of major cardiovascular events (20%), coronary heart disease (17%), stroke (27%), heart failure (28%) and all-cause mortality (13%). A cut-off value of 20 mmHg may allow a significant proportion of patients with partial clinical success to be judged as having absent clinical success. On the other hand, the

consensus definition of reduction in antihypertensive medication use refers to a 50% or greater reduction in defined daily dose (DDD) between preoperative and postoperative periods. Considering only percentages without incorporating absolute changes in actual medication use may result in medication use reductions that are not clinically meaningful being judged as partial clinical success or substantial medication use reductions being judged as absent clinical success. In addition, the PASO lacks an assessment of surgical indicators. For example, the duration of surgery, estimated bleeding, and postoperative complications will significantly affect the patient's recovery and quality of life, and the lack of these indicators does not facilitate the overall assessment of the outcome of the procedure (68).

7.4 New diagnostic method

CT and AVS are commonly used to differentiate between unilateral and bilateral PA, but CT has limitations in the diagnosis of adrenal lesions. It is unable to identify smaller APN or APM_S that are not morphologically distinct from the surrounding tissue, and even when a larger volume of tumor is observed, CT is unable to determine whether it is secretory or not. A systematic review of the diagnostic concordance of CT and MRI with AVS conducted by Kempers, et al. (69) concluded that of the 950 patients included in the 38 studies, 37.8% had CT/MRI findings that were inconsistent with AVS, 14.6% of patients with bilateral PA would undergo adrenalectomy based on CT/MRI findings, 19.1% of patients with unilateral PA could not undergo adrenalectomy, and even 3.9% of patients were diagnosed with the wrong side. The guidelines therefore recommend that AVS should be performed preoperatively in patients with PA who are being considered for surgery, except for young patients (<35 years) with significant aldosterone excess and spontaneous hypokalaemia and a typical unilateral cortical adenoma on CT of the adrenal lesion, who can be crossed over to AVS before adrenalectomy (70).

However, AVS is an invasive procedure that is difficult and complex and carries the risk of complications. Recently, steroid profiling of peripheral veins and functional imaging techniques have provided additional options for differentiating subtypes (16). In a multicenter study of steroid profiling in patients with PA, the subtype could be correctly identified in 172 (80%) of 216 patients with PA based on the analysis of 12 adrenal steroids measured in peripheral blood (71). In addition, steroid profiling can be applied to predict the biochemical outcome of patients after adrenalectomy.Meyer, et al. (60) measured 15 adrenal steroids in the peripheral veins of patients with PA. Of the 70 patients in whom the measurements were performed, biochemical outcomes following adrenalectomy and the diagnosis of bilateral PA could be correctly predicted in 53 (76%) patients using linear discriminant analysis, which further increased the accuracy to 86% using decision tree analysis. As for functional imaging techniques, Heinze, et al. (52) used 68Ga-pentixafor-PET (selectively binds to human CXCR4) in nine patients with APA and found significantly higher tracer uptake on the side of increased aldosterone secretion ($p < 0.01$), which could effectively differentiate APA from non-functioning adenoma. However, more large RCT studies are needed to truly introduce these techniques into clinical practice.

7.5 Partial or total adrenalectomy

The concept of partial adrenalectomy was developed to treat hereditary and sporadic bilateral tumors, to reduce the risk of Addisonian crisis and to avoid the need for steroid replacement (72). Birnbaum, et al. (73) first described partial adrenalectomy to preserve adrenal function in a bilateral pheochromocytoma, and follow-up after 32 months showed that the patient had normal blood pressure and did not require antihypertensive medication or steroid replacement. Walz, et al. (30) first proposed partial adrenalectomy using a retroperitoneoscopic technique in 1996 and performed subtotal resection in five cases of smaller eccentric tumors, demonstrating that with careful selection, endocrine cure could also be achieved in unilateral pheochromocytomas and Conn adenomas. In recent years, the use of minimally invasive adrenal-sparing techniques for PA has increased with increasing experience and the spread of robotic surgery. Theoretically, in multiple occupying lesions or nonclassical PA, micronodules in the residual tissue after partial adrenalectomy have an impact on clinical parameters (blood pressure, plasma renin activity, plasma aldosterone) and they may play a role in PA recurrence. However, a systematic review including four studies (two RCTs) showed no significant differences in clinical and biochemical outcomes and recurrence rates between partial and total adrenalectomy (Table 4) (75). In another systematic review of 60 studies of partial adrenalectomy, the recurrence rate of PA was only 2% and 97% of patients did not require steroid replacement (72). Anceschi, et al. (68) used PASO criteria to assess the outcome of partial adrenalectomy compared to total adrenalectomy and showed that the proportion of patients with complete clinical success was higher in the partial adrenalectomy group than in the total adrenalectomy group (72.4% vs 54.1%) and the success rate of partial clinical success was lower than in the total adrenalectomy group (6.8% vs 23%), but there were differences in the baseline characteristics (patients in the partial adrenalectomy group had a smaller mean tumor diameter) and the surgical approach (most patients in the partial adrenalectomy group were robotic) between the two groups. Billmann, et al. (74) evaluated 234 patients with unilateral PA, 78 (33.3%) underwent minimally invasive partial adrenalectomy and 156 (66.7%) underwent minimally invasive total adrenalectomy. In terms of postoperative morbidity, the incidence of hypocortisolism and hypoglycemia was lower with partial adrenalectomy.

Although the above findings suggest that partial adrenalectomy has similar surgical outcomes to total adrenalectomy and even fewer postoperative complications. However, partial adrenalectomy still has the potential to miss a true source of abnormal aldosterone. Nanba, et al. (50) used CYP11B2 immunostaining to classify postoperative pathology in patients with PA. Preoperative CT in 23 patients showed unilateral adrenal tumors, but in four (17.4%) of these patients the tumors did not show positive CYP11B2 immunostaining, suggesting that the tumors shown on CT may not be the true source of abnormal aldosterone secretion. In a case report by Ito, et al. (76), the patient

TABLE 4 Partial adrenalectomy vs Total adrenalectomy in patients with primary aldosteronism.

First author (year)	Design	Patients (n)	Protocol	Outcome measure	Results	Follow-up
Billmann (2021) (74)	Retrospective	Partial (n = 78) Total (n = 156)	pMIA: the adrenal adenoma was well localized and could be differentiated from adrenal tissue; tMIA: other conditions	Primary outcome: peri- and postoperative complications Secondary outcomes: (1) clinical and biochemical success (2) persistence/ recurrence of the disease (3) operation duration (4) hospital stays (5) blood loss.	(1) Perioperative complications were comparable between both groups; (2) Postoperative hypocortisolism: pMIA (11.5%) vs tMIA (25.0%) ($p < 0.001$); Postoperative hypoglycemia: pMIA (2.6%) vs tMIA (7.1%) ($p = 0.039$); (3) No significant difference could be found between the 2 groups in secondary outcomes; (4) No recurrence was encountered in either the pMIA or the tMIA group.	24 months
Anceschi (2021) (68)	Retrospective	MITA (n = 61) MIPA (n = 29)	MIPA were limited to small tumors (<3 cm)	PASO clinical success Perioperative outcomes	(1) MITA group was higher in the tumor size (4.2 vs 2.7; $p = 0.001$), MIPA group was higher in the preoperative hypertension rate (82.8% vs 57.4%; $p = 0.01$); (2) MIPA group was higher in the complete clinical success rate (72.4% vs 54.1%, $p = 0.097$), MITA group was higher in the partial clinical success (23% vs 6.8%, $p = 0.136$); (3) LOS was increased in the MITA group (4 vs 3 d, $p = 0.038$);The perioperative transfusion rate, 24 h △Hb and overall complications were similar between groups.	42 months
Muth (2015) (75)	Systematic review	2RCTs, Prospective, Retrospective (n = 535)	Total ADX (n = 329) Partial ADX (n = 206	Normalized ARR (%), Hypertension cured/improved (%), Normalized K^+ (%)	No difference in ARR, BP and potassium values improvement between patients randomized to partial or total adrenalectomy.	0.5-5.2 years

ADX, adrenalectomy; LOS, length of hospital stays; MIPA, minimally invasive partial adrenalectomy; MITA, minimally invasive total adrenalectomy; pMIA, partial minimally invasive adrenalectomy; PASO, primary aldosteronism surgical outcome; RCTs, randomized clinical trials; tMIA, total minimally invasive adrenalectomy.

had a preoperative diagnosis of right-sided PA by CT and AVS, etc. Postoperative pathology revealed multiple nodules (up to 6 mm) and hyperplasia of the zona glomerulosa by visual observation, but all these nodules were negative for CYP11B2 immunostaining, while 1 mm-sized micronodule positive for CYP11B2 immunostaining were found at other sites. The authors also encountered a case of a patient with unilateral PA who, 10 years after undergoing partial adrenalectomy on the right side, developed a recurrence in the ipsilateral adrenal gland, rediscovered APA, and underwent total adrenalectomy.Therefore, more large RCT studies and especially long- term follow-up are still needed to verify which surgical approach is of greater benefit.

8 Conclusion

PA is the most common endocrine form of secondary hypertension. Adrenalectomy for unilateral PA is effective. The patient's preoperative factors, vascular and adipose conditions, type of pathology and somatic variants all suggest prognosis to varying degrees. Combining the indicators for analysis can better help the operator manage the patient's prognostic expectations and target patients with potentially poorer prognoses for close monitoring of blood pressure and biochemical indicators. The emergence of the PASO consensus has set a uniform standard for the assessment of surgical outcomes in patients undergoing adrenalectomy for PA, but improvements are still needed. The use of CYP11B2 immunostaining for pathological diagnosis, as advocated by the HISTALDO consensus, can help to better identify potential sources of abnormal aldosterone secretion. Steroid profiling and functional imaging techniques offer new options for determining subtypes of PA as less invasive screening techniques. A combination of techniques and indicators allows for better early diagnosis of PA, better determination of the type of lesion and the selection of the appropriate surgical approach for timely surgical intervention in patients with unilateral PA.

Author contributions

HX: Writing – original draft, Visualization, Software, Methodology, Data curation, Conceptualization. TZ: Writing – original draft, Visualization, Data curation, Conceptualization. WS: Writing – review & editing, Validation, Conceptualization. DY: Writing – review & editing, Visualization, Validation, Methodology, Conceptualization. XZ: Writing – review & editing, Visualization, Validation, Methodology, Conceptualization.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Conn JW. Part I. Painting background. Part II. Primary aldosteronism, a new clinical syndrome, 1954. *J Lab Clin Med*. (1990) 116:253–67.

2. Vorselaars WMCM, Nell S, Postma EL, Zarnegar R, Drake FT, Duh Q-Y, et al. Clinical outcomes after unilateral adrenalectomy for primary aldosteronism. *JAMA Surg*. (2019) 154(4):e185842. doi: 10.1001/jamasurg.2018.5842

3. Burrello J, Burrello A, Stowasser M, Nishikawa T, Quinkler M, Prejbisz A, et al. The primary aldosteronism surgical outcome score for the prediction of clinical outcomes after adrenalectomy for unilateral primary aldosteronism. *Ann Surg*. (2020) 272:1125–32. doi: 10.1097/sla.0000000000003200

4. Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, et al. Prevalence and clinical manifestations of primary aldosteronism encountered in primary care practice. *J Am Coll Cardiol*. (2017) 69:1811–20. doi: 10.1016/j.jacc.2017.01.052

5. Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Sci (New York NY)*. (2011) 331:768–72. doi: 10.1126/science.1198785

6. Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet*. (2013) 45:1055–60. doi: 10.1038/ng.2716

7. Scholl UI, Stölting G, Nelson-Williams C, Vichot AA, Choi M, Loring E, et al. Recurrent gain of function mutation in calcium channel CACNA1H causes early-onset hypertension with primary aldosteronism. *eLife*. (2015) 4:e06315. doi: 10.7554/eLife.06315

8. Fernandes-Rosa FL, Daniil G, Orozco IJ, Göppner C, El Zein R, Jain V, et al. A gain-of-function mutation in the CLCN2 chloride channel gene causes primary aldosteronism. *Nat Genet*. (2018) 50:355–61. doi: 10.1038/s41588–018-0053–8

9. Rege J, Bandulik S, Nanba K, Kosmann C, Blinder AR, Plain A, et al. Somatic SLC30A1 mutations altering zinc transporter ZnT1 cause aldosterone-producing adenomas and primary aldosteronism. *Nat Genet*. (2023) 55:1623–31. doi: 10.1038/s41588–023-01498–5

10. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet*. (2013) 45:440–4, 44e1–2. doi: 10.1038/ng.2550

11. Zennaro M-C, Boulkroun S, Fernandes-Rosa FL. Pathogenesis and treatment of primary aldosteronism. *Nat Rev Endocrinol*. (2020) 16:578–89. doi: 10.1038/s41574–020-0382–4

12. Tetti M, Gong S, Veglio F, Reincke M, Williams TA. Primary aldosteronism: Pathophysiological mechanisms of cell death and proliferation. *Front Endocrinol (Lausanne)*. (2022) 13:934326 [published Online First: 2022/08/26. doi: 10.3389/fendo.2022.934326

13. Rhayem Y, Perez-Rivas LG, Dietz A, Bathon K, Gebhard C, Riester A, et al. PRKACA somatic mutations are rare findings in aldosterone-producing adenomas. *J Clin Endocrinol Metab*. (2016) 101:3010–7. doi: 10.1210/jc.2016–1700

14. Sun L, Jiang Y, Xie J, Zhu H, Wu L, Zhong X, et al. Immunohistochemical analysis of CYP11B2, CYP11B1 and beta-catenin helps subtyping and relates with clinical characteristics of unilateral primary aldosteronism. *Front Mol Biosci*. (2021) 8:751770 [published Online First: 2021/10/12. doi: 10.3389/fmolb.2021.751770

15. Wu X, Azizan EAB, Goodchild E, Garg S, Hagiyama M, Cabrera CP, et al. Somatic mutations of CADM1 in aldosterone-producing adenomas and gap junction-dependent regulation of aldosterone production. *Nat Genet*. (2023) 55:1009–21. doi: 10.1038/s41588–023-01403–0

16. Reincke M, Bancos I, Mulatero P, Scholl UI, Stowasser M, Williams TA. Diagnosis and treatment of primary aldosteronism. *Lancet Diabetes Endocrinol*. (2021) 9:876–92. doi: 10.1016/s2213–8587(21)00210–2

17. Milliez P, Girerd X, Plouin P-F, Blacher J, Safar ME, Mourad J-J. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol*. (2005) 45:1243–48. doi: 10.1016/j.jacc.2005.01.015

18. Rossi G-P, Sechi LA, Giacchetti G, Ronconi V, Strazzullo P, Funder JW. Primary aldosteronism: cardiovascular, renal and metabolic implications. *Trends Endocrinol Metab*. (2008) 19:88–90. doi: 10.1016/j.tem.2008.01.006

19. Akehi Y, Yanase T, Motonaga R, Umakoshi H, Tsuiki M, Takeda Y, et al. High prevalence of diabetes in patients with primary aldosteronism (PA) associated with subclinical hypercortisolism and prediabetes more prevalent in bilateral than unilateral PA: A large, multicenter cohort study in Japan. *Diabetes Care*. (2019) 42:938–45. doi: 10.2337/dc18–1293

20. Williams TA, Reincke M. MANAGEMENT OF ENDOCRINE DISEASE: Diagnosis and management of primary aldosteronism: the Endocrine Society guideline 2016 revisited. *Eur J Endocrinol*. (2018) 179:R19–29. doi: 10.1530/eje-17–0990

21. Masoni A. Catheterisation of the right adrenal vein in man. *Acta Med Scand*. (1957) 159:225–34. doi: 10.1111/j.0954–6820.1957.tb00129.x

22. Williams TA, Burrello J, Sechi LA, Fardella CE, Matrozova J, Adolf C, et al. Computed tomography and adrenal venous sampling in the diagnosis of unilateral primary aldosteronism. *Hypertension*. (2018) 72:641–49. doi: 10.1161/hypertensionaha.118.11382

23. Omura M, Saito J, Matsuzawa Y, Nishikawa T. Supper-selective ACTH-stimulated adrenal vein sampling is necessary for detecting precisely functional state of various lesions in unilateral and bilateral adrenal disorders, inducing primary aldosteronism with subclinical Cushing's syndrome. *Endocr J*. (2011) 58:919–20. doi: 10.1507/endocrj.ej11–0210

24. Thiesmeyer JW, Ullmann TM, Stamatiou AT, Limberg J, Stefanova D, Beninato T, et al. Association of adrenal venous sampling with outcomes in primary aldosteronism for unilateral adenomas. *JAMA Surg*. (2021) 156(2):165–71. doi: 10.1001/jamasurg.2020.5011

25. Wang X, Liu J, Ji A, Liu C, Nahayo S, Wang L, et al. The safety and efficiency of retroperitoneal laparoscopic adrenalectomy via extra and intra perinephric fat approaches: a retrospective clinical study. *BMC Surg*. (2019) 19(1):198. doi: 10.1186/s12893–019-0648–8

26. Higashihara E, Tanaka Y, Horie S, Aruga S, Nutahara K, Homma Y, et al. [A case report of laparoscopic adrenalectomy]. *Nihon Hinyokika Gakkai Zasshi*. (1992) 83:1130–3. doi: 10.5980/jpnjurol1989.83.1130

27. Gagner M, Lacroix A, Bolte E, Pomp A. Laparoscopic adrenalectomy. The importance of a flank approach in the lateral decubitus position. *Surg Endosc*. (1994) 8 (2):135–8. doi: 10.1007/BF00316627

28. Mercan S, Seven R, Ozarmagan S, Tezelman S. Endoscopic retroperitoneal adrenalectomy. *Surgery*. (1995) 118:1071–76. doi: 10.1016/s0039–6060(05)80116–3

29. Lindeman B, Gawande AA, Moore FD, Cho NL, Doherty GM, Nehs MA. The posterior adiposity index: A quantitative selection tool for adrenalectomy approach. *J Surg Res*. (2019) 233:26–31. doi: 10.1016/j.jss.2018.07.003

30. Walz MK, Peitgen K, Hoermann R, Giebler RM, Mann K, Eigler FW. Posterior retroperitoneoscopy as a new minimally invasive approach for adrenalectomy: results of 30 adrenalectomies in 27 patients. *World J Surg*. (1996) 20:769–74. doi: 10.1007/s002689900117

31. Walz MK, Alesina PF, Wenger FA, Deligiannis A, Szuczik E, Petersenn S, et al. Posterior retroperitoneoscopic adrenalectomy—results of 560 procedures in 520 patients. *Surgery*. (2006) 140:943–50. doi: 10.1016/j.surg.2006.07.039

32. Morino M, Benincà G, Giraudo G, Del Genio GM, Rebecchi F, Garrone C. Robot-assisted vs laparoscopic adrenalectomy: a prospective randomized controlled trial. *Surg Endosc*. (2004) 18:1742–46. doi: 10.1007/s00464-004-9046-z

33. Hupe MC, Imkamp F, Merseburger AS. Minimally invasive approaches to adrenal tumors: an up-to-date summary including patient position and port placement of laparoscopic, retroperitoneoscopic, robot-assisted, and single-site adrenalectomy. *Curr Opin Urol*. (2017) 27:56–61. doi: 10.1097/MOU.0000000000000339

34. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, et al. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol*. (2017) 5:689–99. doi: 10.1016/s2213–8587(17)30135–3

35. Sawyer N, Glendenning P, Vasikaran SD, Page MM, van Schie G, Wong SL, et al. The Adrenal Vein Sampling Outcomes Study (AVOS): success rates following adrenalectomy for unilateral primary aldosteronism. *Pathology*. (2023) 55:531–37. doi: 10.1016/j.pathol.2023.02.002

36. Anceschi U, Tufano A, Flammia RS, Mormando M, Fiori C, Zappalà O, et al. Clinical cure vs a novel trifecta system for evaluating long-term outcomes of minimally-invasive partial or total adrenalectomy for unilateral primary aldosteronism: results of a multicentric series. *Cent Eur J Urol*. (2022) 75:345–51. doi: 10.5173/ceju.2022.147

37. Rossi GP, Bolognesi M, Rizzoni D, Seccia TM, Piva A, Porteri E, et al. Vascular remodeling and duration of hypertension predict outcome of adrenalectomy in primary aldosteronism patients. *Hypertension*. (2008) 51:1366–71. doi: 10.1161/HYPERTENSIONAHA.108.111369

38. Bokuda K, Yatabe M, Mizuguchi Y, Niiyama M, Seki Y, Watanabe D, et al. Body mass index and contralateral ratio predict outcome following unilateral adrenalectomy in primary aldosteronism. *Hypertens Res*. (2017) 40:988–93. doi: 10.1038/hr.2017.78

39. Picado O, Whitfield BW, Khan ZF, Jeraq M, Farrá JC, Lew JI. Long-term outcome success after operative treatment for primary aldosteronism. *Surgery*. (2021) 169:528–32. doi: 10.1016/j.surg.2020.07.046

40. Cesmebasi A, Du Plessis M, Iannatuono M, Shah S, Tubbs RS, Loukas M. A review of the anatomy and clinical significance of adrenal veins. *Clin Anat*. (2014) 27:1253–63. doi: 10.1002/ca.22374

41. Scholten A, Cisco RM, Vriens MR, Shen WT, Duh QY. Variant adrenal venous anatomy in 546 laparoscopic adrenalectomies. *JAMA Surg*. (2013) 148:378–83. doi: 10.1001/jamasurg.2013.610

42. Sun F, Zhuo R, Ma W, He H, Ye L, Xu D, et al. Retrospective analysis of variant venous anatomy in 303 laparoscopic adrenalectomies and its clinical implications. *J Surg Oncol*. (2019) 119:801–06. doi: 10.1002/jso.25364

43. Kobayashi K, Alkukhun L, Rey E, Salaskar A, Acharya R. Adrenal vein sampling: tips and tricks. *Radiographics: Rev Publ Radiol Soc North America Inc*. (2024) 44: e230115. doi: 10.1148/rg.230115

44. DePietro DM, Fraker DL, Wachtel H, Cohen DL, Trerotola SO. Double-down" Adrenal vein sampling results in patients with apparent bilateral aldosterone suppression: utility of repeat sampling including super-selective sampling. *J Vasc Intervent Radiol: JVIR*. (2021) 32:656–65. doi: 10.1016/j.jvir.2020.12.029

45. Erbil Y, Barbaros U, Sari S, Agcaoglu O, Salmaslioglu A, Ozarmagan S. The effect of retroperitoneal fat mass on surgical outcomes in patients performing laparoscopic adrenalectomy: the effect of fat tissue in adrenalectomy. *Surg Innov*. (2010) 17:114–9. doi: 10.1177/1553350610365703

46. Pearlstein SS, Kuo JH, Chabot JA, Lee JA. Periadrenal volume is a better predictor of prolonged operative time in laparoscopic retroperitoneal adrenalectomy than BMI. *World J Surg*. (2020) 44:578–84. doi: 10.1007/s00268–019-05324–0

47. Rah C-S, Kim WW, Lee Y-M, Chung K-W, Koh J-M, Lee SH, et al. New predictive factors for prolonged operation time of laparoscopic posterior retroperitoneal adrenalectomy; retrospective cohort study. *Int J Surg*. (2021) 94:106–13. doi: 10.1016/j.ijsu.2021.106113

48. Er LK, Lin M-C, Tsai Y-C, Hsiao J-K, Yang C-Y, Chang C-C, et al. Association of visceral adiposity and clinical outcome among patients with aldosterone producing adenoma. *BMJ Open Diabetes Res Care*. (2020) 8(1):e001153. doi: 10.1136/bmjdrc-2019–001153

49. Williams TA, Gomez-Sanchez CE, Rainey WE, Giordano TJ, Lam AK, Marker A, et al. International histopathology consensus for unilateral primary aldosteronism. *J Clin Endocrinol Metab*. (2021) 106:42–54. doi: 10.1210/clinem/dgaa484

50. Nanba K, Tsuiki M, Sawai K, Mukai K, Nishimoto K, Usui T, et al. Histopathological diagnosis of primary aldosteronism using CYP11B2 immunohistochemistry. *J Clin Endocrinol Metab*. (2013) 98:1567–74. doi: 10.1210/jc.2012–3726

51. Meyer LS, Handgriff L, Lim JS, Udager AM, Kinker I-S, Ladurner R, et al. Single-center prospective cohort study on the histopathology, genotype, and postsurgical outcomes of patients with primary aldosteronism. *Hypertension*. (2021) 78:738–46. doi: 10.1161/hypertensionaha.121.17348

52. Heinze B, Fuss CT, Mulatero P, Beuschlein F, Reincke M, Mustafa M, et al. Targeting CXCR4 (CXC chemokine receptor type 4) for molecular imaging of aldosterone-producing adenoma. *Hypertension*. (2018) 71:317–25. doi: 10.1161/hypertensionaha.117.09975

53. Santana LS, Guimaraes AG, Almeida MQ. Pathogenesis of primary aldosteronism: impact on clinical outcome. *Front Endocrinol*. (2022) 13:927669. doi: 10.3389/fendo.2022.927669

54. Vilela LAP, Rassi-Cruz M, Guimaraes AG, Moises CCS, Freitas TC, Alencar NP, et al. KCNJ5 somatic mutation is a predictor of hypertension remission after adrenalectomy for unilateral primary aldosteronism. *J Clin Endocrinol Metab*. (2019) 104:4695–702. doi: 10.1210/jc.2019–00531

55. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension*. (2014) 64:354–61. doi: 10.1161/HYPERTENSIONAHA.114.03419

56. Wu VC, Wang SM, Chueh SJ, Yang SY, Huang KH, Lin YH, et al. The prevalence of CTNNB1 mutations in primary aldosteronism and consequences for clinical outcomes. *Sci Rep*. (2017) 7:39121. doi: 10.1038/srep39121

57. Williams TA, Reincke M. Pathophysiology and histopathology of primary aldosteronism. *Trends Endocrinol Metab*. (2022) 33:36–49. doi: 10.1016/j.tem.2021.10.002

58. Nanba K, Rainey WE. GENETICS IN ENDOCRINOLOGY: Impact of race and sex on genetic causes of aldosterone-producing adenomas. *Eur J Endocrinol*. (2021) 185:R1–R11. doi: 10.1530/eje-21–0031

59. Kitamoto T, Omura M, Suematsu S, Saito J, Nishikawa T. KCNJ5 mutation as a predictor for resolution of hypertension after surgical treatment of aldosterone-producing adenoma. *J Hypertens*. (2018) 36:619–27. doi: 10.1097/HJH.0000000000001578

60. Meyer LS, Wang X, Sušnik E, Burrello J, Burrello A, Castellano I, et al. Immunohistopathology and steroid profiles associated with biochemical outcomes after adrenalectomy for unilateral primary aldosteronism. *Hypertension*. (2018) 72:650–57. doi: 10.1161/hypertensionaha.118.11465

61. Catena C, Colussi G, Lapenna R, Nadalini E, Chiuch A, Gianfagna P, et al. Long-term cardiac effects of adrenalectomy or mineralocorticoid antagonists in patients with primary aldosteronism. *Hypertension*. (2007) 50:911–8. doi: 10.1161/hypertensionaha.107.095448

62. Nanba K, Omata K, Else T, Beck PCC, Nanba AT, Turcu AF, et al. Targeted molecular characterization of aldosterone-producing adenomas in white Americans. *J Clin Endocrinol Metab*. (2018) 103:3869–76. doi: 10.1210/jc.2018–01004

63. Pauzi FA, Azizan EA. Functional characteristic and significance of aldosterone-producing cell clusters in primary aldosteronism and age-related hypertension. *Front Endocrinol*. (2021) 12:631848. doi: 10.3389/fendo.2021.631848

64. Omata K, Anand SK, Hovelson DH, Liu C-J, Yamazaki Y, Nakamura Y, et al. Aldosterone-producing cell clusters frequently harbor somatic mutations and accumulate with age in normal adrenals. *J Endocr Soc*. (2017) 1:787–99. doi: 10.1210/js.2017–00134

65. Wang H, Wang F, Zhang Y, Wen J, Dong D, Chang X, et al. Surgical outcomes of aldosterone-producing adenoma on the basis of the histopathological findings. *Front Endocrinol*. (2021) 12:663096. doi: 10.3389/fendo.2021.663096

66. Vorselaars WMCM, van Beek D-J, Postma EL, Spiering W, Borel Rinkes IHM, Valk GD, et al. Clinical outcomes after surgery for primary aldosteronism: Evaluation of the PASO-investigators' consensus criteria within a worldwide cohort of patients. *Surgery*. (2019) 166:61–8. doi: 10.1016/j.surg.2019.01.031

67. Ettehad D, Emdin CA, Kiran A, Anderson SG, Callender T, Emberson J, et al. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. *Lancet*. (2016) 387:957–67. doi: 10.1016/s0140–6736(15)01225–8

68. Anceschi U, Tuderti G, Fiori C, Zappalà O, Ferriero MC, Brassetti A, et al. Minimally invasive partial versus total adrenalectomy for the treatment of primary aldosteronism: results of a multicenter series according to the PASO criteria. *Eur Urol Focus*. (2021) 7:1418–23. doi: 10.1016/j.euf.2020.06.023

69. Kempers MJ, Lenders JW, van Outheusden L, van der Wilt GJ, Schultze Kool LJ, Hermus AR, et al. Systematic review: diagnostic procedures to differentiate unilateral from bilateral adrenal abnormality in primary aldosteronism. *Ann Intern Med*. (2009) 151:329–37. doi: 10.7326/0003–4819-151–5-200909010–00007

70. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. (2016) 101:1889–916. doi: 10.1210/jc.2015–4061

71. Eisenhofer G, Dekkers T, Peitzsch M, Dietz AS, Bidlingmaier M, Treitl M, et al. Mass spectrometry-based adrenal and peripheral venous steroid profiling for subtyping primary aldosteronism. *Clin Chem*. (2016) 62:514–24. doi: 10.1373/clinchem.2015.251199

72. Nagaraja V, Eslick GD, Edirimanne S. Recurrence and functional outcomes of partial adrenalectomy: A systematic review and meta-analysis. *Int J Surg*. (2015) 16:7–13. doi: 10.1016/j.ijsu.2015.01.015

73. Birnbaum J, Giuliano A, Van Herle AJ. Partial adrenalectomy for pheochromocytoma with maintenance of adrenocortical function. *J Clin Endocrinol Metab*. (1989) 69:1078–81. doi: 10.1210/jcem-69-5-1078

74. Billmann F, Billeter A, Thomusch O, Keck T, El Shishtawi S, Langan EA, et al. Minimally invasive partial versus total adrenalectomy for unilateral primary hyperaldosteronism-a retrospective, multicenter matched-pair analysis using the new international consensus on outcome measures. *Surgery*. (2021) 169:1361–70. doi: 10.1016/j.surg.2020.09.005

75. Muth A, Ragnarsson O, Johannsson G, Wängberg B. Systematic review of surgery and outcomes in patients with primary aldosteronism. *Br J Surg*. (2015) 102:307–17. doi: 10.1002/bjs.9744

76. Ito A, Yamazaki Y, Sasano H, Matsubara D, Fukushima N, Tamba M, et al. A case of primary aldosteronism caused by unilateral multiple adrenocortical micronodules presenting as muscle cramps at rest: The importance of functional histopathology for identifying a culprit lesion. *Pathol Int*. (2017) 67:214–21. doi: 10.1111/pin.12521

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 07 August 2024
DOI 10.3389/fendo.2024.1423027

OPEN ACCESS

EDITED BY
Mattia Barbot,
University Hospital of Padua, Italy

REVIEWED BY
Irene Tizianel,
University of Padua, Italy
Sergei Tevosian,
University of Florida, United States

*CORRESPONDENCE
Elena Aisha Azizan
elena.azizan@ukm.edu.my

RECEIVED 25 April 2024
ACCEPTED 23 July 2024
PUBLISHED 07 August 2024

CITATION
Aminuddin A, Brown MJ and Azizan EA (2024) Evaluating the role of aldosterone synthesis on adrenal cell fate.
Front. Endocrinol. 15:1423027.
doi: 10.3389/fendo.2024.1423027

Evaluating the role of aldosterone synthesis on adrenal cell fate

Amnani Aminuddin[1], Morris J. Brown [2,3] and Elena Aisha Azizan[1,2,4]*

[1]Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia, [2]Endocrine Hypertension, Department of Clinical Pharmacology and Precision Medicine, William Harvey Research Institute, Queen Mary University of London, London, United Kingdom, [3]National Institute for Health Research (NIHR) Barts Biomedical Research Centre, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom, [4]Research Center, Hospital Tunku Ampuan Besar Tuanku Aishah Rohani, Universiti Kebangsaan Malaysia Specialist Children's Hospital, Kuala Lumpur, Malaysia

Hypertension affects one-third of the adult population worldwide, with primary aldosteronism (PA) accounting for at least 5-10% of these cases. The aldosterone synthase enzyme (CYP11B2) plays a pivotal role in PA manifestation, as increased expression of CYP11B2 leads to excess aldosterone synthesis. Physiological expression of CYP11B2 in humans is normally limited to cells of the adrenal zona glomerulosa under tight homeostatic regulation. In PA, however, there are CYP11B2-positive lesions in the adrenal cortex that autonomously secrete aldosterone, highlighting the dysregulation of adrenal cortex zonation and function as a key aspect of PA pathogenesis. Thus, this review aims to summarize the development of the adrenal glands, the key regulators of adrenal cortex homeostasis, and the dysregulation of this homeostasis. It also discusses the development of CYP11B2 inhibitors for therapeutic use in patients with hypertension, as well as the current knowledge of the effects of CYP11B2 inhibition on adrenal cortex homeostasis and cell fate. Understanding the control of adrenal cell fate may offer valuable insights into both the pathogenesis of PA and the development of alternative treatment approaches for PA.

KEYWORDS

primary aldosteronism, CYP11B2, aldosterone synthesis inhibition, adrenal cell fate, homeostasis of adrenal cortex

1 Introduction

Hypertension is a chronic yet common medical condition that affects one in three adults aged 30 to 79 worldwide (1). Endocrine hypertension accounts for at least 10% of hypertension cases (2). Primary aldosteronism (PA), characterized by excess aldosterone production by the adrenal glands, is one of the common causes of endocrine hypertension. Frequently, the underlying causes of PA include aldosterone-producing adenomas (APA)

and idiopathic hyperaldosteronism (IHA) (3). Aldosterone is normally physiologically synthesized in the zona glomerulosa (ZG) cells of the adrenal cortex. As the rate-limiting enzyme that catalyzes the final steps of aldosterone biosynthesis, aldosterone synthase (CYP11B2), is selectively expressed in the ZG (4). However, in APA and IHA, increased expression and activation of CYP11B2 are commonly observed (5).

Owing to the critical role of CYP11B2 in PA manifestation, research on inhibiting CYP11B2 to suppress aldosterone synthesis has gained much attention (6). In the past few years, several selective CYP11B2 inhibitor drugs have been investigated in clinical trials for treatment of PA (ClinicalTrials.gov). These drugs effectively decreased aldosterone levels without affecting the activity of its closely homologous enzyme, 11β-hydroxylase (CYP11B1), which synthesizes cortisol, a vital hormone for regulating body's stress responses (7–10).

Despite the anticipated positive treatment outcomes of CYP11B2 inhibitors, the effect of inhibiting CYP11B2 on adrenal cell fate is still understudied. Cell fate determination involving centripetal migration and cell differentiation are crucial for zonation and remodeling of ZG, zona fasciculata (ZF) and zona reticularis (ZR) of the adrenal cortex, thus contributing to the proper function of the adrenal glands (11). Could the suppression of CYP11B2, for example, facilitate the differentiation of ZG cells into ZF cells? Or perhaps could the inhibition of CYP11B2 expression lead to the apoptosis of ZG cells? Understanding the potential consequences or compensatory modulations of the steroidogenesis activity and the remodeling or structural changes of the adrenal cortex is thus of profound importance to corroborate the use of these treatments for PA or hypertension in general.

Primarily focusing on the human system, this review will briefly describe an overview of the adrenal glands development and the key regulators involved in adrenal cortex maintenance to provide a clear understanding of the developmental and lineage progression of cells in the adrenal glands. We further highlight the dysregulation in the cellular turnover or homeostasis of the adrenal cortex that may contribute to the onset of PA and endocrine-related hypertension. Additionally, we discuss the development of therapeutic agents that target CYP11B2 directly, considering the role of this enzyme in the pathology of PA. Drawing from both pre-clinical and clinical studies, we delve into the observed effects of CYP11B2 inhibition on the homeostasis and cellular turnover within the adrenal cortex, which seems to significantly influence adrenocortical zonation and function. We suggest that the mechanisms governing adrenal cell fate may offer valuable insights into both the pathogenesis of PA and the development of alternative treatment approaches for PA.

2 Overview of the development of the human adrenal glands

Located at the superior poles of each kidney, the human adult adrenal glands are endocrine glands consisting of two major parts: the adrenal cortex and the adrenal medulla. Each part can be distinctly differentiated by its specific histological structures and biological functions (12). The adrenal cortex is the outer layer of the adrenal glands composed of the ZG, ZF, and ZR. Respectively, each zone is responsible for producing steroid hormones, namely mineralocorticoids, glucocorticoids, and androgenic sex hormones (13). Aldosterone is the main mineralocorticoid produced by the ZG, which is involved in controlling normal electrolyte balance and blood pressure (4). The principal glucocorticoid produced by the ZF is cortisol – a hormone essential for normal metabolic functions and immune responses (14). The ZR, the innermost layer of the adrenal cortex, produces small amounts of sex hormones, specifically dehydroepiandrosterone and androstenedione, which are involved in androgenic activity (15). On the other hand, the adrenal medulla, located at the center of the glands, is responsible for releasing adrenaline and noradrenaline for the fight-or-flight response to various stress factors (16). Prior to evolving into functional adult adrenal glands, the human adrenal glands undergo two crucial stages of development and remodeling – the embryonic and post-natal stages (17, 18).

The embryological origins of mammalian adrenal glands include: 1) the neural crest cells, which give rise to the progenitors of chromaffin cells in the adrenal medulla (16), and 2) the celomic epithelium in the urogenital ridge, which forms the progenitors of the adrenal cortex called the adrenogonadal primordium (AGP) (19). During early gestation, there is a marked increase in expression of steroidogenic factor 1 (Sf-1; nuclear receptor subfamily 5 group A member 1 (NR5A1)), a key regulator for adrenal development and steroidogenesis, in a subset of AGP, leading to the formation of the adrenal fetal zone (FZ) (20). The developing adrenal glands emerge as neural crest cells penetrate the FZ, forming the adrenal medulla at the center of the developing organ (21). Subsequently, mesenchymal cells envelop the developing organ, resulting in the formation of the adrenal capsule (22). The FZ then starts to enlarge, and successively, the adrenal definitive zone (DZ) appears between the adrenal capsule and the FZ (23). The development of the DZ is proposed to be regulated by: 1) NR5A1; 2) the fetal adrenal-specific enhancer (FAdE), the repressor of NR5A1; and 3) the glioma-associated oncogene homolog 1 (Gli1), the activator of hedgehog pathway (20, 23). Later in pregnancy, the DZ expands in size in the fetal adrenal and starts to produce cortisol, marking the development of the ZF of the fetal adrenal cortex.

The maturation of the adrenal cortex begins immediately after birth, whereby the cells within the adrenal FZ start to undergo apoptosis, and the adrenal DZ differentiates into two distinct zones, the ZG and the ZF, under the stimulation of angiotensin II (AngII) and adrenocorticotropic hormone (ACTH) (24). During puberty, the adrenal glands undergo a process called adrenarche, characterized by the increased proliferation of cells that produce adrenal androgens between the ZF and medulla layers. This cell layer makes up the ZR of the adrenal glands, completing the maturation of the adrenal cortex (20, 25).

3 Mature adrenal cortex homeostasis

Following the maturation of the adrenal glands, the homeostasis of the adrenal cortex is constantly maintained throughout life in

response to physiological demands or hormonal feedback regulation for steroid biosynthesis (Figure 1) (17, 23, 25–38). As early as 1883, the 'Standard Model' of homeostasis for the mature adrenal cortex was described as the centripetal migration model of adrenocytes (39). According to this model, adrenal cortex cells derive from adrenocortical stem/progenitor cells in the capsule or subcapsular region of the outer layer of the glands and further migrate centripetally while changing their phenotypes from the ZG, to the ZF and the ZR successively. The cells then undergo apoptosis at the boundary layer between the ZR and the adrenal medulla (26, 39). Until now, this model is yet to be challenged, and lineage tracing studies, along with recent trajectory analyses from single-cell transcriptomic studies, continue to support the model of centripetal migration for the maintenance of homeostasis and tissue renewal of the mature adrenal cortex (40–42).

Aside from its role as the key regulator and stimulator of mineralocorticoid secretion, the renin-angiotensin-aldosterone system (RAAS) also directly controls the proliferation of adrenal cortical cells. Physiologically, in response to low blood pressure and volume, activation of the RAAS leads to the secretion of a critical effector, AngII. The binding of AngII to the AngII receptor type 1 (AT1) activates Gq signaling, which further initiates the steroidogenic pathway for CYP11B2 biosynthesis in the ZG for aldosterone production (22–25). An *in vivo* study by McEwan et al. (1996) demonstrated that AngII induction, as well as low sodium intake, resulted in increased uptake of bromodeoxyuridine (BrdU), a marker of cell proliferation, and hypertrophy of the ZG and ZR, indicating proliferation of adrenal cortical cells (43).

Both cellular environment stimulation and the interaction between activated regulatory proteins among cells of different phenotypes and functions within the adrenal cortex play a crucial role in maintaining the fate or homeostasis of the adrenal cells. A crucial mediator that regulates both the development of the fetal adrenal glands and homeostasis of the adult adrenal cortex is canonical Wnt signaling. The Wnt family member 4 (Wnt4) is a well-known component involved in the activation of canonical Wnt signaling in adrenocortical cells (36). Canonical Wnt signaling activity is highly specific within the ZG, either maintaining the pool of adrenocortical progenitor cells or promoting the differentiation of these progenitor cells into functional steroidogenic CYP11B2-expressing cells upon AngII stimulation (17, 23, 25, 26, 28, 30). Suppression of canonical Wnt signaling leads to the inhibition of ZG zonation and functional control, allowing for the differentiation of ZG into ZF lineage (28, 29, 36, 37). A study by Drelon and colleagues demonstrated that upon stimulation by ACTH, the activation of cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA)/cAMP response element-binding protein (CREB) signaling pathway results in the inhibition of Wnt/β-

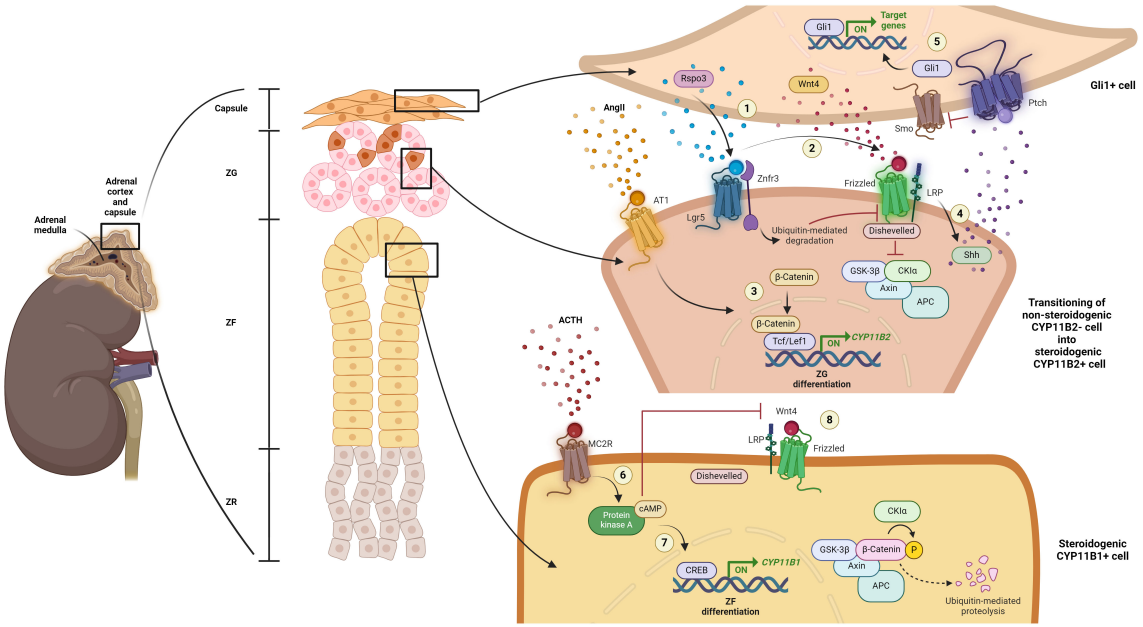


FIGURE 1
Current understanding of mature adrenal cortex regulation. (1) When in demand, capsular Rspo3 is released and binds to its receptor, leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5), located within the cells of the adjacent steroidogenic ZG zone, the CYP11B2-negative/Sonic hedgehog protein (Shh)-expressing progenitor cells of the ZG. Simultaneously, Rspo3 binds to the Znrf3 and promotes its ubiquitin-mediated degradation, thereby inhibiting the turnover of the Wnt4 receptor, Frizzled (FZD). (2) This inhibition allows Wnt4 to bind to FZD, promoting further recruitment of Dishevelled. Consequently, β-catenin is accumulated due to the inactivation of the destruction complex comprising adenomatous polyposis coli (APC), AXIN, casein kinase 1 (CK1) and glycogen synthase kinase 3 protein (GSK3 protein). (3) Along with the stimulation by AngII, further nucleus translocation and interaction of β-catenin with transcription factors, including T-cell factor/lymphoid enhancer factor 1 (Tcf/Lef1), lead to the expression of genes essential for adrenal cortex zonation and function, especially *CYP11B2*, initiating ZG differentiation. (4) Wnt signaling activation also further promotes Shh activation. (5) In turn, Shh interacts with Patched (Ptch) and Smoothened (Smo) to activate Gli1-mediated gene transcription in Gli1-positive capsular cells, facilitating their cellular proliferation or recruitment into the steroidogenic lineage. (6) Meanwhile, upon ACTH stimulation through melanocortin 2 receptor (MC2R), the cAMP/PKA/CREB signaling pathway is activated. This activation (7) suppresses canonical Wnt signaling, inhibiting ZG zonation and function, and (8) promotes the transcription of genes that drives the differentiation of ZG into ZF lineage. Created with BioRender.com.

catenin activation through the repression of Wnt4 and promotes lineage conversion towards ZF differentiation (36). Another study demonstrated that mice with Wnt4 deficiency exhibit disorganized ZG and aldosterone suppression (44). Concurringly, the transmembrane E3 ubiquitin ligase, zinc and ring finger (Znrf3), which antagonizes Wnt/β-catenin signaling, also impact adrenal cortex homeostasis (38). Loss of Znrf3 expression in a mouse model was found to promote the expansion of ZF (35).

Another crucial mediator involves the interplay between adrenal capsule cells and adrenocortical cells. R-spondin 3 (Rspo3), expressed in the Gli1-positive adrenal capsule cells, has been demonstrated to be an important component to ensure the replenishment of damaged and lost cells for maintenance of adrenal zonation throughout life (17, 27, 30). Deletion of Rspo3 in mice results in a complete reversal of the anticipated activation of the cell recruitment process, leading to impaired adrenal cortex zonation (27). These findings could possibly explain the observed reduction in adrenal cellular number when induced with Rspo3 or when Lgr5 was knocked down (45). The reduced cell number might result from the absence of crosstalk signaling between capsular cells and progenitor cells, leading to a lack of cell turnover.

4 Dysregulation in the homeostasis of adrenal cortex

Dysregulation in the adrenal cortex homeostasis can disrupt the control of adrenal glands function, leading to the manifestation of pathological conditions such as Conn's syndrome, Cushing syndrome, and virilization (37). To illustrate, adrenal hyperplasia or enlargement of the adrenal cortex, as seen in aldosterone-producing diffuse hyperplasia (APDH) and congenital adrenal hyperplasia, results in excessive aldosterone, cortisol and/or adrenal androgens production (46). APDH, along with other characterized CYP11B2-positive adrenal cortical lesions including carcinoma, adenoma, nodules, micronodules, are the underlying causes of PA (46, 47). In general, these lesions are characterized by the abnormal growth of aldosterone-producing adrenal cortical cells, also known as CYP11B2-positive adrenal cortical neoplasm. They are mostly benign neoplasms excluding the malignant aldosterone-producing adrenal cortical carcinoma (48).

In most cases, the development of the CYP11B2-positive adrenal cortical neoplasm is attributed to somatic mutations in common aldosterone-driver genes, namely *KCNJ5*, *CACNA1D*, *CTNNB1*, *ATP1A1*, and *ATP2B3* (49–54). In general (except for *CTNNB1*), these mutations affect the regulation of intracellular calcium concentration, leading to the activation of the CYP11B2 biosynthesis and eventually aldosterone production. For example, gain of function mutations in *CACNA1D* produce aberrant L-type voltage-gated calcium channels, $Ca_v1.3$, leading to increased calcium entry due to the mutated channel being activated at much lower potential thresholds of membrane depolarization (49, 50). Similarly, increased intracellular calcium levels also can be caused by the mutated P-type ATPases pumps, Na+/K+ ATPase α subunit and Ca2+ ATPase, which respectively arise from *ATP1A1* and *ATP2B3* mutations. These mutated ATPases promote elevated sodium and calcium permeability, further leading to membrane depolarization and calcium channel opening (49–51). Whereas, mutations in *KCNJ5*, which encodes for the G-protein-activated inward rectifier K+ channel 4 (GIRK4), result in an unselective potassium Kir3.4 channel, leading to increased sodium entry and subsequent cell membrane depolarization and calcium channel opening without the stimulation by AngII (52, 53).

Interestingly, a study by Nanba et al. (2017) found that the prevalence of aldosterone-producing micronodules (APMs), driven by aldosterone-stimulating somatic mutations, is directly proportional to aging (55). They found that the thickness of ZG cells reduced in elderly subjects, corresponding to the suppressed RAAS in older age. Additionally, the expression of CYP11B2 was limited to sporadic micronodules containing mutated genes that cause unregulated aldosterone production. Concurrently, Omata and colleagues demonstrated that the accumulation of computed tomography-undetectable APMs, which mainly harbor *CACNA1D* aldosterone-driver mutations, contributes to the development of idiopathic adrenal hyperplasia (56). This finding challenges the traditional view that idiopathic adrenal hyperplasia results solely from enlargement of the aldosterone-producing zone within the adrenal cortex.

We have previously suggested that the frequency of somatic mutations causing constitutive aldosterone production is due to the selection of cells that are protected from the fate of apoptosis, which we postulate occurs in ZG cells when salt excess suppresses aldosterone synthesis (57). The sharply demarcated, densely stained APMs that have caught the eye since Celso Gomez-Sanchez' development and sharing of an antisera specific for CYP11B2 (58), contrast strikingly with the complete absence of CYP11B2 in the adjacent ZG which comprises of the endocrine cells whose intended cell fate was to make aldosterone. While it is easy to regard age-related somatic mutation as at best neutral, and sometimes harmful process, the high prevalence of mutations causing APMs – at least 20% of all adults in salt-loving societies [based on prevalence of APMs, and proportion of these in which mutations are found] – suggests a physiological rather than pathological process. Thus, it raises the question of whether APMs could be the life-saving emergency supply of aldosterone, in times of catastrophic loss of sodium/water loss or rises in plasma K^+.

Meanwhile, the aldosterone-driver mutation in *CTNNB1* that encodes β-catenin, the critical activator of canonical Wnt signaling pathway, is associated with the development of multiple adrenal cortex disorders (54). Activation of Wnt signaling has been found to promote the proliferation of adrenocortical progenitor cells as well as the differentiation of the progenitor cells into ZG cells (59). Several studies reported the genetic predisposition of the mutations is associated with the demographic (52), gender (60), age (61), or pregnancy-related hormonal imbalance factors (62).

5 Drug inhibition of aldosterone synthesis

In the early section, we described how abnormal adrenal cortex homeostasis promotes the development of CYP11B2-positive adrenal cortical neoplasms, eventually leading to the manifestation of endocrine-related hypertension. Hence, it is of interest to explore therapeutic agents that directly target CYP11B2-positive adrenal cortical cells to control aldosterone levels, thus reversing endocrine hypertension. Several discoveries of compounds that suppress aldosterone production by targeting the CYP11B2-positive cells have been reported. The compounds that selectively target the CYP11B2-positive cells interrupt either the expression or activity of CYP11B2, and hence are known as aldosterone synthase inhibitors.

LCI699 (Osilodrostat) was the first CYP11B2 inhibitor developed for use in PA and hypertension (63). However, its development was mainly challenged by its poor selectivity for CYP11B2. The drug also showed inhibition on the enzymatic action of its homologous protein, CYP11B1, an enzyme that catalyzes the final step of cortisol synthesis from the precursor 11-deoxycortisol, leading to impairments in metabolism, immune function, and stress response (64, 65). In early 2020, Osilodrostat has been approved by the European Medicines Agency and the Food and Drug Administration for the treatment of patients with Cushing's syndrome and Cushing's disease who are not candidates for pituitary surgery or those who have failed surgery respectively (66, 67).

The active development of the selective CYP11B2 inhibitors with undesired inhibition of CYP11B1 has led to several successes (Table 1). Baxdrostat is one of the selective CYP11B2 inhibitors that had completed a phase 2 clinical trial for treatment of patients with treatment-resistant hypertension (7, 68–71). From the finding, the drug lowered serum aldosterone levels without affecting the ACTH-induced change in cortisol in a dose-dependent manner, resulting in significant reduction in both systolic and diastolic blood pressure (7, 71). Another promising selective CYP11B2 inhibitor, namely dexfadrostat phosphate, also known as 5R-fadrozole, had also successfully completed phase 2 clinical trials for treatment in patients with PA (10). The discovery of the off-target CYP11B2 inhibition effect of fadrozole, an approved non-steroidal cytochrome P450 19A1 (CYP19A1) inhibitor for breast cancer management, led to the development of its derivative, 5R-fadrozole (72). Targeting differences in the substrate binding pockets, 5R-fadrozole demonstrated precise inhibitory coordination with the catalytic heme unit of CYP11B2, distinguishing its activity from that against CYP19A1 and CYP11B1 (73).

TABLE 1 List of aldosterone synthase inhibitors or suppressors and their descriptions on the target proteins and mechanism of action, and pre-clinical, clinical trials, or clinical use status.

Compound	Target protein	Mechanism of action	Status of the compound
Osilodrostat (LCI699)	CYP11B1, CYP11B2	Inhibits both enzymatic actions of CYP11B1 and CYP11B2 for catalyzing cortisol and aldosterone synthesis respectively.	• Approved for hypercortisolism in Cushing's syndrome or disease.
LY3045697	CYP11B2	Selectively targets CYP11B2 with 39-fold inhibition effect over CYP11B1.	• Completed phase 1 clinical trial on healthy volunteers for therapeutic use in patients with hypertension, chronic kidney disease, diabetic nephropathy, primary hyperaldosteronism, or cardiac arrhythmias. (ClinicalTrials.gov ID: NCT01750853; NCT01821703)
RO6836191/Baxdrostat (CIN-107)	CYP11B2	Provides selective and competitive blockade of CYP11B2 and inhibit aldosterone production without affecting cortisol level.	• Active phase 3 clinical trial on patients with uncontrolled hypertension on two or more medications and with resistant hypertension. (ClinicalTrials.gov ID: NCT06034743) • Completed phase 2 clinical trial on patients with treatment-resistant hypertension. (ClinicalTrials.gov ID: NCT04519658)
5R-Fadrozole/ Dexfadrostat phosphate (DP13)	CYP11B2	Effectively forms a precise inhibitory coordination with the catalytic heme unit of the CYP11B2, thus reducing the aldosterone level. No specific binding observed with CYP11B1 and CYP19A1.	• Completed phase 2 clinical trial on patients with PA. (ClinicalTrials.gov ID: NCT04007406)
MLS101/Lorundrostat	CYP11B2	Selectively binds to CYP11B2 reducing plasma aldosterone and systolic blood pressure, with no observed cortisol insufficiency observed.	• Completed phase 2 clinical trial on patients with uncontrolled hypertension. (ClinicalTrials.gov ID: NCT05001945)
Atractylenolide-I	CYP11B2	Competitively binds to substrate binding site of CYP11B2 against heme, a catalyst for aldosterone synthesis.	• Only pre-clinical testing available.
YM750, Acyl-coenzyme A: Cholesterol acyltransferase (ACAT) inhibitor	ACAT	Suppresses CYP11B2 expression by inhibiting intracellular calcium signaling activated by KCl-stimulated depolarization.	• Only pre-clinical testing available.
Tacrolimus; Calcineurin inhibitor	Calcineurin	Suppresses CYP11B2 expression by inhibiting calcineurin/NFATc4 downstream signaling.	• Only pre-clinical testing available.

Other than that, Lorundrostat, a well-tolerated and highly selective CYP11B2 inhibitor, effectively decreased aldosterone levels and systolic automated office blood pressure in uncontrolled hypertension patients with obesity or suppressed renin in a phase 2 clinical study. *In vitro* analysis revealed that Lorundrostat reduced aldosterone with a selectivity ratio of 374:1 for the inhibition of CYP11B2 compared to CYP11B1 (8, 74, 75). Similarly, another potent CYP11B2 inhibitor, LY3045697, also exhibited high *in vitro* selectivity for CYP11B2 over CYP11B1, with a 39-fold difference. Moreover, the drug also demonstrated a favorable therapeutic index for effects on CYP11B2 over CYP11B1 in a phase 1 clinical study for the dose safety and tolerability on healthy subjects (9).

Pre-clinical studies have also explored the efficacy of small molecules designed to specifically inhibit aldosterone synthesis without affecting other enzymes involved in steroidogenesis. For instances, *in vitro* and *in vivo* investigations have highlighted atractylenolide-I as a potent compound (76). This compound selectively suppressed the activity of CYP11B2 by competitively binding to its substrate binding sites, Ala320 and Cys450, rather than to heme, an essential catalyst for aldosterone production. Similarly, Shimada and colleagues demonstrated that YM750, an acyl-coenzyme A: cholesterol acyltransferase (ACAT) inhibitor, suppressed aldosterone production through inhibition of intracellular calcium signaling activated by potassium chloride (KCl)-stimulated depolarization in an *in vitro* study using H295R human adrenocortical carcinoma cell line (77). They found that the inhibition suppressed the expression of nuclear receptor related 1 (NURR1) and nerve growth factor-induced subfamily B (NGFIB), important transcription factors that regulate CYP11B2 transcription. Thus, small molecules that indirectly affect the CYP11B2 expression and activity may also offer potential benefits for managing PA.

Another study had also reported the inhibition of CYP11B2 expression stimulated by KCl depolarization using the calcineurin inhibitor, tacrolimus (78). In both *in vitro* and *ex vivo* studies using mouse and human adrenal tissues, tacrolimus blocked calcineurin, a subunit for the calcium ion sensor calmodulin, leading to dephosphorylation of nuclear factor of activated T cell, cytoplasmic 4 (NFATc4). Inactivation of NFATc4 led to downstream effects including the direct suppression of *CYP11B2* transcription as well as indirect suppression through inhibition of NURR1 expression.

6 The effect of CYP11B2 inhibitors on adrenal cortex homeostasis

Pre-clinical studies and clinical trials have demonstrated a promising clinical effectiveness of CYP11B2 inhibitors. However, there is still a gap in literature regarding the impact of these inhibitors on the homeostasis of adrenocortical cells. It is well-established that the maintenance of adrenal cortex is intricately tied to the physiological requirements of the body. Therefore, the question arises – how do CYP11B2 inhibitors influence the overall maintenance or remodeling of adrenal cortex zonation and function or how does adrenal cortex homeostasis adapt to such changes particularly in regard to the cellular turnover of adrenalocortical cells as proposed by the centripetal migration model of adrenal cortex?

A study involving mice with deleted *CYP11B2* demonstrated that when aldosterone was absent, there was an increase in cellular turnover of ZG cells (79). The increased cellular turnover was characterized by the thickening of the ZG layer and the increase in cells that migrated and underwent apoptosis at the boundary layer between the cortex and medulla. Similar cellular turnover effect was demonstrated in the adrenal tissues from monkeys treated with the CYP11B2 inhibitor, baxdrostat (70). Through immunohistochemistry analysis, baxdrostat treatment on monkeys demonstrated increased apoptosis in a dose-dependent manner. Along with the observed increase in apoptosis, the proliferation rate of ZG cells was also increased, associated with the thickening of the ZG. However, this thickening of ZG coincided with an increase in CYP11B2 expression. To note, the cellular turnover of ZG cells continued during the treatment-free period despite the observed reversibility of CYP11B2 expression.

In adult human adrenal glands, a typical finding is of seemingly discontinuous ZG due to a reduction in ZG cell number and the ZG reaching out to the capsule. This appearance is also seen adjacent to many APAs and might be due to the negative feedback of aldosterone from the APA on adjacent ZG cells. By contrast, the ZG adjacent to APAs with either a *KCNJ5* mutation, or with double mutation of *CTNNB1* and either *GNA11* or *GNAQ*, shows prominent ZG hyperplasia, without expression of CYP11B2 (59, 80). It may be that there are two types of maladaptive response to salt-induced suppression of CYP11B2: one which leads to involution of ZG and selection of APM-forming *CACNA1D*-mutant cells that are protected from apoptosis; the other which leads to ZG hyperplasia (as in the $CYP11B2^{-/-}$ mouse) and selection for mutations that confer a proliferative advantage over adjacent cells. The question whether the gain-of-function mutations driving CYP11B2 autonomy are the same as cause the adenomas has not been settled, and there may indeed be a fine balance between cell growth and death depending on the mutation and/or expression level of the mutated channel (81, 82). Double-mutant APAs may prove the rule, with the only unusual feature being the obligatory pairing of CTNNB1 with GNA11/Q mutations. Or they may prove exceptional, with two mutations required to confer a growth advantage over the gross ZG hyperplasia.

Although our recent preliminary *in vitro* findings revealed that transient silencing of CYP11B2 expression in the HAC15 human adrenocortical carcinoma cell line did not significantly induce cellular apoptosis (83), we observed that CYP11B2 silencing activated stress response mechanisms, including autophagy and mitophagy, potentially facilitating cellular adaptation to CYP11B2 modulation through cellular recycling or initiating cellular death response (84). However, further functional studies are required to confirm whether transient silencing of CYP11B2 activates cellular recycling to promote cell survival, initiates subsequent apoptosis, or triggers necrosis.

7 Concluding remarks

In conclusion, exploring CYP11B2 inhibitors presents a promising avenue for managing PA and hypertension. However,

comprehending their effects on adrenal cortex homeostasis is crucial for ensuring their safety and efficacy. Current research suggests that these inhibitors can induce changes in cellular turnover within the adrenal cortex, impacting adrenocortical zonation and function. Further investigations are necessary to elucidate the mechanisms underlying these changes and to optimize therapeutic strategies for better outcomes in patients with endocrine-related hypertension.

Author contributions

AA: Conceptualization, Writing – original draft, Writing – review & editing. MB: Conceptualization, Resources, Supervision, Writing – review & editing. EA: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. AA is supported by the Ministry of Higher Education (MOHE) Malaysia, a Long-term Research Grant Scheme-Jejak Sarjana Ulung (LRGS-JSU), LRGS/1/2021/SKK15/UKM/02/2 of which EA is the principal investigator and MB the co-mentor.

Conflict of interest

MB is a member of a Scientific Advisory Board of AstraZeneca.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. World Health Organization. Global Report on Hypertension: The Race against a Silent Killer (2023). Available online at: https://www.who.int/publications/i/item/9789240081062 (Accessed February 18, 2024).

2. Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens*. (2013) 31:1281–357. doi: 10.1097/01.hjh.0000431740.32696.cc

3. Gomez-Sanchez CE, Gomez-Sanchez CM, Oki K. Aldosterone-producing adenomas. *Hypertension*. (2020) 75:927–9. doi: 10.1161/HYPERTENSIONAHA.119.14534

4. Bollag WB. Regulation of aldosterone synthesis and secretion. In: *Comprehensive Physiology*. New York: Wiley (2014). p. 1017–55. doi: 10.1002/cphy.c130037

5. Nakamura Y, Maekawa T, Felizola SJA, Satoh F, Qi X, Velarde-Miranda C, et al. Adrenal CYP11B1/2 expression in primary aldosteronism: immunohistochemical analysis using novel monoclonal antibodies. *Mol Cell Endocrinol*. (2014) 392:73–9. doi: 10.1016/j.mce.2014.05.002

6. Gomez-Sanchez CE, Gomez-Sanchez EP. Aldosterone synthase inhibitors and the treatment of essential hypertension. *J Clin Endocrinol Metab*. (2023) 108:e638–9. doi: 10.1210/clinem/dgad071

7. Freeman MW, Halvorsen Y-D, Marshall W, Pater M, Isaacsohn J, Pearce C, et al. Phase 2 trial of baxdrostat for treatment-resistant hypertension. *New Engl J Med*. (2023) 388:395–405. doi: 10.1056/NEJMoa2213169

8. Laffin LJ, Rodman D, Luther JM, Vaidya A, Weir MR, Rajicic N, et al. Aldosterone synthase inhibition with lorundrostat for uncontrolled hypertension. *JAMA*. (2023) 330:1140. doi: 10.1001/jama.2023.16029

9. Sloan-Lancaster J, Raddad E, Flynt A, Jin Y, Voelker J, Miller JW. LY3045697: results from two randomized clinical trials of a novel inhibitor of aldosterone synthase. *JRAAS - J Renin-Angiotensin-Aldosterone System*. (2017) 18:147032031771788. doi: 10.1177/1470320317717883

10. Mulatero P, Wuerzner G, Groessl M, Sconfienza E, Damianaki A, Forestiero V, et al. Safety and efficacy of once-daily dexfadrostat phosphate in patients with primary aldosteronism: a randomised, parallel group, multicentre, phase 2 trial. *EClinicalMedicine*. (2024) 71:102576. doi: 10.1016/j.eclinm.2024.102576

11. Lerario AM, Finco I, LaPensee C, Hammer GD. Molecular mechanisms of stem/progenitor cell maintenance in the adrenal cortex. *Front Endocrinol (Lausanne)*. (2017) 8:52. doi: 10.3389/fendo.2017.00052

12. Van Slycke S, Van Den Heede K, Vandenwyngaerden E-A. Adrenal glands: anatomy, physiology, and pathophysiology. In: *Endocrine Surgery Comprehensive Board Exam Guide*. Springer International Publishing, Cham (2021). p. 437–55. doi: 10.1007/978-3-030-84737-1_17

13. Pignatti E, Leng S, Carlone DL, Breault DT. Adrenal zonation and development. In: *Adrenal Disorders: Physiology, Pathophysiology and Treatment*. Cham: Humana Press (2018), 3–13. doi: 10.1007/978-3-319-62470-9_1

14. Su Y, Ge R-S, Xie H. Steroidogenesis of corticosteroids, genetic mutation, and endocrine disruption leading to adrenal insufficiency. In: *Cortisol - Between Physiology and Pathology*. London: IntechOpen (2023). doi: 10.5772/intechopen.1003273

15. Mihai R. Physiology of the pituitary, thyroid and adrenal glands. *Surgery*. (2011) 29:419–27. doi: 10.1016/j.mpsur.2011.06.017

16. Bauer MB, Currie KPM. Adrenal medulla hormones. In: *Hormonal Signaling in Biology and Medicine*. Amsterdam: Elsevier (2020). p. 635–53. doi: 10.1016/B978-0-12-813814-4.00029-8

17. Kim J-H, Choi MH. Embryonic development and adult regeneration of the adrenal gland. *Endocrinol Metab*. (2020) 35:765–73. doi: 10.3803/EnM.2020.403

18. Pignatti E, du Toit T, Flück CE. Development and function of the fetal adrenal. *Rev Endocr Metab Disord*. (2023) 24:5–21. doi: 10.1007/s11154-022-09756-3

19. Oikonomakos I, Weerasinghe Arachchige LC, Schedl A. Developmental mechanisms of adrenal cortex formation and their links with adult progenitor populations. *Mol Cell Endocrinol*. (2021) 524:111172. doi: 10.1016/j.mce.2021.111172

20. Xing Y, Lerario AM, Rainey W, Hammer GD. Development of adrenal cortex zonation. *Endocrinol Metab Clin North Am*. (2015) 44:243–74. doi: 10.1016/j.ecl.2015.02.001

21. Shanahan D, Jay Lennard TW. Adrenal embryology, anatomy, and physiology. In: *Endocrine Surgery*. Springer, London (2009). p. 337–42. doi: 10.1007/978-1-84628-881-4_24

22. Wood MA, Acharya A, Finco I, Swonger JM, Elston MJ, Tallquist MD, et al. Fetal adrenal capsular cells serve as progenitor cells for steroidogenic and stromal adrenocortical cell lineages in M. *musculus*. *Dev*. (2013) 140:4522–32. doi: 10.1242/dev.092775

23. Kim AC, Barlaskar FM, Heaton JH, Else T, Kelly VR, Krill KT, et al. In search of adrenocortical stem and progenitor cells. *Endocr Rev*. (2009) 30:241–63. doi: 10.1210/er.2008-0039

24. Ishimoto H, Jaffe RB. Development and function of the human fetal adrenal cortex: a key component in the feto-placental unit. *Endocr Rev*. (2011) 32:317–55. doi: 10.1210/er.2010-0001

25. Lotfi CFP, Kremer JL, Passaia BDS, Cavalcante IP. The human adrenal cortex: growth control and disorders. *Clinics*. (2018) 73:e473s. doi: 10.6061/clinics/2018/e473s

26. Vinson GP. Functional zonation of the adult mammalian adrenal cortex. *Front Neurosci*. (2016) 10:238. doi: 10.3389/fnins.2016.00238

27. Vidal V, Sacco S, Rocha AS, Da Silva F, Panzolini C, Dumontet T, et al. The adrenal capsule is a signaling center controlling cell renewal and zonation through Rspo3. *Genes Dev*. (2016) 30:1389–94. doi: 10.1101/gad.277756.116

28. Walczak EM, Kuick R, Finco I, Bohin N, Hrycaj SM, Wellik DM, et al. Wnt signaling inhibits adrenal steroidogenesis by cell-autonomous and non–cell-autonomous mechanisms. *Mol Endocrinol*. (2014) 28:1471–86. doi: 10.1210/me.2014-1060

29. Zhou T, Luo P, Wang L, Yang S, Qin S, Wei Z, et al. CTNNB1 knockdown inhibits cell proliferation and aldosterone secretion through inhibiting Wnt/β-catenin signaling in H295R cells. *Technol Cancer Res Treat*. (2020) 19:153303382097968. doi: 10.1177/1533033820979685

30. Little DW, Dumontet T, LaPensee CR, Hammer GD. β-catenin in adrenal zonation and disease. *Mol Cell Endocrinol*. (2021) 522:111120. doi: 10.1016/j.mce.2020.111120

31. King P, Paul A, Laufer E. Shh signaling regulates adrenocortical development and identifies progenitors of steroidogenic lineages. *Proc Natl Acad Sci*. (2009) 106:21185–90. doi: 10.1073/pnas.0909471106

32. Laufer E, Kesper D, Vortkamp A, King P. Sonic hedgehog signaling during adrenal development. *Mol Cell Endocrinol*. (2012) 351:19–27. doi: 10.1016/j.mce.2011.10.002

33. Mateska I. Primary cilia and Sonic hedgehog signaling in adrenal gland physiology and cancer. *Curr Opin Endocr Metab Res*. (2024) 34:100507. doi: 10.1016/j.coemr.2024.100507

34. Shimada H, Yamazaki Y, Sugawara A, Sasano H, Nakamura Y. Molecular mechanisms of functional adrenocortical adenoma and carcinoma: genetic characterization and intracellular signaling pathway. *Biomedicines*. (2021) 9:892. doi: 10.3390/biomedicines9080892

35. Basham KJ, Rodriguez S, Turcu AF, Lerario AM, Logan CY, Rysztak MR, et al. A ZNRF3-dependent Wnt/β-catenin signaling gradient is required for adrenal homeostasis. *Genes Dev*. (2019) 33:209–20. doi: 10.1101/gad.317412.118

36. Drelon C, Berthon A, Sahut-Barnola I, Mathieu M, Dumontet T, Rodriguez S, et al. PKA inhibits WNT signalling in adrenal cortex zonation and prevents Malignant tumour development. *Nat Commun*. (2016) 7:12751. doi: 10.1038/ncomms12751

37. Pitsava G, Maria AG, Faucz FR. Disorders of the adrenal cortex: Genetic and molecular aspects. *Front Endocrinol (Lausanne)*. (2022) 13:931389. doi: 10.3389/fendo.2022.931389

38. Hao H-X, Jiang X, Cong F. Control of Wnt receptor turnover by R-spondin-ZNRF3/RNF43 signaling module and its dysregulation in cancer. *Cancers (Basel)*. (2016) 8:54. doi: 10.3390/cancers8060054

39. Salmon TN, Zwemer RL. Life history of cortico-adrenal gland cells of the rat by means of trypan blue injections. *Anat Rec*. (1941) 80:421–9. doi: 10.1002/ar.1090800404

40. Freedman BD, Kempna PB, Carlone DL, Shah MS, Guagliardo NA, Barrett PQ, et al. Adrenocortical zonation results from lineage conversion of differentiated zona glomerulosa cells. *Dev Cell*. (2013) 26:666–73. doi: 10.1016/j.devcel.2013.07.016

41. Bandiera R, Vidal VPI, Motamedi FJ, Clarkson M, Sahut-Barnola I, vonGise A, et al. WT1 maintains adrenal-gonadal primordium identity and marks a population of AGP-like progenitors within the adrenal gland. *Dev Cell*. (2013) 27:5–18. doi: 10.1016/j.devcel.2013.09.003

42. del Valle I, Young MD, Kildisiute G, Ogunbiyi OK, Buonocore F, Simcock IC, et al. An integrated single-cell analysis of human adrenal cortex development. *JCI Insight*. (2023) 8:e168177. doi: 10.1172/jci.insight.168177

43. McEwan PE, Lindop GB, Kenyon CJ. Control of cell proliferation in the rat adrenal gland in *vivo* by the renin-angiotensin system. *Am J Physiology-Endocrinology Metab*. (1996) 271:E192–8. doi: 10.1152/ajpendo.1996.271.1.E192

44. Heikkilä M, Peltoketo H, Leppäluoto J, Ilves M, Vuolteenaho O, Vainio S. Wnt-4 deficiency alters mouse adrenal cortex function, reducing aldosterone production. *Endocrinology*. (2002) 143:4358–65. doi: 10.1210/en.2002-220275

45. Shaikh LH, Zhou J, Teo AED, Garg S, Neogi SG, Figg N, et al. LGR5 activates noncanonical Wnt signaling and inhibits aldosterone production in the human adrenal. *J Clin Endocrinol Metab*. (2015) 100:E836–44. doi: 10.1210/jc.2015-1734

46. Mete O, Erickson LA, Juhlin CC, de Krijger RR, Sasano H, Volante M, et al. Overview of the 2022 WHO classification of adrenal cortical tumors. *Endocr Pathol*. (2022) 33:155–96. doi: 10.1007/s12022-022-09710-8

47. Juhlin CC, Bertherat J, Giordano TJ, Hammer GD, Sasano H, Mete O. What did we learn from the molecular biology of adrenal cortical neoplasia? From histopathology to translational genomics. *Endocr Pathol*. (2021) 32:102–33. doi: 10.1007/s12022-021-09667-0

48. Hodgson A, Pakbaz S, Mete O. A diagnostic approach to adrenocortical tumors. *Surg Pathol Clin*. (2019) 12:967–95. doi: 10.1016/j.path.2019.08.005

49. Pons Fernández N, Moreno F, Morata J, Moriano A, León S, De Mingo C, et al. Familial hyperaldosteronism type III a novel case and review of literature. *Rev Endocr Metab Disord*. (2019) 20:27–36. doi: 10.1007/s11154-018-9481-0

50. Azizan EAB, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet*. (2013) 45:1055–60. doi: 10.1038/ng.2716

51. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet*. (2013) 45:440–4. doi: 10.1038/ng.2550

52. Mohideen SK, Mustangin M, Kamaruddin NA, Muhammad R, Jamal ARA, Sukor N, et al. Prevalence and histopathological characteristics of KCNJ5 mutant aldosterone-producing adenomas in a multi-ethnic Malaysian cohort. *Front Endocrinol (Lausanne)*. (2019) 10:666. doi: 10.3389/fendo.2019.00666

53. Mulatero P, Monticone S, Rainey WE, Veglio F, Williams TA. Role of KCNJ5 in familial and sporadic primary aldosteronism. *Nat Rev Endocrinol*. (2013) 9:104–12. doi: 10.1038/nrendo.2012.230

54. Berthon A, Sahut-Barnola I, Lambert-Langlais S, de Joussineau C, Damon-Soubeyrand C, Louiset E, et al. Constitutive β-catenin activation induces adrenal hyperplasia and promotes adrenal cancer development. *Hum Mol Genet*. (2010) 19:1561–76. doi: 10.1093/hmg/ddq029

55. Nanba K, Vaidya A, Williams GH, Zheng I, Else T, Rainey WE. Age-related autonomous aldosteronism. *Circulation*. (2017) 136:347–55. doi: 10.1161/CIRCULATIONAHA.117.028201

56. Omata K, Satoh F, Morimoto R, Ito S, Yamazaki Y, Nakamura Y, et al. Cellular and genetic causes of idiopathic hyperaldosteronism. *Hypertension*. (2018) 72:874–80. doi: 10.1161/HYPERTENSIONAHA.118.11086

57. Azizan EAB, Drake WM, Brown MJ. Primary aldosteronism: molecular medicine meets public health. *Nat Rev Nephrol*. (2023) 19:788–806. doi: 10.1038/s41581-023-00753-6

58. Gomez-Sanchez CE, Qi X, Velarde-Miranda C, Plonczynski MW, Parker CR, Rainey W, et al. Development of monoclonal antibodies against human CYP11B1 and CYP11B2. *Mol Cell Endocrinol*. (2014) 383:111–7. doi: 10.1016/j.mce.2013.11.022

59. Zhou J, Azizan EAB, Cabrera CP, Fernandes-Rosa FL, Boulkroun S, Argentesi G, et al. Somatic mutations of GNA11 and GNAQ in CTNNB1-mutant aldosterone-producing adenomas presenting in puberty, pregnancy or menopause. *Nat Genet*. (2021) 53:1360–72. doi: 10.1038/s41588-021-00906-y

60. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension*. (2014) 64:354–61. doi: 10.1161/HYPERTENSIONAHA.114.03419

61. Pauzi FA, Azizan EA. Functional characteristic and significance of aldosterone-producing cell clusters in primary aldosteronism and age-related hypertension. *Front Endocrinol (Lausanne)*. (2021) 12:631848. doi: 10.3389/fendo.2021.631848

62. Teo AED, Garg S, Haris Shaikh L, Zhou J, Karet Frankl FE, Gurnell M, et al. Pregnancy, primary aldosteronism, and adrenal CTNNB1 mutations. *New Engl J Med*. (2015) 373:1429–36. doi: 10.1056/NEJMoa1504869

63. Calhoun DA, White WB, Krum H, Guo W, Bermann G, Trapani A, et al. Effects of a novel aldosterone synthase inhibitor for treatment of primary hypertension. *Circulation*. (2011) 124:1945–55. doi: 10.1161/CIRCULATIONAHA.111.029892

64. Creemers SG, Feelders RA, De Jong FH, Franssen GJH, De Rijke YB, Van Koetsveld PM, et al. Osilodrostat is a potential novel steroidogenesis inhibitor for the treatment of cushing syndrome: an in *vitro* study. *J Clin Endocrinol Metab*. (2019) 104:3437–49. doi: 10.1210/jc.2019-00217

65. Brixius-Anderko S, Scott EE. Aldosterone synthase structure with cushing disease drug LCI699 highlights avenues for selective CYP11B drug design. *Hypertension*. (2021) 78:751–9. doi: 10.1161/HYPERTENSIONAHA.121.17615

66. Duggan S. Osilodrostat: first approval. *Drugs*. (2020) 80:495–500. doi: 10.1007/s40265-020-01277-0

67. Perosevic M, Tritos NA. Clinical utility of osilodrostat in cushing's disease: review of currently available literature. *Drug Des Devel Ther*. (2023) 17:1303–12. doi: 10.2147/DDDT.S315359

68. Freeman MW, Bond M, Murphy B, Hui J, Isaacsohn J. Results from a randomized, open-label, crossover study evaluating the effect of the aldosterone synthase inhibitor baxdrostat on the pharmacokinetics of metformin in healthy human subjects. *Am J Cardiovasc Drugs*. (2023) 23:277–86. doi: 10.1007/s40256-023-00572-x

69. Freeman M, Bond M, Murphy B, Isaacsohn J. Results from a phase 1 multiple ascending dose study demonstrating safety and selectivity of aldosterone synthase inhibitor CIN-107. *Eur Heart J*. (2022) 43:ehac544.2200. doi: 10.1093/eurheartj/ehac544.2200

70. Bogman K, Schwab D, Delporte ML, Palermo G, Amrein K, Mohr S, et al. Preclinical and early clinical profile of a highly selective and potent oral inhibitor of aldosterone synthase (CYP11B2). *Hypertension*. (2017) 69:189–96. doi: 10.1161/HYPERTENSIONAHA.116.07716

71. Forzano I, Mone P, Varzideh F, Jankauskas SS, Kansakar U, De Luca A, et al. The selective aldosterone synthase inhibitor baxdrostat significantly lowers blood pressure in patients with resistant hypertension. *Front Endocrinol (Lausanne)*. (2022) 13:1097968. doi: 10.3389/fendo.2022.1097968

72. Lamberts SWJ, Bruining HA, Marzouk H, Zuiderwijk J, Uitterlinden P, Blidj JJ, et al. The new aromatase inhibitor CGS-16949A suppresses aldosterone and cortisol production by human adrenal cells. *vitro*. *J Clin Endocrinol Metab*. (1989) 69:896–901. doi: 10.1210/jcem-69-4-896

73. Pignatti E, Kollar J, Hafele E, Schuster D, Steele RE, Vogt B, et al. Structural and clinical characterization of CYP11B2 inhibition by dexfadrostat phosphate. *J Steroid Biochem Mol Biol*. (2023) 235:106409. doi: 10.1016/j.jsbmb.2023.106409

74. Rodman D, Weir MR, Slingsby B, Laffin L, Nissen SE. Highly effective blood pressure lowering with MLS-101, a new aldosterone synthase inhibitor, in individuals with obesity and RAAS dysregulation. *J Am Coll Cardiol*. (2023) 81:306. doi: 10.1016/S0735-1097(23)00750-7

75. Fujii A, Hiraga Y, Kawai M, Ogawa K, Ohta Y, Rahman SMA, et al. First-in-human study of MLS-101, a potent and highly selective aldosterone synthase inhibitor. *J Am Coll Cardiol*. (2023) 81:616. doi: 10.1016/S0735-1097(23)01060-4

76. Liu W, Li Z, Chu S, Ma X, Wang X, Jiang M, et al. Atractylenolide-I covalently binds to CYP11B2, selectively inhibits aldosterone synthesis, and improves hyperaldosteronism. *Acta Pharm Sin B*. (2022) 12:135–48. doi: 10.1016/j.apsb.2021.09.013

77. Shimada H, Hata S, Yamazaki Y, Otsubo Y, Sato I, Ise K, et al. YM750, an ACAT Inhibitor, acts on adrenocortical cells to inhibit aldosterone secretion due to depolarization. *Int J Mol Sci*. (2022) 23:12803. doi: 10.3390/ijms232112803

78. Berber M, Leng S, Wengi A, Winter DV, Odermatt A, Beuschlein F, et al. Calcineurin regulates aldosterone production via dephosphorylation of NFATC4. *JCI Insight*. (2023) 8:e157027. doi: 10.1172/jci.insight.157027

79. Lee G, Makhanova N, Caron K, Sequeira Lopez ML, Gomez RA, Smithies O, et al. Homeostatic responses in the adrenal cortex to the absence of aldosterone in mice. *Endocrinology*. (2005) 146:2650–6. doi: 10.1210/en.2004-1102

80. De Sousa K, Boulkroun S, Baron S, Nanba K, Wack M, Rainey WE, et al. Genetic, cellular, and molecular heterogeneity in adrenals with aldosterone-producing adenoma. *Hypertension*. (2020) 75:1034–44. doi: 10.1161/HYPERTENSIONAHA.119.14177

81. Scholl UI, Nelson-Williams C, Yue P, Grekin R, Wyatt RJ, Dillon MJ, et al. Hypertension with or without adrenal hyperplasia due to different inherited mutations in the potassium channel KCNJ5. *Proc Natl Acad Sci U.S.A*. (2012) 109:2533–8. doi: 10.1073/pnas.1121407109

82. Yang Y, Gomez-Sanchez CE, Jaquin D, Aristizabal Prada ET, Meyer LS, Knösel T, et al. Primary aldosteronism: KCNJ5 mutations and adrenocortical cell growth. *Hypertension*. (2019) 74:809–16. doi: 10.1161/HYPERTENSIONAHA.119.13476

83. Long KC, Sukor N, Tan GC, Murad NAA, Brown MJ, Azizan EA. PS-B04-9: Elucidating effect of inhibiting aldosterone synthesis on human adrenocortical cells. *J Hypertens*. (2023) 41:e362. doi: 10.1097/01.hjh.0000916468.72978.94

84. Fan YJ, Zong WX. The cellular decision between apoptosis and autophagy. *Chin J Cancer*. (2013) 32:121–9. doi: 10.5732/cjc.012.10106

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 21 August 2024
DOI 10.3389/fendo.2024.1419028

OPEN ACCESS

EDITED BY
Carmela Maniero,
Barts Health NHS Trust, United Kingdom

REVIEWED BY
Dimitrios Chantzichristos,
University of Gothenburg, Sweden
Anli Tong,
Peking Union Medical College Hospital (CAMS), China

*CORRESPONDENCE
Wolfgang Raber
wolfgang.raber@meduniwien.ac.at

RECEIVED 17 April 2024
ACCEPTED 29 July 2024
PUBLISHED 21 August 2024

CITATION
Raber W, Schendl R, Arikan M, Scheuba A, Mazal P, Stadlmann V, Lehner R, Zeitlhofer P, Baumgartner-Parzer S, Gabler C and Esterbauer H (2024) Metastatic disease and major adverse cardiovascular events preceding diagnosis are the main determinants of disease-specific survival of pheochromocytoma/paraganglioma: long-term follow-up of 303 patients. *Front. Endocrinol.* 15:1419028. doi: 10.3389/fendo.2024.1419028

Metastatic disease and major adverse cardiovascular events preceding diagnosis are the main determinants of disease-specific survival of pheochromocytoma/paraganglioma: long-term follow-up of 303 patients

Wolfgang Raber[1]*, Raphael Schendl[1], Melisa Arikan[2], Andreas Scheuba[2], Peter Mazal[3], Valerie Stadlmann[4], Reinhard Lehner[4], Petra Zeitlhofer[5], Sabina Baumgartner-Parzer[1], Cornelia Gabler[6] and Harald Esterbauer[7]

[1]Department of Medicine III, Clinical Division of Endocrinology and Metabolism, Medical University of Vienna, Vienna, Austria, [2]Department of Surgery, Medical University of Vienna, Vienna, Austria, [3]Department of Clinical Pathology, Medical University of Vienna, Vienna, Austria, [4]Department of Medical Genetics, Medical University of Vienna, Vienna, Austria, [5]Labdia Labordiagnostik, and St. Anna Children's Cancer Research Institute (CCRI), Vienna, Austria, [6]Department of IT Systems and Communications, Medical University of Vienna, Vienna, Austria, [7]Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria

Purpose: The natural history in unselected cohorts of patients with pheochromocytoma/ paraganglioma (PPGL) followed for a period >10 years remains limited. We aimed to describe baseline characteristics and outcome of a large cohort and to identify predictors of shorter survival.

Methods: This retrospective single-center study included 303 patients with newly diagnosed PPGL from 1968 to December 31, 2023, in 199 prospectively supplemented since July 2020. Mean follow-up was 11.4 (range 0.3-50) years, germline genetic analyses were available in 92.1%. The main outcome measures were overall (OAS), disease-specific (DSS), recurrence-free (RFS) survival and predictors of shorter survival evaluated in patients with metastases at first diagnosis (n=12), metastatic (n=24) and nonmetastatic (n=33) recurrences and without evidence of PPGL after first surgery (n=234).

Results: Age at study begin was 49.4 ± 16.3 years. There were 72 (23.8%) deaths, 15 (5.0%), 29 (9.6%) and 28 (9.2%) due to PPGL, cardiovascular disease (CVD) and malignant or other diseases, respectively. Median OAS, DSS1 (tumor-related) and DSS2 (DSS1 and death caused by CVD) were 4.8, 5.9 and 5.2 years (patients with metastases at first diagnosis), 21.2, 21.2 and 19.9 years, and 38.0, undefined and 38.0 years (patients with metastatic and with nonmetastatic recurrences, respectively). Major adverse cardiovascular events (MACE) preceded the first diagnosis in 15% (n=44). Shorter DSS2 correlated with older age ($P \leq 0.001$), male sex ($P \leq 0.02$), MACE ($P \leq 0.01$) and primary metastases ($P<0.0001$, also for DSS1).

Conclusion: The clinical course of unselected patients with PPGL is rather benign. Survival rates remain high for decades, unless there are MACE before diagnosis or metastatic disease.

KEYWORDS

pheochromocytoma, paraganglioma, recurrence, survival, genetics, natural history, long-term follow-up

1 Introduction

Pheochromocytoma (PCC) and paraganglioma (PGL), together PPGL, are rare neuro-endocrine tumors occurring with an annual incidence of 0.04-0.66 per 100.000 individuals (1, 2). These tumors have the highest degree of heritability of all human neoplasias. Approximately 40% of patients harbor a pathogenic germline mutation in one of the about 20 driver genes discovered so far (3). Discovery may be either incidental, due to screening procedures or because of adrenergic symptoms. Many patients with PPGL may in fact have symptoms suggestive of catecholamine excess, yet these may remain unrecognized by patients and/or physicians even for years (4, 5). Clinical presentation of PPGL with life-threatening major cardiovascular events (MACE) are dramatic challenges to patients and physicians (6–10). The prognostic impact to long-term prognosis of different modes of clinical presentation is not known, however.

Knowledge of the natural history of PPGL in unselected cohorts followed for a period >10 years remains limited. Most studies report overall survival (OAS). The primary measure of interest, the disease-specific survival (11), has, however, rarely been assessed and if so, cohorts were either highly selected or included patients from multiple centers of many countries and definitions of ´disease-specific´ were not uniform (12–14). Reported independent prognostic factors of survival differ greatly, and no study has included the mode of presentation, especially the occurrence of MACE prior to first diagnosis, in the survival analyses (12–19).

We report a cohort of 303 patients with newly diagnosed PPGL followed for up to 50 years and aimed to assess detailed baseline characteristics, OAS, disease-specific survival (DSS) and recurrence-free (RFS) survival, in addition to predictors of shorter survival in patients with primary metastases (n=12), metastatic (n=24) and nonmetastatic (n=33) recurrences, as well as without evidence of PPGL after first surgery (n=234). At least one molecular genetic analysis for germline variants associated with hereditary disease was available in 279 patients (92.1%) - in 153 (50.5% of the total cohort) by state-of-the-art next generation sequencing (NGS). In July 2020, the study was approved by the Ethics Board of the Medical University of Vienna (Nr. 1022/2020).

2 Materials and methods

2.1 Patients

A thorough search of the electronic patient management system of the Vienna General Hospital for the years 1992 to 2023 and of the electronic coding system of the Clinical Division of Endocrinology & Metabolism, Department of Medicine III of the Vienna General Hospital for the years 1968 to 2017, were performed. The former database covers all patients admitted to the Vienna General Hospital, the latter all visits to the out-patient unit of the Division of Endocrinology. The database query was for Codes of the International Classification of Disease Version 10 and 9 (ICD-10 and ICD-9) with known association to PPGL that had been entered into PDF documents of the discharge and surgical reports (Details in the Supplementary Material). Inclusion criteria were either, histopathological proof of PPGL after surgery (n=299) or the diagnosis of PPGL by (18) Fluoro-dihydrophenylalanine (F-DOPA) positron-emission tomography (PET)-CT (n=3) or biopsy (n=1) in four multimorbid patients refusing surgery. Patients treated for a malignant comorbidity at the time of first diagnosis of PPGL were excluded.

The first systematic search of the Austrian Death Registry in July 2020 identified 58 (19.1%) deceased patients (data current until December 31, 2019). According to Austrian law, health data after death may be used for scientific purpose without informed consent. Thus, the data of these 58 deceased patients were included in the analyses. All survivors by January 1, 2020 were then invited for further prospective evaluation, which 199 patients (81.2%) accepted. All 199 have since been seen on a regular out-patient basis by one of the authors (W.R.) until today. The second consultation of the Austrian Death Registry in October 2023 (data current through December 31, 2022) resulted in additional ten nonsurvivors. The remaining 32 patients were contacted by telephone and four additional deaths were identified. The other 28 of these 32 patients refused to present for FU examination, but missing data of their medical history could be collected. By the end of the study, there were 72 nonsurvivors (23.8%) and 231 (76.2%) survivors. One hundred fifty-nine (68.8%) survivors were last seen in 2023, an additional 40 (17.3%) patients in 2022. A flowchart of the study cohort is shown in Figure 1.

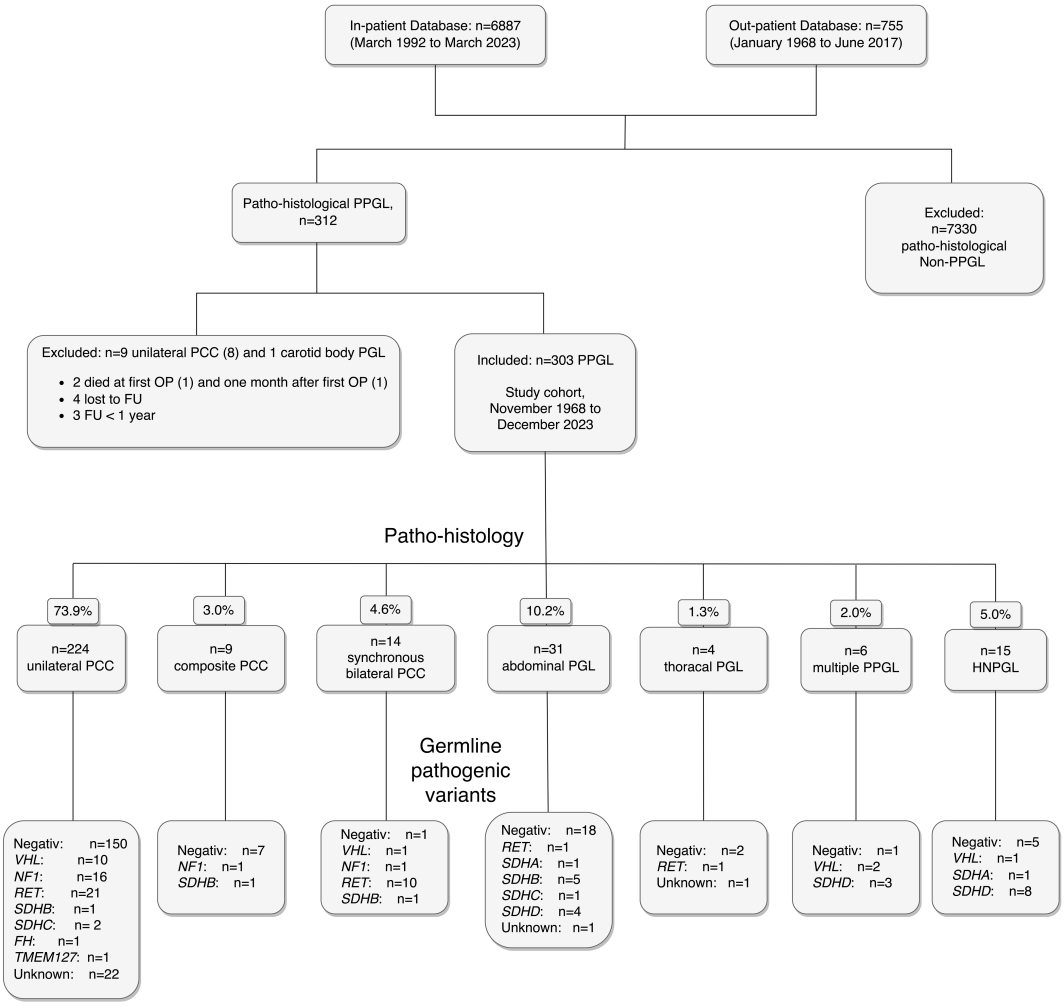


FIGURE 1
Flowchart of the study cohort of 303 patients with PPGL, including histopathological diagnoses and pathogenic germline genetic findings. Abbreviations are those used in the text.

2.2 Methods

2.2.1 Clinical presentation and comorbidities

Nine clinical scenarios leading to the diagnosis of PPGL were identified in 291 (96.0%) patients: (a) incidental diagnosis during imaging or laboratory procedures performed for reasons unrelated to PPGL, (b) screening in the context of familial syndromes, (c) adrenergic symptoms (at least two of the typical triad of paroxysmal headache, palpitations and diaphoresis), diagnostic work-up for (d) uncontrolled or (e) suspected secondary hypertension, (f) signs and symptoms (growing lump in the neck, hearing problems) suggestive of head-and-neck PGL (HNPGL), (g) incidental despite MACE within five years preceding the first diagnosis of PPGL, (h) uncontrolled hypertension despite MACE within five years preceding the first diagnosis and (i) MACE leading to the diagnosis (Table 1). For the purposes of this study, MACE were defined as a life threatening event of the cerebrovascular, cardiovascular or peripheral arterial system including transient ischemic attacks (TIA), prolonged reversible ischemic deficits (PRIND), ischemic or hemorrhagic stroke, acute coronary syndrome (ACS), non ST-elevation myocardial infarction (NSTEMI), ST-elevation myocardial infarction (STEMI), non-specified acute MI (AMI), dissecting aortic aneurysm or critical peripheral artery ischemia.

To allow sufficient power for the Cox regression analyses, the nine parameters were combined to establish three groups: all MACE together (the MACE group), patients with adrenergic symptoms, uncontrolled hypertension or the suspicion of secondary hypertension (the group of non-MACE symptoms) and patients diagnosed incidentally or due to screening procedures (the oligosymptomatic group). The MACE group included both MACE before first diagnosis and during FU, provided plasma metanephrines (P-MNs) or 24-hour unrinary metanephrines (U-MNs) were diagnostic of recurrence of PPGL (20) around the time of MACE. Of note, incidental diagnosis did not mean, that these patients were asymptomatic. The number of incidentally diagnosed patients suffering from various symptoms that had gone unnoticed to patients and/or physicians or those with MACE were significant and are detailed in Supplementary Tables 1 and 2, respectively.

TABLE 1 Characteristics of the study cohort (n=303 PPGL) followed for up to 50 years according to survivors (n=231) and nonsurvivors (n=72).

	All (n=303)	Survivors (n=231)	Non-survivors (n=72)	p-value
Age at 1st surgery in years, mean ± SD	49.4 ± 16.3	46.7 ± 15.3	58.0 ± 16.6	<0.0001
>49a, n (%)	156 (51.5)	105 (45.5)	51 (70.8)	0.0002
Female sex, n (%)	167 (55.1)	135 (58.4)	32 (44.4)	0.04
Histopathological diagnosis				**0.26**
PCC group, all, n (%)	247 (81.5)	190 (82.3)	57 (79.2)	
uPCC unilateral, n (%)	224 (73.9)	173 (74.9)	51 (70.8)	
cPCC unilateral, n (%)	9 (3.0)	6 (7.5)	3 (4.2)	
bPCC synchronous, n (%)	14 (4.6)	11 (4.8)	3 (4.2)	
PGL group, all, n (%)	41 (13.5)	28 (12.1)	13 (18.1)	
aPGL, n (% PGL not HNPGL)	31 (88.6)	21 (95.5)	10 (76.9)	
tPGL, n (% PGL not HNNPGL)	4 (11.4)	1 (4.5)	3 (23.1)	
mPPGL synchronous	6 (2.0)	6 (2.6)	0	
HNPGL	15 (5.0)	13 (5.6)	2 (2.8)	
Clinical scenarios at presentation				**0.0004**
Oligosymptomatic group, n (%)	103 (34.0)	84 (36.4)	19 (26.4)	
a) Incidental, n (%)	89 (29.4)	71 (30.7)	18 (25.0)	0.65 (incidental vs. all other)
≥2 classical symptoms, n (% incidental)	13 (14.6)	11	2	
Weight loss, n	5	4	1	
Abdominal or back pain, n	5	2	3	
Hypertension, n	21	17	4	
Really no symptoms, n	45	37	8	
b) Screening, n (%)	14 (4.6)	13 (5.6)	1 (1.4)	0.21 (all screening vs. all other presentations)
≥2 classical symptoms, n	1	1	0	
Weight loss, n	1	1	0	
Hearing problems, n	1	1	0	
Really no symptoms, n	12	11	1	
Non-MACE symptomatic group, n (%)	144 (47.5)	119 (51.5)	25 (34.7)	0.004 (adrenergic sympt vs. all other pres)
c) Adrenergic symptoms, n (%)	96 (31.7)	84 (36.4)	12 (16.7)	0.59 (uncontr hypertens vs. all other pres)
d) Uncontrolled hypertension, n (%)	22 (7.3)	16 (6.9)	6 (8.3)	0.34 (susp of 2nd hypertens vs. all other pres)
e) Suspicion of secondary hypertension, n (%)	15 (5.0)	10 (32.2)	5 (6.9)	0.0003 (MACE leading to dx vs. all other pres)
f) Lump in the neck/hearing problems, n (%)	11 (3.6)	9 (3.9)	2 (2.8)	
MACE group, all, n (%)	44 (14.5)	23 (10.0)	21 (29.2)	0.19 (MACE not leading to dx vs. all other pres)
g) Incidental despite prior MACE, n (%)	22 (7.3)	14 (6.1)	8 (11.1)	<0.0001 (all MACE vs. all other presentations)
h) Uncontr hypertension desp prior MACE, n (%)	4 (1.3)	2 (0.8)	2 (2.8)	0.0005 (MACE vs. asympt)
k) MACE, n (%)	18 (5.9)	7 (3.0)	11 (15.3)	0.0001 (MACE vs. non-MACE sympt)
Unknown, n (%)	12 (4.0)	5 (2.2)	7 (9.7)	0.87 (Asympt vs. non-MACE sympt)
Comorbidities				**<0.0001**
No comorbid disease group, n (%)	151 (49.8)	135 (58.4)	16 (22.2)	

(Continued)

TABLE 1 Continued

	All (n=303)	Survivors (n=231)	Non-survivors (n=72)	p-value
Comorbidities				<0.0001
No comorbid disease, n (%)	44 (14.5)	37 (16.0)	7 (9.7)	<0.0001 (No comorb. vs all comorb comb)
≥ 2CV risk factors (CV RF), n (%)	107 (35.3)	98 (42.4)	9 (12.5)	<0.0001 (≥ 2CV RF vs. all comorb comb)
CV disease (CVD), n (%)	81 (26.7)	50 (21.6)	31 (43.1)	0.86 (CVD vs. other comorbidities)
Malignancy dominated group, n (%)	52 (17.2)	38 (16.5)	14 (19.4)	0.02 (CVD vs. all other comb)
Malignant disease without CV RF, n (%)	22 (7.3)	15 (6.5)	7 (9.7)	0.02 (Mal+CVD vs. Mal dom)
Malignant disease with CV RF, n (%)	19 (6.3)	14 (6.1)	5 (6.9)	
Other*, n (%)	11 (3.6)	9 (3.9)	2 (2.8)	0.13 (CVD vs. Mal+CVD)
Malignant disease +CVD, n (%)	19 (6.3)	8 (3.5)	11 (15.3)	0.19 (CVD vs. Mal dom)
Genetic results				<0.0001
Positive, n (%)	95 (31.4)	76 (32.9)	19 (26.4)	<0.0001 (pos vs. unknown)
Cluster 1A, n (% of positive)	29 (30.2)	25 (33.3)	4 (21.1)	<0.0001 (neg vs. unknown)
Cluster 1B, n (% of positive)	14 (14.6)	14 (18.7)	0	0.88 (pos vs. neg)
Cluster 2, n (% of positive)	52 (55.2)	37 (48.7)	15 (78.9)	
Negative, n (%)	184 (60.7)	148 (64.1)	36 (50.0)	0.02 (Cluster 2 vs. Cluster 1)
Unknown, n (%)	24 (7.9)	7 (3.0)	17 (23.6)	
Death in the 1990s, 2000s, 2010s, 2020s, n			6, 7, 2, 2	
Tumor size in cm, mean ± SD	5.3 ± 2.8	4.8 ± 2.3	6.9 ± 3.5	<0.0001
<6cm, n (% of data available)	186 (65.3)	157 (71.4)	29 (44.6)	0.0003
≥6cm, n (% of data available)	99 (34.7)	63 (28.6)	36 (55.4)	
Primary metastatic, n (%)	12 (4.0)	1 (0.4)	11 (15.3)	<0.0001
of which local Lnn., n (%)	6 (50.0)	1 (100.0)	5 (45.5)	
of which distant sites, n (%)	6 (50.0)	0	6 (54.5)	

Cluster 1A included pathogenic germline variants of succinate dehydrogenase subunits A-D (SDHA-D) and fumarate hydratase (FH), cluster 1B of von Hippel-Lindau (VHL) tumor suppressor gene and cluster 2 of rearranged-during-transfection (RET) proto-oncogene, neurofibromin 1 (NF1) tumor suppressor gene and transmembrane protein 127 (TMEM127). More detailed characteristics of patients are given in Supplementary Table 4. *Details of "other" comorbidities are given in Supplementary Table 3.

The following comorbidities were assessed for all 303 patients: no symptoms; at least three of the following typical cardiovascular (CV) risk factors (RF): type-2 diabetes, hypertension, hypercholesterolemia, smoking or obesity; patients with established CVD or pulmonary disease, including atrial fibrillation or chronic obstructive pulmonary disease (COPD); patients with malignant disease; with malignant disease and CV RF; with malignant disease and concurrent CVD together; and patients with other illnesses (Table 1). Stage I tumors according to the TNM classification (21) were not considered malignant comorbidities. The detailed comorbidities of patients are given in Supplementary Table 3.

For the Cox regression, the comorbidity parameters were grouped as follows: no comorbidities and patients with CV RFs together (the non-disease group), patients with CVD or COPD as outlined above (the CVD group), malignancies with or without CV RF including other illness (the malignancy dominated group) and patients with malignancies and concurrent CVD, suspected to represent patients with the most serious comorbidities. For the prognostic model of the 291 patients with nonmetastatic disease at the begin of the study, the first two (the non-disease and the CVD) groups of patients and the latter two (those with malignant comorbidities) were considered together, respectively.

2.2.2 Biochemical evaluation and imaging

Preoperative biochemical test results were available for 259 patients (85.5%). U-MNs were determined in 159 (52.5%), P-MNs in 132 (43.6%) and simultaneously in 112 (37.0%). Current assays for the measurements of U-MNs and P-MNs in our hospital were described previously (22). Further details as to biochemical testing and imaging procedures are described in the Supplementary Material (Supplementary Text and Supplementary Table 4).

2.2.3 Surgery, histopathological diagnosis, PASS- and GAPP scores

The surgical approach to the first operation was available for 299 (98.7%) patients. Four multimorbid patients diagnosed with

PPGL by F-DOPA PET-CT (n=3) or biopsy (n=1) refused surgery but were included in the analyses.

For 285 patients (94.1% of the total cohort) with histopathological confirmation of PPGL, the surgical specimens were examined by pathologists of the Vienna General Hospital with extensive experience in the diagnosis of neuro-endocrine tumors, for 14 patients (4.6%) the examination was performed elsewhere. The histopathological diagnoses were unilateral PCC (uPCC), unilateral composite PCC (PCC with variable proportions of ganglioneuroma cells, cPCC), synchronous bilateral PCC (bPCC), abdominal and intrathoracic PGL (aPGL and tPGL), synchronous multiple PGL (mPPGL) with (n=3) or without (n=3) PCC and HNPGL (Figure 1). Bilateral PCC was defined by either, tumorous lesions in both adrenals (n=12) or a unilateral tumor with contralateral diffuse or nodular hyperplasia of the adrenal medulla (n=2) (23). Occurrence was defined as synchronous, when PPGL were diagnosed simultaneously or within three months of each other and as recurrent PPGL, when there were more than three months in between the diagnoses. Twelve patients (3.4% of the study cohort) had primary metastatic PPGL. The remaining 291 patients were disease free after the first operation, as assessed by postoperative biochemical results within the reference range (n=245), by normal postoperative imaging (n=25) or both (n=31). For the Cox regression, patients with uPCC, cPCC and bPCC (the PCC group) and those with aPGL, tPGL and mPGL with or without PCC (the PGL group) were combined to create 3 variables (PCC, PGL and HNPGL) (Table 1). Pheochromocytoma of the Adrenal gland Scaled Score (PASS) was available for 214 (80.6%) patients, the Grading for Adrenal Pheochromocytoma and Paraganglioma (GAPP) score for 73 (24.1%). Patients were assessed for PASS score <4 and ≥4 points and for GAPP score <3, 3-6 and ≥7 points, equivalent to suggested low and high risk (PASS), and low, medium and high risk (GAPP) for metastatic recurrence, respectively (24, 25).

2.2.4 Family history and germline genetic analysis (Table 1)

2.2.4.1 Family history

Family history was available for 277 patients (91.4%). Great attention was paid to assess diseases related to multiple endocrine neoplasia type 2A (MEN-2A), von Hippel-Lindau syndrome (VHL), neurofibromatosis type 1 (NF1) and familial PGL syndromes. Family history was considered positive when patients reported either the established diagnosis of PPGL or an associated genetic syndrome in their family. Premature CVD or symptoms suggestive of PPGL in relatives were not considered positive family history.

2.2.4.2 Germline genetic testing

Germline genetic analyses were performed in 279 patients (92.1%) of our study cohort. Four additional patients (1.3%) with NF-1 were diagnosed on clinical grounds (26). During the study period, there have been four different laboratories involved, two between the mid-1990s and mid-2010s responsible for the germline analyses of RET and of RET, SDHB, SDHC, SDHD, VHL, respectively, and two currently performing NGS and the neuroendocrine gene panel, respectively (Supplementary Table 5). More than 100 patients had NGS testing complementary to their sequencing of RET, SDHB, SDHC, SDHD and VHL genes in the past. Currently, NGS results are available for 153 patients (50.5%) of the cohort (in 126 simultaneously with neuroendocrine gene panel analyses), with the trend increasing. The close cooperation and regular boards between the endocrinologist (W.R.) and the genetic specialists (H.E., V.St. and R.L.) ensured best possible clinical interpretation of identified variants, leading to additional in-depth analyses upon availability of new clinical information. For the Cox regression, pathogenic genetic variants of individual genes are considered within distinct clusters (cluster 1A, 1B and 2) according to current definitions (27). The genetic methods used are described in detail in the Supplementary Text.

2.3 Statistics

Categorical variables are presented using number (%) of subjects, continuous data as mean ± SD or as median (range) depending on data type and distribution. Whenever possible, comparisons were made with the Chi Square test, the Fishers´ exact test, the one-sided t-test, the Mann-Whitney U test, one-way analysis of variance (ANOVA) followed by Tukey´s multiple comparisons test or the Kruskal-Wallis test followed by Dunn´s multiple comparisons test, as appropriate.

The length of FU for the OAS was defined as the number of years from the date of first surgery to that of death from any cause or to the date last known alive. Four patients of our cohort did not have surgery and were included from the date of first diagnosis of PPGL. The date of death and the cause of death (ICD-code) were obtained from the death registry of Statistik Austria, the national death registry of Austria, from hospital charts or from relatives of the patients. The latter two were contributing to less than 5% of all identified deaths as the only source. Data of the Austrian death registry are current until December 31, 2022. An additional four deceased patients were identified between January 1, 2023 and December 31, 2023 (the end of the study period) through personal contact with family members. Given that this number was not lower than the mean annual death rate in our cohort, we have chosen to include these four patients in the analyses. Patients with PPGL are at risk of MACE and death from acute cardiovascular events (6–10). DSS as defined by the number of years from first surgery to the disease-specific death or the date last known alive, was therefore analyzed using two definitions: first, death due to PPGL-related oncological causes (DSS1), second, DSS1 plus death due to CVD (DSS2).

The length of RFS was determined as the number of years from first surgery to the date of first occurrence of metastatic or nonmetastatic recurrence of PPGL or to the date last seen recurrence-free. The post-recurrence survival (PRS) was calculated as the difference of OAS minus RFS in relapsing patients. Recurrence was diagnosed by histopathological examination (either at autopsy or of a biopsy or surgical specimen) or by F-DOPA based PET-CT scans. Elevated P-MNs or U-MNs by themselves were not considered evidence of recurrence, unless confirmed by functional imaging or histopathology. Malignant recurrence was defined by

histopathological (autopsy or surgery) and F-DOPA positive evidence of PPGL in organs where chromaffin cells are not present physiologically, such as lymph nodes, bone and – as multiple lesions - the liver, nonmalignant recurrence by evidence of PPGL in the adrenals, sympathetic or parasympathetic ganglia. There was no occurrence of a single liver lesion in any patient which could have given rise to misinterpretation (23). The Kaplan-Meier method was used to estimate survival as a function of time after first diagnosis or after first recurrence and comparisons of curves were made using the log-rank test. Cox proportional hazard regression was utilized to examine potential predictor variables of OAS, DSS1, DSS2 and RFS. Additional statistical considerations are given in the Supplementary Material.

P-values <0.05 were considered significant. All computations were performed using GraphPad Prism version 10.1.1 for Mac, GraphPad Software, San Diego, California, USA, www.graphpad.com.

3 Results

3.1 Characteristics of patients (Table 1)

A total of 6887 in-patients and 755 out-patients were assessed for eligibility, of which 312 patients with a histopathological diagnosis of PPGL were identified. In a study of 165 operations of patients with PCC from France, including 23 patients with malignant tumors at the time of surgery, perioperative mortality and morbidity were significant (4 deaths, 38 other complications including 13 spleen resections and hematomas), but mortality was not related to PCC (28). In our hospital, two patients with PPGL died within 90 days of first surgery (one due to air embolism during minimal invasive approach and one due to heart attack one month after the operation). They were excluded, as were seven additional patients (three with FU of less than 3 months and four lost to FU within 3 months after first surgery). The remaining 303 patients were followed for up to 50 years (mean ± SD 11.4 ± 9.2) years and represent the cohort of this study.

The age of our patients was 49.4 ± 16.3 years at study begin. Nonsurvivors were older than survivors (p<0.0001). There were slightly, but not significantly, more females in the entire cohort. Survivors were more often female (p=0.04).

The proportions of PCC (173 vs. 51 uPCC, 6 vs. 3 cPCC, 11 vs. 3 bPCC) and of PGL not HNPGL (21 vs. 10 aPGL, 1 vs 3 tPGL) were not different between survivors and nonsurvivors (p=0.26). There were more mPGL and more HNPGL in survivors than in nonsurvivors (6 vs zero and 13 vs 2, respectively).

Incidental discovery and diagnosis by screening were observed in 103 (34.0%) and 14 (4.6%) of the cohort, respectively, with no difference between survivors and nonsurvivors. Adrenergic symptoms with or without hypertension were leading to the diagnosis of PPGL in 96 patients (31.7%), more often in survivors than in nonsurvivors (p=0.04). Evaluation for uncontrolled or secondary hypertension gave rise to the diagnosis of PPGL in 22 (7.3%) and 15 (5.0%) of patients and no difference was observed between survivors and nonsurvivors. MACE lead to the diagnosis of PPGL in 18 patients (5.9%), more often in nonsurvivors than in survivors (p<0.0001). MACE not leading to the diagnosis of PPGL jeopardized the life of 26 patients (8.6%), not different in survivors versus nonsurvivors. All MACE together occurred in 44 patients (14.5%), more often in nonsurvivors than in survivors (p<0.0001). Details of patients with incidentally detected PPGL and those with MACE are given in Supplementary Tables 1, 2.

Comorbidities were detected in 152 patients (50.2%), no comorbid disease and RF of CVD in 44 (14.5%) and 107 (35.3%) patients, respectively. CVD, patients with malignancy dominated disorders and those with both CVD and malignant disease were prevalent in 81 (26.7%), 52 (17.2%) and 19 (6.3%) of the cohort. Patients without comorbidities and CV RF (but no established CVD) were more common in survivors than in nonsurvivors (p<0.0001 for both comparisons), whereas CVD was more frequent in nonsurvivors than in survivors (p=0.02). When the prevalence of CVD was compared to the other comorbidities combined, that difference became nonsignificant. Details of comorbidities before first diagnosis of the total cohort are given in Supplementary Table 3.

Details of biochemical test results and imaging data are given in the Supplementary Material (Supplementary Text and Supplementary Table 4).

Family history was positive in 48 patients (15.8%) and negative in 228 (75.2%). No difference was observed between survivors and nonsurvivors regarding both the prevalence of positive and negative family history. The number of patients with unknown, as compared to positive and negative family history was smaller in survivors than in nonsurvivors (<0.0001 for both comparisons). Pathogenic variants in one of the driver genes were detected in 47 of the 48 patients (97.9%) with a positive family history and in 36 (15.8%) of those with negative family history (Supplementary Table 4).

Germline genetic analyses were available for 279 patients (92.1%), positive in 96 (31.4%) and negative in 184 (60.7%). Four additional patients were diagnosed with NF1 due to clinical criteria. There was no difference of positive or negative findings between survivors and nonsurvivors. 17 nonsurvivors (23.6%) and 7 survivors (3%) had missing genetic information (p<0.0001), excluding the four with clinically diagnosed NF1. Pathogenic variants of cluster 1A, 1B and 2 genes were detected in 29, 14 and 53 patients with positive genetic results (30.2%, 14.6% and 55.2%), respectively (Table 1 and Supplementary Table 7).

Details of radiological imaging results, surgical approaches, experience of surgeons, duration of surgery, PASS and GAPP-score, decades of surgery and years of last FU of survivors or death of nonsurvivors, as appropriate, are given in the Supplementary Material (Supplementary Text and Supplementary Table 4).

Details of metastatic and nonmetastatic recurrences are given in the Supplementary Material.

3.2 Survival

3.2.1 Overall survival, disease-specific survival 1 and 2

A total of 72 patients (23.8%) died during the study period. The duration of FU was not different (p=n.s.) between survivors and nonsurvivors (11.4 ± 9.4 vs. 11.1 ± 8.7 years, respectively) nor between patients with PPGL-associated oncological death

(n=15), death from cardiovascular disease (n=29) or from malignant and other causes (n=28), respectively (6.9 ± 5.6 years vs. 11.7 ± 10.3 years vs. 12.7 ± 7.8 years). More survivors than nonsurvivors were seen in the 2020s (p<0.0001). Most nonsurvivors died in the 2010s.

Nonsurvivors died with comparable relative frequency (p=0.40) from PPGL, CVD or malignant disease in the 1990s, 2000s, 2010s and 2020s (Supplementary Table 11). Patients with primary metastatic disease had the worst prognosis: 11 of 12 died after a FU of 5.2 ± 2.9 (median 4.8) years. DSS1 was better than DSS2 (p=0.02) and both better than OAS (p<0.0001 and p=0.036, for the comparison DSS1 vs. OAS and DSS2 vs. OAS, respectively), when the total cohort was considered (Figure 2). At 50 years of FU, the DSS1 was still 88% (95% CI 80-93). The 5-, 10-, 20- and 30-year rates of OAS, DSS1 and DSS2 are summarized in Table 2. The Kaplan Meier product limit estimates of the entire cohort are shown in Figure 2, those of patients with primary metastatic disease vs. metastatic recurrence and of metastatic vs. nonmetastatic recurrences in Figure 3. A Waterfall plot displaying the length of OAS, RFS and PRS of all 303 patients divided by primary metastatic disease, metastatic and nonmetastatic recurrence, no recurrences as well as unknown recurrence is shown in Figure 4. Details of causes of death of the 72 nonsurvivors are given in Supplementary Table 8.

Details as to recurrence free survival (RFS) and post recurrence survival (PRS) are given in the Supplementary Material.

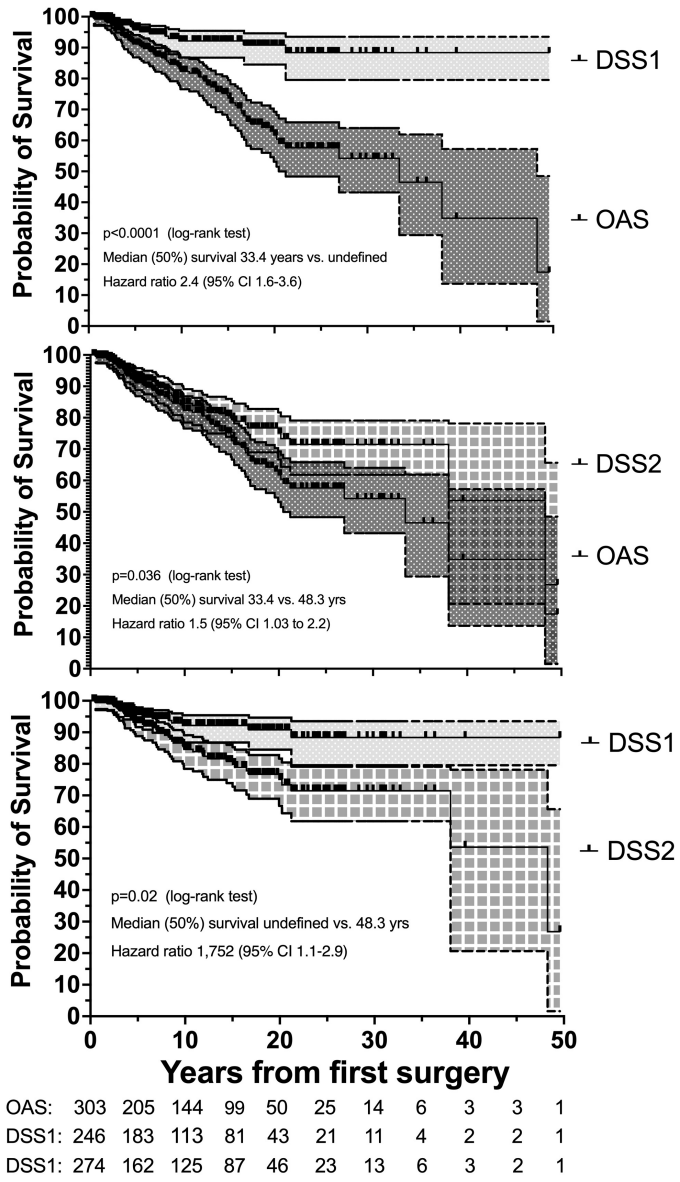


FIGURE 2
Disease-specific survival 1 (DSS1 with 95% CI) is better than DSS1 plus cardiovascular survival (DSS2 with 95% CI), and both are better than overall survival (OAS with 95% CI) in 303 patients with PPGL with a follow-up of up to 50 years. Number (#) at risk denote the number of patients at risk to be censored due to death or loss to FU at the start of the study and after each of the given 5-year intervals.

TABLE 2 Summary of the 5-, 10-, 20- and 30-year survival rates for death of all causes (OAS), PPGL-related death (DSS1) and death due to DSS1 and cardiovascular diseases (CVD) combined (DSS2), including 95% confidence intervals (95% CI) of the total cohort, as well as of patients with metastatic and with nonmetastatic recurrences.

Survival	Total cohort (n=303)		Metast rec (n=24)		Nonmetast rec (n=33)	
	% (95% CI)	# at risk	% (95% CI)	# at risk	% (95% CI)	# at risk
5-year OAS	91 (87-94)	205	82 (58-93)	31	100 (100-100)	18
5-year DSS1	96 (94-98)	162	79 (53-92)	15	100 (100-100)	25
5-year DSS2	93 (89-96)	183	80 (55-92)	16	100 (100-100)	29
10-year OAS	82 (77-87)	144	67 (43-83)	15	96 (76-99)	27
10-year DSS1	92 (87-95)	113	62 (37-80)	12	100 (100-100)	22
10-year DSS2	85 (78-89)	125	64 (39-81)	13	100 (100-100)	26
20-year OAS	63 (54-70)	50	55 (34-74)	9	81 (56-92)	12
20-year DSS1	91 (85-95)	43	56 (31-75)	7	100 (100-100)	10
20-year DSS2	75 (69-82)	46	50 (25-71)	7	89 (64-97)	12
30-year OAS	54 (43-64)	14	39 (15-62)	5	80 (56-92)	8
30-year DSS1	88 (80-93)	11	46 (21-69)	4	100 (100-100)	6
30-year DSS2	71 (62-79)	13	42 (18-65)	4	89 (64-97)	8

Number (#) at risk denote the number of patients at risk to be censored due to death or loss to FU after the 5-, 10-, 20- and 30-year time points.

3.3 Prognostic factors for survival

After fitting the Cox proportional hazard model, the key predictor parameters of shorter OAS probability included primary metastatic disease (HR 11.1, 95% CI 4.1-27.9), MACE (HR 2.7, 95% CI 1.2-6.1), comorbid malignancies irrespective of potential additional CVD (HR 2.5, 95% CI 1.3-4.9, male sex (HR 2.0, 95% CI 1.1-3.6) and higher age (HR 1.05, 95% CI 1.03-1.07). The key predictor parameters of shorter DSS2 were primary metastatic disease (HR 23.3, 95% CI 8.1-62.5), male sex (HR 2.6, 95% CI 1.2-5.6), MACE (HR 4.5, 95% CI 1.7-12.2) and higher age (HR 1.06, 95% CI 1.03-1.09). The small number of tumor-related deaths (15 patients) and the overwhelming prognostic influence of primary metastatic disease on DSS1 (HR 41.8, 95% CI 13.2-129) precluded the evaluation of potential additional risk factors (Table 3).

When only considering patients at risk for metastasis after first surgery (e.g. excluding those with metastatic disease at first diagnosis), independent prognostic factors changed only slightly. Comorbid malignant disease (HR 4.6, 95% CI 2.0-11.0) instead of malignant with or without CVD was predictive of shorter OAS and metastatic recurrence (HR 2.9, 95% CI 1.02-7.8) of shorter DSS2. All nine deaths due to PPGL-related causes could be predicted by metastatic recurrence, precluding the evaluation of any other variable (Supplementary Table 12).

Predictive factors for recurrence-free survival are given in the Supplementary Material (Supplementary Text and Supplementary Table 13).

4 Discussion

Our single-center cohort of 303 patients with newly diagnosed PPGL was followed for 11.4 ± 9.2 (mean ± SD, range 0.3-50) years. We present the first study, that meets the FU duration of at least 10 years recommended by the European Society of Endocrinology for patients with PPGL (29). To the best of our knowledge, this is the first single-center study assessing comorbidities, detailed symptoms/presentations leading to the diagnosis of PPGL and other characteristics in a large unselected cohort of patients with PPGL and using these parameters for analysis of overall and disease specific survival, as well as recurrence-free survival during a sufficiently long FU.

4.1 Clinical presentation and prognostic implications

In our cohort, there were 111 (36.6%) patients with incidentally discovered PPGL, 14 (4.6%) patients diagnosed due to screening and 165 (54.5%) patients detected due to clinical suspicion. Those identified by chance were older and had genetic abnormalities less frequently as compared to PPGL detected due to either clinical suspicion or screening, which is in line with other authors (3). PPGL have recently reported to be most frequently discovered incidentally (4, 30, 31), with up to 69% of 132 patients with PCC in one series from Great Britain. Others, on the other hand, have reported lower rates of incidentally discovered patients (32–34). Our patients were most commonly diagnosed due to clinical suspicion. Tumor size did not differ irrespective of the mode of discovery which is in contrast to others reporting that incidentally discovered PPGL were smaller than those detected due to clinical suspicion, but larger than tumors identified due to screening (30). In our study, larger tumor size was predictive of metastatic recurrence and DSS1, while smaller tumor size was predictive of nonmetastatic recurrence. Larger tumor size has been reported to be predictive of recurrent PPGL by some (35),

10.3389/fendo.2024.1419028

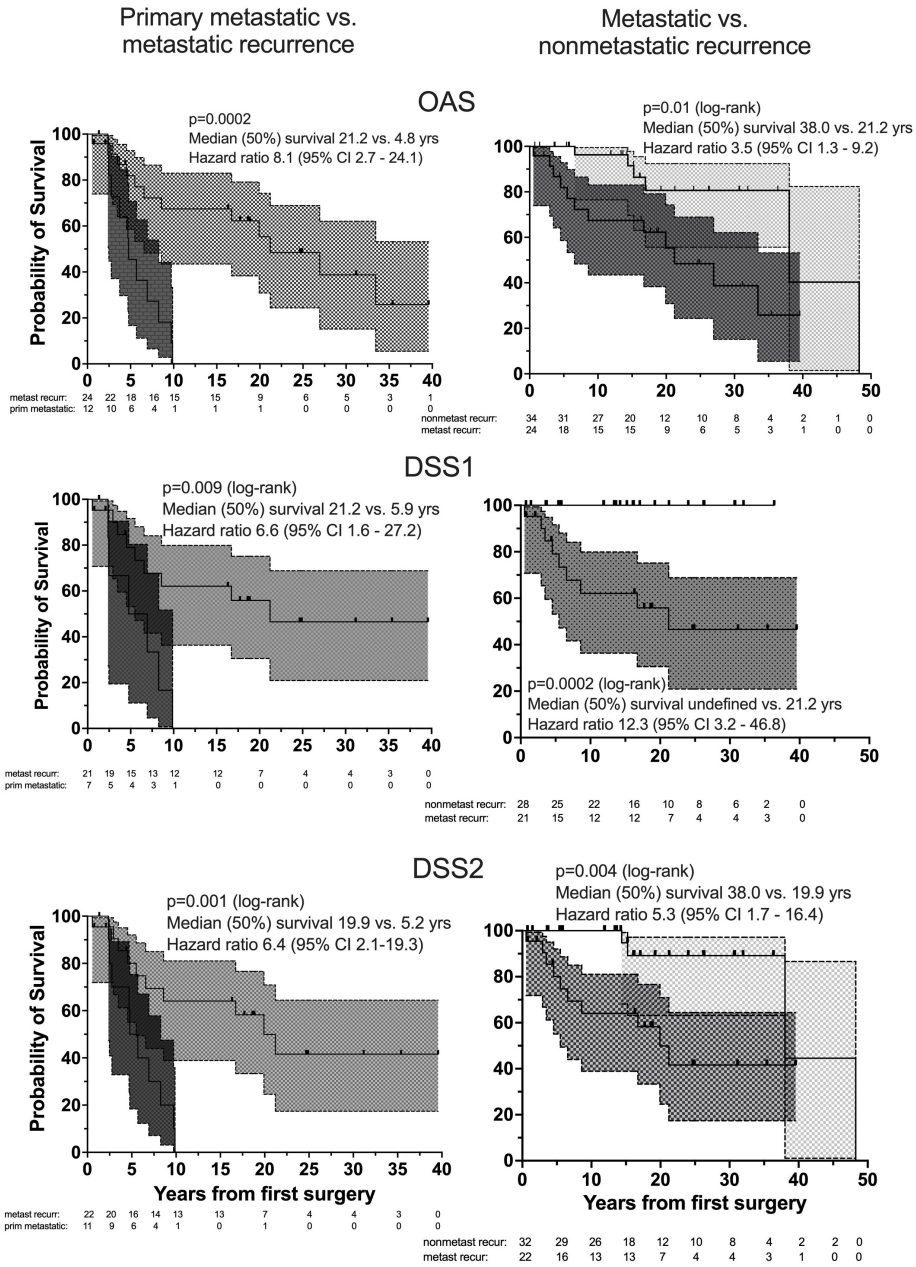


FIGURE 3
Overall survival, OAS (top), DSS1 (middle) and DSS2 (bottom) of patients with primary metastatic (dark gray), metastatic recurrent (medium gray) and nonmetastatic recurrent (light gray) PPGL by Kaplan Meier curves with 95% CI bands (left: comparison between patients with primary metastatic disease and metastatic recurrence, right: between metastatic and nonmetastatic recurrent PPGL). Number (#) at risk denote the number of patients at risk to be censored due to death or loss to FU at the start of the study and after each of the given 5-year intervals.

but not all (18, 36), of shorter OAS by some (37–39), but not all (16, 18, 35, 36, 40, 41) and of shorter DSS by some (12, 38), but not all (14, 16, 41) authors. The heterogenous design as to patient selection, some studies pulling from national or regional cancer databases, others being single center or multicenter studies, with variable proportions of patients with malignant PPGL either at diagnosis or during FU, differences in the frequency of genetic testing and of positive genetic results or distinct pathogenic gene variants and the highly variable duration of FU limit the comparability among different studies. It has been acknowledged that up to 40% of patients with incidentally detected PPGL in fact suffered from adrenergic symptoms prior to diagnosis, symptoms that had apparently gone unnoticed by patients and/or their physicians (30, 42). The proportion of patients with PPGL suffering from MACE prior to diagnosis or the impact of different modes of clinical presentations on DSS have not yet been studied. In our study, 36 of the 103 patients (35.0%) with incidentally discovered PPGL were in fact highly symptomatic, 14 with ≥2 typical adrenergic symptoms

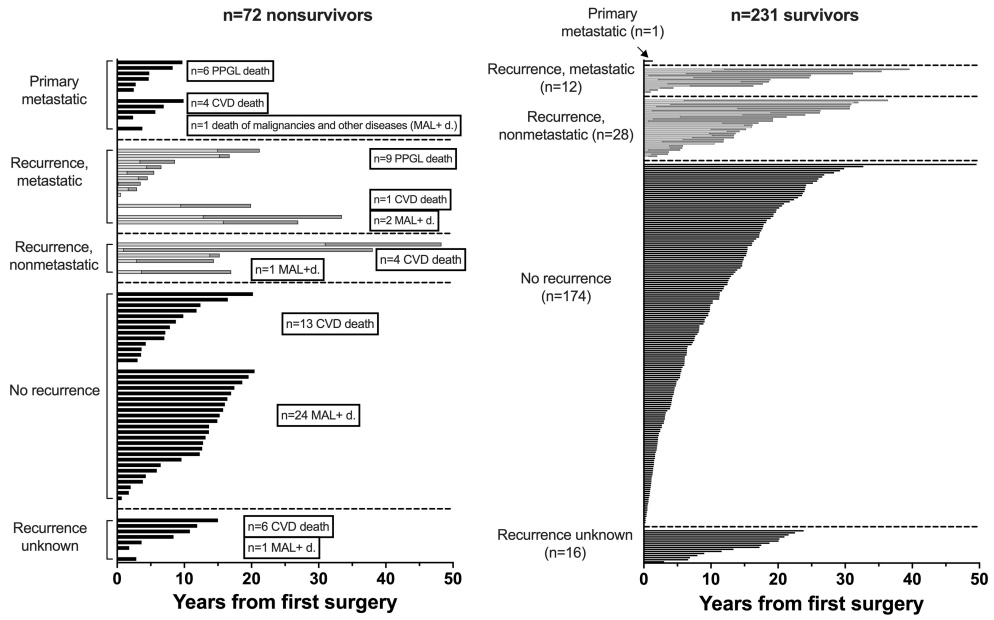


FIGURE 4
Waterfall plot of overall survival (OAS, black bars), recurrence free survival (RFS, bright bars) and post recurrence survival (PRS, grey bars) over a follow-up of 50 years of 303 patients with PPGL (RFS+PRS=OAS in recurrent disease).

and 22 with MACE a median time of 6 (range 0.5-60) months prior to first surgery. A total of 44 patients (14.5% of the study cohort) suffered from life-threatening MACE prior to the discovery of the tumors. This incidence is in line with others reporting a frequency of 18-19% life-threatening cardiovascular complications in patients with PPGL (7, 43). In 26 of these 44 patients (59.1%), the MACE did not lead to clinical suspicion of PPGL (the diagnosis established by chance in 22 and due to diagnostic work-up for uncontrolled hypertension in 4 patients), not more frequently in nonsurvivors than in survivors. Four of these 26 patients survived a second MACE 12, 18, 26 and 35 months after the first, respectively (all prior to first diagnosis of PPGL). In 18 patients, the MACE was the key factor for the discovery of PPGL, more frequently identified in nonsurvivors than in survivors. Two of the 44 MACE (two transient ischemic attacks during hypertensive crisis) occurred during core needle biopsy of the adrenal tumor prior to biochemical assessment. A recent systematic review of 56 studies including a total of 86 patients (34% with metastatic disease) with a history of core needle biopsy (CNB) reported a 23.1% incidence of complications. No CNB related death was described, but complications requiring hospitalization or intervention occurred in 4 of 27 patients (two AMI, one Takotsubo syndrome, one temporal duodenal obstruction caused by hematoma) and CNB related catecholamine symptoms including hypertensive crisis in 8 of 25 patients (44). In our cohort, MACE was the risk factor with the second largest hazard ratio for death due to all causes (HR 2.7, 95% CI 1.2-6.1) as well as due to PPGL and CVD together (HR 4.5, 95% CI 1.7-12.2). Incidental diagnosis did not confer any prediction of survival. Of note, adrenergic symptoms that to physicians may be most suggestive of PPGL was chosen as reference in the Cox regression (Table 3). Data regarding missed clinical clues and a delayed diagnosis of PPGL have been published previously (45), but the prognostic significance as to survival of both has not been evaluated. In a population-based study from Denmark, median duration of symptoms prior to the diagnosis of PPGL of a cohort of 192 patients was 1.7 years, with 26.4% having symptoms for ≥5 years (46). In our study, MACE not leading to the diagnosis of PPGL occurred well within 5 years before first diagnosis. Thus, the high catecholamine serum concentrations due to undiagnosed secretory PPGL over a period of months or even years may have been at least contributory to the MACE. Catecholamine induced damage to the heart and vessels is well known in PPGL (6, 47) and is, in part, reversible after adrenalectomy for PCC (48, 49). Left ventricular ejection fraction improves after surgical therapy, but systolic and diastolic myocardial strain impairment, as well as focal and diffuse myocardial necrosis identifiable as cardiac MRI abnormalities persist beyond those seen in hypertensive patients alone (50).

4.2 Survival

Overall mortality of our cohort was 23.8% (72 of 303 patients). This is comparable to the mortality of adrenal adenomas in a population-based setting (51). Although the proportion of patients with CVD in our cohort (26.7%) was similar to that at time zero of those of the adrenal adenoma study (all different CV entities together 16-32%), patients with adrenal adenomas were older, individuals <20 years excluded and FU duration was shorter than in our cohort. In addition, the patterns of survival curves differ. The

10.3389/fendo.2024.1419028

TABLE 3 Predictive factors for overall survival (OAS), disease-specific survival 1 (DSS 1), disease-specific survival 2 (DSS 2) and recurrence-free survival (RFS, all recurrences) for 303 patients with PPGL by Cox regression.

	Reference	OAS		DSS 1		DSS 2		RFS	
		HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	per year	1.05 (1.03-1.07)	<0.0001			1.06 (1.03-1.09)	<0.0001	0.96 (0.93-0.99)	0.0006
Sex	female	2.0 (1.1-3.6)	0.02			2.6 (1.2-5.6)	0.01		
Location	PCC								
	PGL			~~3.5 (0.5-9.0)~~**	~~0.20~~				
	HNPGL			~~0.2 (0.02-1.3)~~**	~~0.13~~				
Comorbidity	No disease								
	CVD							4.0 (1.7-9.2)	0.001
	Mal	2.5 (1.3-4.9)	0.005					1.3 (0.7-2.7)	0.41
	Mal+CVD							0.7 (0.1.2.6)	0.65
Symptoms	Typical								
	MACE	2.7 (1.2-6.1)	0.01	~~3.6 (0.73-14.6)~~**	~~0.08~~	4.5 (1.7-12.2)	0.002		
	Oligosympt	1.6 (0.8-3.2)	0.19	~~1.02 (0.2-4.1)~~**	~~0.98~~	2.0 (0.8-5.1)	0.14		
Secretory	yes								
Tumor size	per cm								
Meta at first diagnosis	no	11.1 (4.1-27.0)	<0.0001	41.8 (13.2-129)*	<0.0001	23.3 (8.1-62.5)	<0.0001		
Metast rec	no								
Genetics	neg							3.1 (1.5-6.8)	0.002
Genetic cluster	neg								
	Cluster 1A							11.1 (4.7-27.5)§	<0.0001
	Cluster 1B							0.9 (0.2-3.2)§	0.87
	Cluster 2							1.7 (0.7-4.1)§	0.26

§ HR for RFS of genetics (all positive vs. all negative findings) and of the three clusters are from two different models, keeping the other parameters constant. These two models included 9.6 and 8.7 parameters per event, respectively. When all positive genetic findings were replaced by the three clusters (RFS), the significance of the other parameters remained unchanged. *Given the low number of events (n=15 deaths from PPGL), the predictive model for DSS1 precluded the analysis of more than one factor. ** Exchanging symptoms before diagnosis (or other dependent variables) for primary metastatic disease led to statistical overfitting for DSS1 and was not better than the simpler model containing no covariates (the crossed-out HR and p-values for location and symptoms before diagnosis are for illustrative purposes only).

linear increase of mortality of adrenal adenomas contrasts with the comparatively steeper curves of our patients with PPGL. In our study, the death rate due to all causes was higher in first 20 years of FU, in the first 5-10 years of patients with metastatic recurrence and highest in the first 5 years in patients with primary metastatic disease (Figure 3). In our cohort, OAS was independently predicted by age, sex, malignant comorbidities, MACE and primary metastatic disease. When assessing survival of patients with PPGL, most studies refer to overall survival. However, 28 of 72 nonsurvivors (38.9%) of our study died of causes unrelated to PPGL and in an additional 29 patients (40.3%) the cause of death was cardiovascular, leaving a tumor-related (oncological) mortality of 5.0% of the total cohort.

There is a great heterogeneity in disease-specific outcome studies. Between 1997 and 2023, we have identified 17 studies (12–17, 32, 33, 52–60) reporting number and proportion of patients with disease-specific death (DSD), seven that included 5- and 10-year DSS (12, 14, 17, 38, 56, 59, 60) and three with analyses of risk factors of DSS by Cox regression analysis (12, 14, 16). In the four studies including patients with malignant PPGL only, DSD was uniformly defined as tumor-associated and ranged from 26.8% to 46.2% (12, 13, 16, 56). The participants in a study from the NIH were highly selected, included 27 (20.5%) children <19 years and 73 of 132 patients had pathogenic variants of the SDHB gene (56). Two studies including patients with bilateral PCC (53, 54) and one with locally advanced PPGL (15) described ≤5 patients with DSD, corresponding to a DSD rate of 4.8% to 5.5% over a median FU period of 4.5 to 8.5 years. Another two studies of PCC only and of secreting PPGL reported ≤6 DSD, corresponding to a DSD rate of 2.6% to 5.1% (17, 32, 59). These findings are in line with the results of our DSD rate (15 PPGL-related oncological deaths, 5.0%). The 5-year and 10-year DSS (including 95% CI) in studies of patients with

exclusively malignant PPGL ranged from 62% to 96.2% (12, 13, 38, 56) and from 63.5 to 86.4% (12, 38, 56) respectively. In studies, that also included patients without metastatic disease at the beginning, the 5- and 10-year DSS (including 95% CI) ranged from 92.2 to 100% and 86.7 to 100%, respectively (17, 59, 60). The DSS of our study (Table 3) are in line with these results. RF for DSD in studies investigating only patients with metastatic PPGL were higher age (12, 16), larger tumor size (12), primary metastatic (12) or all distant metastatic recurrent (16) disease and no surgical therapy (16). In an analysis of the SEER database, Mei L et al. reported hazard ratios of 14 independent variables and of six significant risk factors for DSS (higher age, male sex, a second primary malignancy, aortic/carotid body PGL, distant metastasis and higher TNM stage), but did not describe the number of events nor whether the analysis was univariate or multivariate (41). A systematic review and meta-analysis of patients with metastatic PPGL of 20 retrospective noncomparative studies identified 1338 patients with a mean FU of 6.3 ± 3.2 years. The 5-year (7 studies, n=738 patients) and 10-year (2 studies, n=55 patients) overall mortality rates were 37% (95% CI, 24%-51%) and 29% (95% CI, 17-42%). Higher mortality was associated with male sex and synchronous metastases (61).

There is only one study including death due to CVD in the DSS and reporting predictive factors for that definition of DSS in a cohort of patients with and without metastatic PPGL (14). This study comprised of 639 patients (407 PCC, 175 PGL not HNPGL and 57 HNPGL) from 6 tertiary European centers and from one quaternary referral center in the USA. DSD was defined differently to our study, however, including not only death due to events, that could have been associated with previous long-term or current catecholamine excess (e.g. CV manifestations), but also death caused by peri- or postsurgical complications, metastatic disease or treatment complications. The respective contributions of these causes of death to the 5- and 10-year DSS of 86.1% and 59.8% of 209 (190 PPGL not HNPGL, 19 HNPGL) patients with metastatic (35.9% of the study cohort) and 98.6% and 97.2% of 430 (392 PPGL not HNPGL, 38 HNPGL) patients without metastatic disease were not given. 15% of the cohort presented with a history of recurrent disease at the beginning of the study, more patients (100 of 549 PPGL not HNPGL, 6 of 57 HNPGL, together 16.5%) than of our cohort harbored pathogenic mutations in the SDHB gene, median FU was shorter and the definition of DSD differed from those of DSS1 (PPGL-related) and of DSS2 (PPGL- and CVD-related) of our study. The results are thus not entirely comparable to our study. Still, the long-term DSS after 5, 10 or 20 years were comparable.

Our study has limitations, including the lack of determination of dopamine metabolites, that have been shown to be predictive of DSS in some (14), but not all (12) studies. Elevated dopamine concentrations in urine provide a poor marker of a dopaminergic phenotype (14). However, less than 5% of our patients presented with urinary dopamine concentrations greater than the upper limit of the normal reference range and the biochemical phenotype failed to show a significant association with either recurrence or survival in our study. In addition, the number of events remained rather small, preventing the analysis of more than a few potential risk factors. This is an inherent dilemma of a rare disease, which may be overcome with a multicenter design, yet at the expense of generalizability to clinical practice. Also, we did not assess therapeutic measures other than surgical interventions. However, there is no cure for metastatic PPGL and surgery remains the only effective therapy until today (62). Despite these limitations, our study has unparalleled strengths. We were able to retrieve detailed clinical, biochemical and genetic data and present largely unbiased detailed outcome of the largest single-center cohort reported to date. Our study presents outcome data over the longest follow-up period of patients with PPGL, a study strength that minimized misclassification of patients with metastatic potential among those without evidence of metastases. Of note, two thirds of all patients repeatedly presented to one of us (W.R.) during the last 3 ½ years of the study. We were thus able to prospectively assess comprehensive clinical and up-to-date germline genetic data. Our results are representative of a tertiary referral hospital serving a population of approximately two million.

5 Conclusions

In summary, this study identified major adverse cardiovascular events such as acute myocardial infarctions or stroke prior to diagnosis, occurring in 44 (14.5%) of our patients, as predictors of both shorter overall and disease-specific survival, defined as death due to PPGL-related causes and cardiovascular disease (CVD) for the first time. Primary metastatic disease was the predominant predictive factor for death due to PPGL-related causes, metastatic recurrence predictive of shorter survival due to oncological causes in patients with nonmetastatic disease after the first operation. Higher age and male sex remained independent predictors of death due to all causes and due to PPGL plus CVD. Shorter recurrence free survival was predicted by lower age, CVD prior to first surgery and cluster 1 pathogenic germline variants. There was no disease-specific death in any patient with nonmetastatic recurrences.

Author's note

Presented in part at the 2022 Annual Meeting of the Endocrine Society: Raphael Schendl, Christoph Krall, Wolfgang Raber, OR12-5 | LBSAT57 Long-term Follow-Up of Pheochromocytoma/Paraganglioma (PPGL) after first diagnosis: a retrospective single-center study of 173 patients, Journal of the Endocrine Society, Volume 6, Issue Supplement_1, November-December 2022, Page A85, https://doi.org/10.1210/jendso/bvac150.175.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Ethics Committee, Medical University of Vienna, Borschkegasse 8b/6, 1090 Vienna, Austria. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

WR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. RS: Data curation, Investigation, Writing – review & editing. MA: Data curation, Writing – review & editing. AS: Writing – review & editing. PM: Investigation, Writing – review & editing. VS: Investigation, Writing – review & editing. RL: Investigation, Writing – review & editing. PZ: Investigation, Writing – review & editing. SB-P: Investigation, Writing – review & editing. CG: Data curation, Resources, Writing – review & editing. HE: Investigation, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The constructive criticism of Jonas Stritzker, M.D. is gratefully acknowledged.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1419028/full#supplementary-material

References

1. Al Subhi AR, Boyle V, Elston MS. Systematic review: incidence of pheochromocytoma and paraganglioma over 70 years. *J Endocr Soc*. (2022) 6:1–9. doi: 10.1210/jendso/bvac105

2. Berends AMA, Buitenwerf E, de Krijger RR, Veeger NJGM, van der Horst-Schrivers ANA, Links TP, et al. Incidence of pheochromocytoma and sympathetic paraganglioma in the Netherlands: A nationwide study and systematic review. *Eur J Intern Med*. (2018) 51:68–73. doi: 10.1016/j.ejim.2018.01.015

3. Cascón A, Calsina B, Monteagudo M, Mellid S, Díaz-Talavera A, Currás-Freixes M, et al. Genetic bases of pheochromocytoma and paraganglioma. *J Mol Endocrinol*. (2023) 70:e220167. doi: 10.1530/JME-22-0167

4. Gruber LM, Hartman RP, Thompson GB, McKenzie TJ, Lyden ML, Dy BM, et al. Pheochromocytoma characteristics and behavior differ depending on method of discovery. *J Clin Endocrinol Metab*. (2019) 104:1386–93. doi: 10.1210/jc.2018-01707

5. Falhammar H, Kjellman M, Calissendorff J. Initial clinical presentation and spectrum of pheochromocytoma: a study of 94 cases from a single center. *Endocr Connect*. (2018) 7:186–92. doi: 10.1530/EC-17-0321

6. Y-Hassan S, Falhammar H. Cardiovascular manifestations and complications of pheochromocytomas and paragangliomas. *J Clin Med*. (2020) 9:1–19. doi: 10.3390/jcm9082435

7. Zelinka T, Petrák O, Turková H, Holaj R, Štrauch B, Kršek M, et al. High incidence of cardiovascular complications in pheochromocytoma. *Horm Metab Res*. (2012) 44:379–84. doi: 10.1055/s-0032-1306294

8. Ando Y, Ono Y, Sano A, Fujita N, Ono S, Tanaka Y. Clinical characteristics and outcomes of pheochromocytoma crisis: a literature review of 200 cases. *J Endocrinol Invest*. (2022) 45:2313–28. doi: 10.1007/s40618-022-01868-6

9. De Angelis E, Bochaton T, Ammirati E, Tedeschi A, Polito MV, Pieroni M, et al. Pheochromocytoma-induced cardiogenic shock: A multicentre analysis of clinical profiles, management and outcomes. *Int J Cardiol*. (2023) 383:82–8. doi: 10.1016/j.ijcard.2023.05.004

10. Riester A, Weismann D, Quinkler M, Lichtenauer UD, Sommerey S, Halbritter R, et al. Life-threatening events in patients with pheochromocytoma. *Eur J Endocrinol*. (2015) 173:757–64. doi: 10.1530/EJE-15-0483

11. Mariotto AB, Noone AM, Howlader N, Cho H, Keel GE, Garshell J, et al. Cancer survival: an overview of measures, uses, and interpretation. *J Natl Cancer Inst Monogr*. (2014) 2014:145–86. doi: 10.1093/jncimonographs/lgu024

12. Hamidi O, Young WF, Iñiguez-Ariza NM, Kittah NE, Gruber L, Bancos C, et al. Malignant pheochromocytoma and paraganglioma: 272 patients over 55 years. *J Clin Endocrinol Metab*. (2017) 102:3296–305. doi: 10.1210/jc.2017-00992

13. Hescot S, Curras-Freixes M, Deutschbein T, Van Berkel A, Vezzosi D, Amar L, et al. Prognosis of Malignant pheochromocytoma and paraganglioma (MAPP-prono study): A European network for the study of adrenal tumors retrospective study. *J Clin Endocrinol Metab*. (2019) 104:2367–74. doi: 10.1210/jc.2018-01968

14. Pamporaki C, Prodanov T, Meuter L, Berends AMA, Bechmann N, Constantinescu G, et al. Determinants of disease-specific survival in patients with and without metastatic pheochromocytoma and paraganglioma. *Eur J Cancer*. (2022) 169:32–41. doi: 10.1016/j.ejca.2022.03.032

15. Moog S, Castinetti F, Docao C, Amar L, Hadoux J, Lussey-Lepoutre C, et al. Recurrence-free survival analysis in locally advanced pheochromocytoma: first appraisal. *J Clin Endocrinol Metab*. (2021) 106:2726–37. doi: 10.1210/clinem/dgab202

16. Zheng L, Gu Y, Silang J, Wang J, Luo F, Zhang B, et al. Prognostic nomograms for predicting overall survival and cancer-specific survival of patients with Malignant pheochromocytoma and paraganglioma. *Front Endocrinol (Lausanne)*. (2021) 12:684668. doi: 10.3389/fendo.2021.684668

17. O'Dwyer PJ, Chew C, Zino S, Serpell MG. Long-term follow-up of patients undergoing laparoscopic surgery for phaeochromocytoma. *BJS Open*. (2022) 6:1–5. doi: 10.1093/bjsopen/zrac076

18. Torresan F, Beber A, Schiavone D, Zovato S, Galuppini F, Crimì F, et al. Long-term outcomes after surgery for pheochromocytoma and sympathetic paraganglioma. *Cancers (Basel)*. (2023) 15:1–11. doi: 10.3390/cancers15112890

19. Wang H, Papachristos AJ, Gill AJ, Clifton-Bligh R, Aniss AM, Glover A, et al. Genotype-phenotype correlations and clinical outcomes in 155 cases of pheochromocytoma and paraganglioma. *World J Surg*. (2023) 47:690–8. doi: 10.1007/s00268-022-06862-w

20. Eisenhofer G, Pamporaki C, Lenders JWM. Biochemical assessment of pheochromocytoma and paraganglioma. *Endocr Rev*. (2023) 44:862–909. doi: 10.1210/endrev/bnad011

21. Bertero L, Massa F, Metovic J, Zanetti R, Castellano I, Ricardi U, et al. Eighth Edition of the UICC Classification of Malignant Tumours: an overview of the changes in the pathological TNM classification criteria-What has changed and why? *Virchows Arch*. (2018) 472:519–31. doi: 10.1007/s00428-017-2276-y

22. Raber W, Kotal H, Marculescu R, Scheuba C, Niederle MB, Kautzky-Willer A, et al. Measurements of plasma-free metanephrines by immunoassay versus urinary metanephrines and catecholamines by liquid chromatography with amperometric detection for the diagnosis of pheochromocytoma/paraganglioma. *J Clin Med*. (2020) 9:1–16. doi: 10.3390/jcm9103108

23. Mete O, Asa SL, Gill AJ, Kimura N, de Krijger RR, Tischler A. Overview of the 2022 WHO classification of paragangliomas and pheochromocytomas. *Endocr Pathol*. (2022) 33:90–114. doi: 10.1007/s12022-022-09704-6

24. Koh JM, Ahn SH, Kim H, Kim BJ, Sung TY, Kim YH, et al. Validation of pathological grading systems for predicting metastatic potential in pheochromocytoma and paraganglioma. *PloS One*. (2017) 12:1–14. doi: 10.1371/journal.pone.0187398

25. Wachtel H, Hutchens T, Baraban E, Schwartz LE, Montone K, Baloch Z, et al. Predicting metastatic potential in pheochromocytoma and paraganglioma: A comparison of PASS and GAPP scoring systems. *J Clin Endocrinol Metab*. (2020) 105(12):e4661–70. doi: 10.1210/clinem/dgaa608

26. Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, Evans DG, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. *J Med Genet*. (2007) 44:81–8. doi: 10.1136/jmg.2006.045906

27. Nölting S, Bechmann N, Taieb D, Beuschlein F, Fassnacht M, Kroiss M, et al. Personalized management of pheochromocytoma and paraganglioma. *Endocr Rev*. (2022) 43:199–239. doi: 10.1210/endrev/bnab019

28. Plouin P-F, Duclos J-M, Soppelsa F, Boublil G, Chatellier G. Factors associated with perioperative morbidity and mortality in patients with pheochromocytoma: analysis of 165 operations at a single center. *J Clin Endocrinol Metab*. (2001) 86:1480–6. doi: 10.1210/jc.86.4.1480

29. Plouin PF, Amar L, Dekkers OM, Fassnacht M, Gimenez-Roqueplo AP, Lenders JWM, et al. European Society of Endocrinology Clinical Practice Guideline for long-term follow-up of patients operated on for a phaeochromocytoma or a paraganglioma. *Eur J Endocrinol*. (2016) 174:G1–G10. doi: 10.1530/EJE-16-0033

30. Aggarwal S, Prete A, Chortis V, Asia M, Sutcliffe RP, Arlt W, et al. Pheochromocytomas most commonly present as adrenal incidentalomas: A large tertiary center experience. *J Clin Endocrinol Metab*. (2023) 109(1):e389–96. doi: 10.1210/clinem/dgad401

31. Cvasciuc IT, Gull S, Oprean R, Lim KH, Eatock F. Changing pattern of pheochromocytoma and paraganglioma in a stable UK population. *Acta Endocrinol (Buchar)*. (2020) 16:78–85. doi: 10.4183/aeb.2020.78

32. Kim KH, Chung JS, Kim WT, Oh CK, Chae YB, Yu HS, et al. Clinical experiences of pheochromocytoma in Korea. *Yonsei Med J*. (2011) 52:45–50. doi: 10.3349/ymj.2011.52.1.45

33. Noshiro T, Shimizu K, Watanabe T, Akama H, Shibukawa S, Miura W, et al. Changes in clinical features and long-term prognosis in patients with pheochromocytoma. *Am J Hypertens*. (2000) 13:35–43. doi: 10.1016/S0895-7061(99)00139-9

34. Amar L, Servais A, Gimenez-Roqueplo AP, Zinzindohoue F, Chatellier G, Plouin PF. Year of diagnosis, features at presentation, and risk of recurrence in patients with pheochromocytoma or secreting paraganglioma. *J Clin Endocrinol Metab*. (2005) 90:2110–6. doi: 10.1210/jc.2004-1398

35. Ebbehoj AL, Sondergaard E, Jacobsen SF, Trolle C, Robaczyk MG, Rasmussen AK, et al. Mortality in patients with Pheochromocytoma: a population-based study 1977-2016. *Endocrine Abstracts*. (2018) 56 GP44. doi: 10.1530/endoabs.56.GP44

36. Jimenez C, Ma J, Gonzalez AR, Varghese J, Zhang M, Perrier N, et al. TNM staging and overall survival in patients with pheochromocytoma and sympathetic paraganglioma. *J Clin Endocrinol Metab*. (2023) 108:1132–42. doi: 10.1210/clinem/dgac677

37. Hue JJ, Alvarado C, Bachman K, Wilhelm SM, Ammori JB, Towe CW, et al. Outcomes of Malignant pheochromocytoma based on operative approach: A National Cancer Database analysis. *Surgery*. (2021) 170:1093–8. doi: 10.1016/j.surg.2021.04.001

38. Goffredo P, Sosa JA, Roman SA. Malignant pheochromocytoma and paraganglioma: a population level analysis of long-term survival over two decades. *J Surg Oncol*. (2013) 107:659–64. doi: 10.1002/jso.23297

39. Ayala-Ramirez M, Feng L, Johnson MM, Ejaz S, Habra MA, Rich T, et al. Clinical risk factors for Malignancy and overall survival in patients with pheochromocytomas and sympathetic paragangliomas: primary tumor size and primary tumor location as prognostic indicators. *J Clin Endocrinol Metab*. (2011) 96:717–25. doi: 10.1210/jc.2010-1946

40. Kim JH, Moon H, Noh J, Lee J, Kim SG. Epidemiology and prognosis of pheochromocytoma/paraganglioma in Korea: A nationwide study based on the national health insurance service. *Endocrinol Metab (Seoul)*. (2020) 35:157–64. doi: 10.3803/EnM.2020.35.1.157

41. Mei L, Khurana A, Al-Juhaishi T, Faber A, Celi F, Smith S, et al. Prognostic factors of Malignant pheochromocytoma and paraganglioma: A combined SEER and TCGA databases review. *Horm Metab Res*. (2019) 51:451–7. doi: 10.1055/a-0851-3275

42. Uslar T, San Francisco IF, Olmos R, Macchiavelo S, Zuñiga A, Rojas P, et al. Clinical presentation and perioperative management of pheochromocytomas and paragangliomas: A 4-decade experience. *J Endocr Soc*. (2021) 5:1–7. doi: 10.1210/jendso/bvab073

43. Scholten A, Cisco RM, Vriens MR, Cohen JK, Mitmaker EJ, Liu C, et al. Pheochromocytoma crisis is not a surgical emergency. *J Clin Endocrinol Metab*. (2013) 98:581–91. doi: 10.1210/jc.2012-3020

44. Zhang L, Åkerström T, Mollazadegan K, Beuschlein F, Pacak K, Skogseid B, et al. Risk of complications after core needle biopsy in pheochromocytoma/paraganglioma. *Endocr Relat Cancer*. (2023) 30:e220354. doi: 10.1530/ERC-22-0354

45. Rogowski-Lehmann N, Geroula A, Prejbisz A, Timmers HJLM, Megerle F, Robledo M, et al. Missed clinical clues in patients with pheochromocytoma/paraganglioma discovered by imaging. *Endocr Connect*. (2018) 7:1168–77. doi: 10.1530/EC-18-0318

46. Ebbehoj A, Stochholm K, Jacobsen SF, Trolle C, Jepsen P, Robaczyk MG, et al. Incidence and clinical presentation of pheochromocytoma and sympathetic paraganglioma: A population-based study. *J Clin Endocrinol Metab*. (2021) 106:E2251–61. doi: 10.1210/clinem/dgaa965

47. Szatko A, Glinicki P, Gietka-Czernel M. Pheochromocytoma/paraganglioma-associated cardiomyopathy. *Front Endocrinol (Lausanne)*. (2023) 14:1204851. doi: 10.3389/fendo.2023.1204851

48. Dobrowolski P, Januszewicz A, Klisiewicz A, Gosk-Przybyłek M, Pęczkowska M, Kabat M, et al. Left ventricular structural and functional alterations in patients with pheochromocytoma/paraganglioma before and after surgery. *JACC Cardiovasc Imaging*. (2020) 13:2498–509. doi: 10.1016/j.jcmg.2020.07.017

49. Kvasnička J, Petrák O, Zelinka T, Klímová J, Kološová B, Novák K, et al. Effect of adrenalectomy on remission of subclinical left ventricular dysfunction in patients with pheochromocytoma: a speckle-tracking echocardiography study. *Endocr Connect*. (2021) 10:1538–49. doi: 10.1530/EC-21-0462

50. Ferreira VM, Marcelino M, Piechnik SK, Marini C, Karamitsos TD, Ntusi NAB, et al. Pheochromocytoma is characterized by catecholamine-mediated myocarditis, focal and diffuse myocardial fibrosis, and myocardial dysfunction. *J Am Coll Cardiol*. (2016) 67:2364–74. doi: 10.1016/j.jacc.2016.03.543

51. Zhang CD, Li D, Kaur RJ, Ebbehoj A, Singh S, Atkinson EJ, et al. Cardiometabolic outcomes and mortality in patients with adrenal adenomas in a population-based setting. *J Clin Endocrinol Metab*. (2021) 106:3320–30. doi: 10.1210/clinem/dgab468

52. Falhammar H, Kjellman M, Calissendorff J. Treatment and outcomes in pheochromocytomas and paragangliomas: a study of 110 cases from a single center. *Endocrine*. (2018) 62:566–75. doi: 10.1007/s12020-018-1734-x

53. Kittah NE, Gruber LM, Bancos I, Hamidi O, Tamhane S, Iñiguez-Ariza N, et al. Bilateral pheochromocytoma: Clinical characteristics, treatment and longitudinal follow-up. *Clin Endocrinol (Oxf)*. (2020) 93:288–95. doi: 10.1111/cen.14222

54. Neumann HPH, Tsoy U, Bancos I, Amodru V, Walz MK, Tirosh A, et al. Comparison of pheochromocytoma-specific morbidity and mortality among adults with bilateral pheochromocytomas undergoing total adrenalectomy vs cortical-sparing adrenalectomy. *JAMA Netw Open*. (2019) 2(8):e198898. doi: 10.1001/jamanetworkopen.2019.8898

55. Grubbs EG, Rich TA, Ng C, Bhosale PR, Jimenez C, Evans DB, et al. Long-term outcomes of surgical treatment for hereditary pheochromocytoma. *J Am Coll Surg*. (2013) 216:280–9. doi: 10.1016/j.jamcollsurg.2012.10.012

56. Turkova H, Prodanov T, Maly M, Martucci V, Adams K, Widimsky J, et al. CHARACTERISTICS AND OUTCOMES OF METASTATIC SDHB AND SPORADIC PHEOCHROMOCYTOMA/PARAGANGLIOMA: AN NATIONAL INSTITUTES OF HEALTH STUDY. *Endocr Pract*. (2016) 22:302–14. doi: 10.4158/EP15725.OR

57. Timmers HJLM, Brouwers FM, Hermus ARMM, Sweep FCGJ, Verhofstad AAJ, Verbeek ALM, et al. Metastases but not cardiovascular mortality reduces life expectancy following surgical resection of apparently benign pheochromocytoma. *Endocr Relat Cancer*. (2008) 15:1127–33. doi: 10.1677/ERC-08-0049

58. Plouin PF, Chatellier G, Fofol I, Corvol P. Tumor recurrence and hypertension persistence after successful pheochromocytoma operation. *Hypertension*. (1997) 29:1133–9. doi: 10.1161/01.HYP.29.5.1133

59. Khorram-Manesh A, Ahlman H, Nilsson O, Friberg P, Odén A, Stenström G, et al. Long-term outcome of a large series of patients surgically treated for pheochromocytoma. *J Intern Med*. (2005) 258:55–66. doi: 10.1111/j.1365-2796.2005.01504.x

60. Goldstein RE, O'Neill JA, Holcomb GW, Morgan WM, Neblett WW, Oates JA, et al. Clinical experience over 48 years with pheochromocytoma. *Ann Surg*. (1999) 229:755–66. doi: 10.1097/00000658-199906000-00001

61. Hamidi O, Young WF, Gruber L, Smestad J, Yan Q, Ponce OJ, et al. Outcomes of patients with metastatic phaeochromocytoma and paraganglioma: A systematic review and meta-analysis. *Clin Endocrinol (Oxf)*. (2017) 87:440–50. doi: 10.1111/cen.13434

62. Young WF. Metastatic pheochromocytoma: in search of a cure. *Endocrinology*. (2020) 161(3):1–4. doi: 10.1210/endocr/bqz019

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 14 January 2025
DOI 10.3389/fendo.2024.1372683

OPEN ACCESS

EDITED BY
Elena Aisha Azizan,
National University of Malaysia, Malaysia

REVIEWED BY
Piotr Glinicki,
Centre of Postgraduate Medical Education, Poland
Chiara Lopez,
University of Turin, Italy

*CORRESPONDENCE
Takumi Kitamoto
t.kitamoto@chiba-u.jp

RECEIVED 18 January 2024
ACCEPTED 16 December 2024
PUBLISHED 14 January 2025

CITATION
Kitamoto T, Ruike Y, Koide H, Inoue K, Maezawa Y, Omura M, Nakai K, Tsurutani Y, Saito J, Kuwa K, Yokote K and Nishikawa T (2025) Shifting paradigms in primary aldosteronism: reconsideration of screening strategy via integrating pathophysiological insights.
Front. Endocrinol. 15:1372683.
doi: 10.3389/fendo.2024.1372683

Shifting paradigms in primary aldosteronism: reconsideration of screening strategy via integrating pathophysiological insights

Takumi Kitamoto[1]*, Yutaro Ruike[1], Hisashi Koide[1], Kosuke Inoue[2], Yoshiro Maezawa[1], Masao Omura[3], Kazuki Nakai[3], Yuya Tsurutani[3], Jun Saito[3], Katsuhiko Kuwa[4], Koutaro Yokote[1] and Tetsuo Nishikawa[3]

[1]Department of Diabetes, Metabolism and Endocrinology, Chiba University Hospital, Chiba, Japan, [2]Department of Social Epidemiology, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [3]Endocrinology and Diabetes Center, Yokohama Rosai Hospital, Yokohama, Japan, [4]National Metrology Institute of Japan, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan

Several decades have passed since the description of the first patient with primary aldosteronism (PA). PA was initially classified in two main forms: aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA). However, the pathogenesis of PA has now been shown to be far more complex. For this reason, the traditional classification needs to be updated. Given the recent advancements in our understanding of PA pathogenesis, we should reevaluate how frequent PA cases are, beginning with the reconstruction of the screening strategy. Recent studies consistently indicated that PA has been identified in 22% of patients with resistant hypertension and 11% even in normotensives. The frequency is influenced by the screening strategy and should be based on understanding the pathogenesis of PA. Progress has been made to promote our understanding of the pathogenesis of PA by the findings of aldosterone driver mutations, which have been found in normotensives and hypertensives. In addition, much clinical evidence has been accumulated to indicate that there is a spectrum in PA pathogenesis. In this review, we will summarize the recent progress in aldosterone measurement methods based on LC-MS/MS and the current screening strategy. Then, we will discuss the progress of our understanding of PA, focusing on aldosterone driver mutations and the natural history of PA. Finally, we will discuss the optimal strategy to improve screening rate and case detection.

KEYWORDS

primary aldosteronism, aldosterone measurement, screening test, low renin hypertensive, somatic mutation

Search strategy and selection criteria

We searched MEDLINE for articles published from 1 January 1955 to 29 February 2024, using the search terms "primary aldosteronism," "Conn's syndrome,' "hyperaldosteronism," "screening test," and "aldosterone measurement". We mainly focused on English-language publications of the past 5 years (1 June 2019 to 29 February 2024) and selected relevant and highly referenced studies published before this timeframe.

Introduction

Several decades have passed since the first description of primary aldosteronism (PA) due to aldosterone-producing adenomas (APA) (1). Subsequently, it was observed that some cases of PA lack the classical biological characteristics of hypokalemia or high plasma (or serum) aldosterone concentration (2). Most cases of PA are classified as APA or bilateral adrenal hyperplasia, usually diagnosed as idiopathic hyperaldosteronism (IHA). The former is a surgically curable form of PA, representing more than 5% of patients with hypertension (3), whereas the latter is treated with a mineralocorticoid receptor antagonist (MRA). Additionally, patients with PA exhibit a 1.7- to 3.5-fold increased risk of cardiovascular and cerebrovascular complications (4–7) than essential hypertensives, and early subtype diagnosis is crucial to reversing the excess risk of vascular complications and achieving a better prognosis. To simplify the initial step in diagnosing PA, from 1981, the plasma aldosterone-to-renin activity ratio (ARR) or plasma aldosterone/direct renin ratio (ADRR) has been introduced, given their superiority over the isolated measurements of plasma (or serum) aldosterone concentration and plasma renin activity (PRA) or direct renin concentration (DRC) (8). Since then, ARR and ADRR have played a primary role in screening tests for PA (9–15). In decades, numerous robust prospective studies have established that the prevalence of PA is 3%–19% in all patients with hypertension (3, 16–36), and more patients showed positive results of screening tests in referral centers than in primary care clinics (3, 22, 30, 37). Several models have demonstrated that screening all patients with resistant hypertension for PA is cost-effective (38–40). However, merely less than 2%–5% of patients expected to have PA had been screened (41–44). The factors contributing to this low screening rate may vary across countries. However, the following points seem to be shared: 1) low awareness of PA, 2) the difficulty of changing medications before screening tests, and 3) the need to consider dietary salt intake and the position for blood collection. All these factors contribute to the complexity of the screening phase.

Therefore, this review aimed to restructure the case detection strategy for PA based on numerous novel discoveries over the last few decades, particularly aldosterone driver mutations and the natural history of PA. Moreover, we aimed to discuss an alternative screening strategy that focuses on renin suppression as a biomarker of PA.

Pathophysiology of PA

PA is a state of hypertension caused by inappropriate aldosterone secretion. Here, "inappropriate" refers to an inappropriate secretory response to salt intake. The body maintains the sodium and fluid balance through the renin–angiotensin–aldosterone system (RAAS) in response to salt intake. PA is a state of hypertension caused by inappropriate aldosterone secretion in response to salt intake, which is independent of renin secretion. Renin secretion is affected by 1) salt restriction (45), 2) fluid volume depletion with diuretics, 3) the use of RAAS inhibitors, such as MRA, angiotensin-II receptor blockers (ARB), and angiotensin-converting enzyme inhibitors, and 4) other hormones, such as glucocorticoids, estrogens, and progestogens (46, 47). Other factors that increase renin levels include renovascular hypertension and pregnancy (high levels of progesterone antagonize aldosterone action in the mineralocorticoid receptor [MR]). Factors suppressing renin secretion include renal failure, β-adrenergic blockers, a-methyldopa, clonidine, and nonsteroidal anti-inflammatory agents. Antidepressants such as selective serotonin reuptake inhibitors elevate aldosterone and renin; however, whether this results in lowering ARR is debatable (48, 49). An overview of the clinically significant factors affecting aldosterone, renin, and ARR levels is summarized in Table 1.

In PA, stimulation of renin secretion is blunted because of the feedback effects of aldosterone hypersecretion. In other words, the renin levels remained relatively low in response to these stimuli (50). Regardless of the fluctuations in aldosterone concentration, extracellular fluid volume expansion persists, resulting in continuous renin suppression. If renin-independent aldosterone excess persists, the distal nephron will reabsorb sodium into the body, and potassium will flow out, resulting in hypertension and hypokalemia as the typical PA phenotype.

TABLE 1 Parameters affecting aldosterone, renin, and ARR.

Parameters	Dietary sodium restriction	Hypokalemia	β-Adrenergic blockers	K^+-wasting diuretics	K^+-sparing diuretics	ACE inhibitors	ARBs	Ca^{2+} blockers	α-Blockers	ENaC inhibitors	MR antagonist
Aldosterone	↑	↓	↓	→/↑	↑	↓	↓	→/↓	→	↑	↑
Renin	↑↑	→/↑	↓↓	↑↑	↑↑	↑↑	↑↑	↑	→	↑↑	↑↑
ARR	↓	↓	↑	↓	↓	↓	↓	↓	→	→/↓	→/↓

ARR, aldosterone to renin ratio; ACE, angiotensin-converting enzyme; ARBs, angiotensin II type 1 receptor blockers; ENaC, epithelial sodium channel; MR, mineralocorticoid receptor
[Adapted from *Funder JW*, et al. *J Clin Endocrinol Metab. 2016;101* (5):*1889-1916* (9).; *Naruse M*, et al. *Endocrine Journal. 2022;69* (4):*327-359* (15)]
↑, Elevated; ↓, Suppressed; →, Not affected.

10.3389/fendo.2024.1372683

Reliable methods for aldosterone measurement

Since screening tests rely on plasma aldosterone and renin levels, measurement reliability is crucial for interpreting clinical outcomes.

The reliability of routine tests depends on the measurement performance. Among the common methods for measuring aldosterone, radioimmunoassays (RIA), liquid chromatography–mass spectrometry (LC-MS/MS), and chemiluminescent enzyme immunoassays (CLEIA) are widely used because of their unique strengths and limitations. RIA, a long-established method, offers practicality and ease of use; however, it suffers from cross-reactivity and variability due to low antibody specificity. LC-MS/MS, which is considered the gold standard, provides unparalleled accuracy and sensitivity (51–53), yet its high cost, time demands, and technical requirements limit its feasibility for high-throughput testing. CLEIA improves upon RIA, with better specificity and compatibility with standardized reference materials, making it suitable for routine clinical use. Each method plays a valuable role in the clinical and research settings, catering to different accuracy and accessibility requirements.

We have undertaken standardization of aldosterone concentration measurements traceable to the International System of Units. We have assembled a Certified Reference Materials (CRM) and a Designated Comparison Method (DCM) (54). A new CLEIA-based test kit, approved for *in vitro* diagnostics, was established using these standards and demonstrated alignment with LC-MS/MS results, supporting CLEIA's reliability as a routine method (55). Despite variations in RIA due to antibody specificity (55), LC-MS/MS provided a stable reference.

Notably, the median LC-MS/MS value was 48.5 pg/mL compared with 120 pg/mL of RIA (SPAC-S®), prompting a proposal to lower the ARR screening cutoff to 55 pmol/mU ($PAC_{LC\text{-}MS/MS}$/DRC) compared with 70 pmol/mU (PAC_{RIA}/DRC) and set a cutoff value after saline infusion test to 83 pmol/L ($PAC_{LC\text{-}MS/MS}$) (56, 57).

Two types of renin measurement, PRA and DRC, are currently available (9). Both PRA and DRC have methodological limitations. First of all, it is important to highlight that low renin activity could not be accurately measured in the specimens obtained from patients with PA (58).

In particular, PRA is dependent on the generation of angiotensin I, which can result in significant variability in low-renin states owing to reduced renin secretion (58). This often leads to an underestimation of renin activity (59). However, DRC provides a more stable measurement, as it directly quantifies renin concentration without relying on angiotensin I production (60). Consequently, the DRC is less affected by fluctuations in substrate levels and remains relatively stable even in low-renin states. In contrast, the PRA tends to show greater variability and is more susceptible to pre-analytical errors (61).

The poor correlation between the PRA and DRC in low-renin states is particularly relevant for PA screening (62). In such cases, PRA may underestimate renin levels due to its dependence on angiotensin I, which can affect the ARR used in screening. DRC's stability makes it a potentially more reliable indicator of renin levels in these cases, thus potentially improving the accuracy of PA screening by reducing false-negative results when ARR is used.

Furthermore, the correlation between the PRA and DRC can be poor, particularly in low-renin states (62). This is because PRA reflects the overall activity of the renin–angiotensin system (RAS) and is more sensitive to feedback control, whereas DRC measures renin levels more directly. This distinction is crucial in PA screening as each method has different implications for accuracy and reliability depending on the renin levels.

Additionally, the variability in the renin substrate among the samples can lead to decreased PRA when the substrate concentration is insufficient for a 90-min reaction (63). If specimens are left at room temperature after collection, angiotensin levels can increase, causing inaccuracies in the PRA. PRA measurements are most accurate when specimens are chilled; however, this can lead to inaccurate DRC values. Therefore, proper handling conditions are essential to obtain reliable renin measurements and improve the overall accuracy of PA screening.

Understanding these possible errors is necessary before testing to interpret the results accurately. PRA measurements are affected by several conditions, resulting in poor reproducibility between laboratories (64–66). The DRC procedure is inexpensive, requires a short test time, and has superior specimen handling and reproducibility. However, PRA is currently the mainstream test because of low correlation of DRC with PRA ≤ 1.0 ng/mL/h. Some studies have demonstrated that DRC showed lower sensitivity than PRA when used as a screening test in a ratio to aldosterone levels compared with PRA (58, 67, 68). Nevertheless, a method that adequately correlates with PRA over a wide range has been developed (69) and may be widely used in the future because of its simplicity.

The current screening strategy, along with the guidelines

Each guideline from a different country has specific criteria for screening tests. The Japan Endocrine Society (JES) revised its guidelines for PA in 2021 (15). In Table 2, we summarize the current statements for screening strategies compared with the Endocrine Society guidelines (9), which have been adopted in many research reports. The Endocrine Society guideline recommends targeted screening for PA in specific high-risk groups, such as patients with resistant hypertension, hypokalemia, or adrenal incidentalomas, as well as those with a family history of early-onset hypertension. This selective approach aimed to efficiently identify PA in patients most likely to have the condition based on clinical indicators. In contrast, the JES guidelines advocate a broader screening approach and recommend testing for all patients with hypertension. This inclusive strategy reflects the findings that PA often presents without hypokalemia (70) and poses a higher cardiovascular risk (7, 71, 72) and that early diagnosis and treatment can be more cost-effective over time (38, 39). By screening all patients with hypertensions, the JES aims to improve PA detection rates and address the underdiagnosis of PA in the general hypertensive population.

10.3389/fendo.2024.1372683

TABLE 2 Comparisons of screening strategy between ES and JES guidelines.

	US	Japan
Screening target population	Patients with 1) Sustained BP above 150/100 mm Hg on each of three measurements obtained on different days 2) With hypertension resistant to three conventional antihypertensive drugs (including a diuretic), or controlled BP (<140/90 mm Hg) on four or more antihypertensive drugs 3) Hypertension and spontaneous or diuretic-induced hypokalemia 4) Hypertension and adrenal incidentaloma 5) Hypertension and sleep apnea 6) Hypertension and a family history of early onset hypertension or cerebrovascular accident at a young age (<40 years) 7) All hypertensive first-degree relatives of patients with PA.	A) All hypertensive patients, especially those with a high prevalence of PA B) Clinical features suspicious for PA include 1) Spontaneous hypokalemia 2) Resistant hypertension 3) Hypertension onset before 40 years of age 4) Adrenal tumor 5) Stroke at a young age 6) Sleep apnea syndrome
Preparation	Diet: liberalize sodium intake Potassium replacement: to achieve plasma [K+] of 4.0 mmol/L Antihypertensive agents: Withdraw at least 4 weeks: Spironolactone, eplerenone, amiloride, and triamterene Potassium-wasting diuretics Products derived from licorice root withdraw at least 2 weeks: β-Adrenergic blockers, central α-2 agonists, and non-steroidal anti-inflammatory drugs Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, renin inhibitors, and dihydropyridine calcium channel antagonists Establish oral contraceptives and hormone replacement therapy when direct renin concentration is measured	Diet: not mentioned Potassium replacement: preferred to normalize potassium level (target value not stated) Antihypertensive agents: Withdraw at least 4 weeks Mineralocorticoid receptor antagonists (spironolactone, eplerenone, esaxerenone) Switching anti-hypertensive medicines to calcium channel blockers, alpha-blockers, or combinations whenever possible
Blood collection	Collect blood 1) After the patient has been up (sitting, standing, or walking) for at least 2 h and seated for 5 min–15 min 2) Carefully, avoiding stasis and hemolysis 3) Maintain sample at room temperature during delivery to laboratory and prior to centrifugation	Collect blood 1) Obtained at any time in the sitting position is acceptable for screening 2) Desirable to conduct blood sampling early in the morning in the supine position after overnight fasting 3)* PRA (in a container of ice), DRC (at room temperature): PRA or DRC less than 30 min of transport to laboratory.

The target population, preparation, and blood collection procedure for PA screening tests recommended in clinical practice guidelines from Endocrine Society (9) and Japan Endocrine Society (15) are summarized. * We added point#3, as handling the samples properly for accurate results is important.

Both guidelines converge on prioritizing high-prevalence groups but differ in the scope of initial screening due to varied emphasis on risk factors, cost-effectiveness, and the broader impact of PA on cardiovascular health.

Each guideline has recommended the use of ARR or ADRR as screening tests (9, 13–15, 73). Since ARR and ADRR are strongly influenced by renin value, the combined value of PAC_{RIA} (≥120 pg/mL in JES, ≥150 pg/mL in the United State [U.S.]) and ARR (≥200 pg/mL per ng/mL/h) or ADRR (≥24 pg/mL per mU/L) are recommended. However, we should be aware that more than 35% of patients with PA, particularly those with bilateral PA, have a PAC_{RIA} < 150 pg/mL (74). PAC_{CLEIA} cutoff is also mentioned in the JES guidelines. They recommended judging the screening test positive when PAC_{CLEIA} ≥ 60 pg/mL and ARR ≥ 200 as positive. An ARR between 100 and 200 is provisionally positive and set as a borderline range until the PAC_{CLEIA} is generalized and its optimal cutoff is established. When the active renin concentration (ARC) is measured instead of PRA, they recommended judging the screening test positive when ARR (PAC_{CLEIA}/ARC) ≥40 and PAC ≥60 pg/mL and ARR between 20 and 40 set as a borderline range.

Japanese and U.S. PA experts now specify that screening with normal blood collection is acceptable to enhance the screening rate. Many drugs and conditions do not hinder the detection of typical PA (75). In cases where the initial test is inconclusive or strongly suspicious, adjustment of interfering anti-hypertensive drugs to the ARR/ADRR thresholds (see Table 2 for the withdrawal period of each drug) and blood collection early in the morning, in the supine position after overnight fasting, are recommended (76–79). Confirmatory tests should be performed to confirm inappropriate aldosterone secretion and to exclude false-negative results. Evidence regarding the number of tests to be performed or the superiority of any confirmatory test is unavailable. When patients desire surgical treatment, performing adrenal venous sampling (AVS) is recommended for the subtype diagnosis. From these comparisons, a general agreement on the issues and methods of screening tests is observed.

Variable prevalence rates of PA as determined by the ARR screening strategy

Several studies have evaluated the prevalence of PA using the ARR (or ADRR) as a screening strategy, as summarized in Table 3. The prevalence of positive screening results was variable across the studies (3, 16–36, 80–82), ranging from 3% to 19% when considering the general hypertensive population and from 21% to 68% in patients

with resistant hypertension, whereas the prevalence of confirmed PA cases was 3%–19% and 7%–22%, respectively. The factors causing the variable prevalence of PA are unstandardized screening strategies (i.e., different thresholds, and collection in different positions) and methodological issues (as discussed early). In addition to medications, several factors influence the ARR, potentially impacting on the accuracy of PA screening, such as pulsatility of aldosterone secretion, sodium intake, ethnicity, and posture (Table 1). Aldosterone secretion is not constant but rather pulsatile, with natural fluctuations throughout the day and night. One study demonstrated the high reproducibility of the ARR in multiple measurements taken on the same patient over time (83); however, many studies have indicated a large variability in plasma aldosterone levels in patients with or without PA (84–88). Dietary sodium restriction led to a misinterpretation of screening test, with normal results in 52% of patients with PA (89). Ethnic differences can influence the baseline aldosterone and renin levels. Certain ethnic groups, such as African Americans, tend to have lower renin levels (90). Body position affects aldosterone and renin levels. For example, standing increases renin secretion due to decreased renal perfusion, which raises renin levels and lowers the ARR. Prevalence studies have shown a wide range in the proportion of APAs in patients with PA, which impacts the aldosterone and renin values and serum potassium levels in each cohort. These factors can affect ARR measurements and screening accuracy for PA. A recent study using LC-MS/MS or CLEIA showed that more than half of the patients had an ARR ≤ 30 ng/mL/h at least once, below the screening threshold (91). PAC is highly variable, and the boundary between normal and abnormal cannot be determined independently.

A better screening strategy is required for accurate PA case detection. Numerous discussions are available regarding the need for standardization. However, international agreements on screening tests are yet to be reached. The reasons seem to be the following: 1) matching the measurement system of each institution is almost impossible, 2) setting body position and time for each measurement in patients is challenging in daily practice; and 3) serum or plasma aldosterone cutoff values vary according to measurement conditions and probably according to ethnicity. For example, the *KCNJ5* somatic mutations, an aldosterone driver mutation causing severe forms of PA is known to show large ethnic differences in frequency (92–98). As we will discuss in the next section, we will delve into aldosterone driver mutations, which will advance our understanding of PA's pathophysiology of PA to better discuss screening strategies.

Distinctive clinical presentation of PA by aldosterone driver mutations

Our understanding of the pathophysiology of PA has significantly advanced since the discovery of aldosterone driver mutations, which have been observed even in the adrenals of normotensive individuals and APAs and have shown sex and ethnic differences. Somatic mutations in the gene encoding *KCNJ5* in APA (99) cells were first reported in 2011. Following this discovery, *ATP2B3* and *ATP1A1* (100), and *CACNA1D* (101) somatic mutations were identified. Recent work has demonstrated that more APAs carry *CACNA1D* mutations in *KCNJ5* wild-type APAs when CYP11B2 immunohistochemistry-guided high-throughput sequencing is used instead of Sanger sequencing (102). More than 90% of APAs harbored any of these aldosterone driver mutations. Since 2011, several studies have reported the frequency of *KCNJ5* mutations (92–96, 99, 103–119) (Table 4). The frequency of *KCNJ5* mutation in APA is higher in eastern countries [70.5 (43.2–74.7) (%) (95, 96, 103–110)] than in western nations [41.0 (35.5–51.8%) (92–94, 99, 102, 111–119)]; however, *KCNJ5* mutation is commonly the dominant mutation across the countries. We previously discussed the clinical impact of *KCNJ5* mutations on APAs (120).

In summary, the typical clinical characteristics of APAs harboring *KCNJ5* mutations are female dominance, higher aldosterone production capacity, and induction of hypokalemia, compared with *KCNJ5*-wild APAs. While the frequency of *KCNJ5* mutations in APAs differs between Asians and Westerners, the plasma aldosterone levels of *KCNJ5*-mutated APAs are similar between the two groups [*KCNJ5* mutated vs. wild APA: PAC_{RIA} 46.9 (40.1–59.8) and PRA 0.3 (0.2–0.4) in Asia, and PAC_{RIA} 48.0 (31.2–116.2) and PRA 0.3 (0.2–0.5) in Western countries] (Table 4). The distribution of somatic mutations in APAs might have caused variability in the aldosterone values of patients with PA in these studies. The underlying cause of the distinctive frequency of *KCNJ5* mutations has not yet been elucidated. Whether this is due to a selection bias among patients with APA enrolled in the studies or environmental factors, such as ethnicity, remains to be investigated. In parallel with the research on somatic mutations in APA, efforts have been extended to normotensive and IHA cases. Approximately half of the adrenals from normotensive participants contained aldosterone-producing micronodules (APMs; formerly known as aldosterone-producing cell clusters), termed by CYP11B2-positive clusters (121), and more than 40% of APM harbored *CACNA1D* or *ATP1A1* somatic mutations (122, 123). The most frequent mutation identified in these patients was that of the *CACNA1D* gene, which was found to almost exclusively cause IHA (124), even if this interpretation is limited by the scarcity of surgically resected IHA samples. The differential distribution of somatic mutations, such as *KCNJ5* mutations that appeared uniquely in APAs and *CACNA1D*, which was exclusively observed in IHA, might partly explain the distinctive clinical characteristics of APA and IHA (125). Moreover, APMs in the adrenal glands of normotensive increase with aging (123, 126, 127). These findings support the concept of a continuum pathophysiology of PA from normotensive to participants with hypertension (128). A recent study using expression quantitative trait loci analysis identified the risk loci for PA (129). These discoveries have led us to conceive novel ideas for the methods for early diagnosis of PA (130–132). Aldosterone driver mutations that increase with age have been observed in normotensive patients, and some mutations display sex differences. Furthermore, different mutations demonstrate different clinical behaviors in aldosterone overproduction. In light of these points, defining a certain threshold for absolute aldosterone values for the boundary between PA and non-PA cases should be complicated.

TABLE 3 Prevalence of confirmed PA cases and positive screening results in patients with hypertension.

Author (Year, region)	Design	Center	Population	Cases screened (n)	Scr. test	Test position	Conf. test	Subtyping	Positive Scr. test	Positive Conf. test	APA (%)	Aldosterone (ng/dL)	Renin (ng/mL/h)
Lim PO, et al. (2000, UK) (16)	Ret-Sin	Ref	HT	465	PAC/PRA ≥ 27	Seated	FST	n.a.	17%	9%	n.a.	13.3 (10.5–17.7)	0.3 (0.2–0.4)
Loh KC, et al. (2000, Singapore) (17)	Pro-Mul	Pri	HT	350	PAC/PRA > 20, PAC > 15	Seated	SIT	CT/AVS	18.0%	4.6%	50%	22.0 ± 1.1*	0.02–1.04
Calhoun et al. (2002, U.S.) (80)	Pro-Sin	Ref	R-HT	88	U-Ald > 12, PRA < 1.0	Not specified	U-Ald PRA	n.a.	20.0%	20.0%	n.a.	19.2 (5.0–47.0)	0.3 (0.2–0.8)
Rossi E, et al. (2002, Italy) (3)	Pro-Sin	Ref	HT	1,046	CCT, PAC/PRA ≥ 35	Seated	SIT	n.a.	12.8%	6.3%	n.a.	n.a	n.a
Mosso L, et al. (2003, Chile) (19)	Ret-Mul	Pri	HT	609	SAC/PRA > 25	Seated	FST	n.a.	10.2%	6.1%	n.a.	16.9 ± 6.8	0.3 ± 0.2
Stowasser M, et al. (2003, Australia) (20)	Ret-Sin	Ref	HT	300	PAC/PRA > 30	Seated	FST	CT/AVS	20%	18%	31%	21.1 ± 1.6	0.4 ± 0.04
Strauch B, et al. (2003, Germany) (21)	Pro-Sin	Ref	HT	402	PAC/PRA ≥ 50	Upright	SIT	CT/AVS	22%	19%	36%	42.2 ± 36.9	0.2 ± 0.2
Omura M, et al. (2004, Japan) (22)	Pro-Sin	Ref	HT	1,020	PAC > 12, PRA < 1.0	Supine	FUT	CT/AVS	12%	6%	74%	n.a.	n.a.
Nishizaka MK, et al. (2005, U.S.) (23)	Pro-Sin	Ref	R-HT	265	PRA < 1.0	Not specified	PRA, U-Ald	n.a.	58%	22%	n.a.	n.a.	n.a.
Rossi GP, et al. (2006, Italy) (14)	Pro-Mul	Ref	HT	1,125	CCT ¶	Seated	SIT	CT/AVS	20%	11%	63%	29.7 (17.0–226)	0.62 (0.02–0.96)
Fogari R, et al. (2007, Italy) (24)	Pro-Sin	Ref	HT	3,000	PAC/PRA ≥ 25	Upright	SIT	CT	23%	6%	30%	13.6 ± 6.2	0.3 ± 0.2

(Continued)

10.3389/fendo.2024.1372683

TABLE 3 Continued

Author (Year, region)	Design	Center	Population	Cases screened (n)	Scr. test	Test position	Conf. test	Subtyping	Positive Scr. test	Positive Conf. test	APA (%)	Aldosterone (ng/dL)	Renin (ng/mL/h)
Douma S, et al. (2008, Greece) (25)	Ret-Sin	Ref	R-HT	1,616	SAC/PRA ≥ 37, SAC ≥ 15	Supine	PRA, PAC	CT/AVS	21%	11%	n.a.	22.9 (15.1–1503)	0.14 (0.01–0.65)
Westerdahl C, et al. (2011, Sweden) (26)	Pro-Mul	Pri	HT	200	SAC/PRC > 2.34	Seated	FST	CT/AVS	18%	6%	27%	11.8 ± 8.7	4.0 ± 2.2†
Sigurjonsdottir HA, et al. (2012, Sweden) (27)	Pro-Mul	Ref, Pri	HT	353	SAC/PRC > 4.61, SAC > 15.5	Seated	OSLT	CT/AVS	13%	6%	60%	24.0 (20.0–33.4)	n.a. †
Sang X, et al. (2013, China) (28)	Pro-Mul	Ref, Pri	R-HT	1,656	PAC/PRA ≥ 20	Seated	SIT	CT/AVS	30%	7%	51%	32.0 (24.0–49.7)	0.4 (0.1–0.6)
Galati SJ, et al. (2016, U.S.) (29)	Pro-Sin	Ref	HT	296	PAC/PRA ≥ 20.0, PAC ≥ 10.0 PRA < 1.0	Seated	OSLT	CT/AVS	5%	1%	n.a.	41, 17‡	0.2, 0.3‡
Monticone S, et al. (2017, Italy) (30)	Pro-Mul	Pri	HT	1,672	SAC/PRA ≥ 30.0, SAC ≥ 10.0	Seated	SIT, CCT	n.a.	14%	6%	27%	31.0 (22.0–42.9)	0.3 (0.2–0.5)
Kayser SC, et al. (2018, Netherlands) (31)	Ret-Mul	Pri	HT	361	PAC/PRC ≥ 4.0, PAC ≥ 40.0	Not specified	SIT	n.a.	26%	3%	n.a.	66.8 ± 11.8	0.5 (0.3–0.7)§
Brown JM, et al. (2020, U.S.) (32)	Ret-Mul	Ref	Nor, HT, R-HT	1,015	PRA < 1.0, < 0.6 (seated/supine),	Seated or supine	OSLT	n.a.	68%	11.3%, 22.0% ‖	n.a.	8.3 (6.9–15.0), 25.0 (14.2–38.8) ‖	0.5 (0.2–0.6), 1.1 (0.6–3.1) ‖
Burrello, et al. (2020, Italy) (81)	Ret-Sin	Ref	HT	5,100	PAC/PRA ≥ 30.0, PAC ≥ 10.0	Not specified	SIT, CCT	CT/AVS	37%	8%	n.a.	n.a.	n.a.
Parasiliti-Caprino, et al. (2020, Italy) (82)	Ret-Sin	Ref	R-HT	170	Resistant hypertensives	n.a.	SIT	n.a.	40%	19	n.a.	35.8 (24.7–44.2)	0.3 (0.2–0.8)

(Continued)

10.3389/fendo.2024.1372683

TABLE 3 Continued

Author (Year, region)	Design	Center	Population	Cases screened (n)	Scr. test	Test position	Conf. test	Subtyping	Positive Scr. test	Positive Conf. test	APA (%)	Aldosterone (ng/dL)	Renin (ng/mL/h)
Xu Z, et al. (2020, China) (33)	Pro-Mul	Pri	HT	1,020	PAC/PRC > 20.0, PAC > 20	Upright	CCT, SIT	CT/AVS	9%	4%	20%	16.5 (13.2–21.5)	3.6 (1.1–6.6) †
Xu F, et al. (2021, China) (34)	Pro-Sin	Ref	HT	7,594	PAC/PRC ≥ 3.7, PAC ≥ 10.0	Upright	SIT, CCT	CT/AVS	5%	3%	39%	n.a.	n.a.
Asbach E, et al. (2022, Germany) (35)	Pro-Mul	Pri	HT	200	SAC/PRC ≥ 12.0, PAC ≥ 5.0	Seated	SIT, CCT	CT/AVS	21%	6%	9%	11.2 (8.3–15.9)	4.4 (2.0–6.5)†
Yoon M, et al. (2022, Korean) (36)	Ret-Sin	Ref	HT	1,173	PAC/PRA ≥ 30 or PAC/PRA > 20, PAC > 15	Not specified	SIT	CT/AVS	31%	6%	27%	25.4 (20.0–32.6)	0.4 (0.3–0.7)

The prevalence of PA in hypertensive patients was summarized.

Scr. test, screening test; Conf. test, confirmatory test; Ret, retrospective analysis; Pro, prospective cohort; Sin, single center; Mul, multicenters; Pri, primary care; Ref, referral center; HT, hypertensive patients; R-HT, resistant hypertensive patients; Nor, normotensive patients; PAC, plasma aldosterone concentration (ng/dL); SAC, serum aldosterone concentration (ng/dL); PRA, plasma renin activity (ng/mL/h); PRC, plasma renin concentration (mIU/L, pmol/L, specified at †, §); FST, fludrocortisone suppression test; SIT, saline infusion test; FUT, furosemide upright test; OSLT, oral salt loading test; CCT, captopril challenge test; U-Ald, urinary aldosterone in a day (μg/24 h) *; Mean ± SE, †; (mIU/L), ‡; Only two cases were diagnosed PA, §; (pmol/L), ||; (normotension), (resistant hypertension), ¶; In the Captopril Challenge Test (CCT), an ARR ≥ 40.0 at baseline, ≥ 30.0 after captopril administration, or a logistic discriminant function (LDF) score ≥ 0.50 indicated a positive result (see details in the original paper). A case was considered to have screened positive for PA if any of these three criteria were met. A SIT was conducted after a positive screening to confirm autonomous aldosterone secretion.

10.3389/fendo.2024.1372683

TABLE 4 Prevalence of *KCNJ5* mutation in APAs from Asia, the U.S., and European countries.

Author (Year, region)	Design	APA (n)	*KCNJ5* Seq	*KCNJ5* (%)	*KCNJ5*-mutated APA			*KCNJ5*-wild APA		
					Aldosterone (ng/dL)	Renin (ng/mL/h)	Potassium (mEq/L)	Aldosterone (ng/dL)	Renin (ng/mL/h)	Potassium (mEq/L)
Choi M, et al (2011, Sweden) (99)	Ret-Sin	22	Conv	36.4%	n.a.	n.a.	3.5 ± 0.6	n.a.	n.a.	3.5 ± 0.4
Akerstrom T, et al (2012, Sweden, Germany, Australia, France) (111)	Ret-Mul	348	Conv	45.1%	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Azizan EA, et al (2012, UK, Australia) (92)	Ret-Mul	73	Conv	41.1%	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Boulkroun S, et al (2012, France, Germany, Italy) (93)	Ret-Mul	380	Conv	33.9%	38.7 (24.9–61.9)	0.30 (0.12–0.49)	4.7 ± 0.6	31.8 (20.1–47.9)	0.27 (0.15–0.60)	5.2 ± 0.4
Taguchi R, et al (2012, Japan) (108)	Ret-Sin	23	Conv	65.2%	6.05 (4.68–9.78)	0.40 (0.30–0.80)	4.2 ± 1.1	4.75 (4.25–7.18)	0.70 (0.45–0.98)	4.5 ± 0.4
Arnesen T, et al (2013, Norway) (113)	Ret-Sin	28	Conv	35.7%	31.2 (21.6–38.3)	0.50 (0.22–0.77)	3.2 (3.0–3.6)	32.3 (24.2–45.2)	0.20 (0.20–0.50)	3.3 (3.0–3.4)
Fernandes-Rosa FL, et al (2014, France, Germany, Italy) (114)	Ret-Sin	474	Conv	38.0%†‡§	29.8 (22.6–41.4)	1.7 (1.0–2.7)*	3.3 (3.0–3.6)	29.3 (18.4–42.4)	1.7 (1.0–3.1)*	3.0 (2.7–3.3)
Kitamoto T, et al (2014, Japan) (105)	Ret-Sin	108	Conv	69.4%†‡§	43.6 (30.0–61.1)	0.2 (0.1–0.4)	3.2 ± 0.5	24.7 (18.5–38.1)	0.2 (0.1–0.5)	3.3 ± 0.5
Williams TA, et al (2014, Italy) (94)	Ret-Mul	112	Conv	39.3%‡§	48.0 (32.0–66.0)	0.2 (0.1–0.3)	2.9 ± 0.7	47.0 (35.0–60.0)	0.2 (0.20–0.39)	3.1 ± 0.7
Akerstrom T, et al (2015, Sweden, Germany, Australia) (112)	Ret-Mul	165	Conv	54.5%	136.5 ± 21.9	n.a.	n.a.	135.5 ± 14.5	n.a.	n.a.
Cheng CJ, et al (2015, Taiwan) (103)	Ret-Sin	69	Conv	37.7%	49.4 ± 29.2	0.45 ± 0.32	2.6 ± 0.6	42.1 ± 29.5	0.66 ± 1.19	2.9 ± 0.6
Scholl UI, et al (2015, U.S., Germany) (119)	Ret-Mul	90	Conv	37.1%†‡§‖	n.a.	n.a.	3.1 ± 0.6	n.a.	n.a.	3.4 ± 0.6
Wang B, et al (2015, China) (109)	Ret-Mul	114	Conv	75.4%	25.1 ± 6.8	n.a.	2.9 (2.6–3.2)	18.6 ± 5.5	n.a.	3.5 (3.1–3.9)
Wu VC, et al (2015, Taiwan) (95)	Ret-Mul	148	Conv	59.5%†‡§	59.7 ± 32.9	n.a.	3.2 ± 0.7	40.7 ± 25.1	n.a.	3.8 ± 0.6
Zheng FF, et al (2015, China) (96)	Ret-Mul	168	Conv	76.8%†‡§	36.5 (22.3–47.7)	0.3 (0.1–0.7)	2.6 (2.2–2.8)	31.5 (21.2–44.5)	0.6 (0.2–2.4)	2.9 (2.5–3.0)

(Continued)

10.3389/fendo.2024.1372683

TABLE 4 Continued

Author (Year, region)	Design	APA (n)	*KCNJ5* Seq	*KCNJ5* (%)	*KCNJ5*-mutated APA			*KCNJ5*-wild APA		
					Aldosterone (ng/dL)	Renin (ng/mL/h)	Potassium (mEq/L)	Aldosterone (ng/dL)	Renin (ng/mL/h)	Potassium (mEq/L)
Hong AR, et al (2016, Korean) (104)	Ret-Sin	66	Conv	71.2%†‡§	41.3 (31.7–52.5)	0.10 (0.10–0.19)	2.8 (2.5–3.1)	48.2 (33.0–55.2)	0.10 (0.10–0.10)	2.9 (2.6–3.0)
Nanba K, et al (2018, U.S.) (117)	Ret-Sin	75	Cyp11b2-g	42.7%†‡§	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Warachit W, et al (2018, Thailand) (110)	Ret-Sin	96	Conv	69.8%	54.9 (33.2–76.5)	0.37 (0.20–0.67)	2.6 ± 0.6	34.7 (24.5–62.9)	0.39 (0.19–0.62)	2.4 ± 0.6
Mohideen SK, et al (2019, Malaysia) (106)	Ret-Sin	54	Conv	31.5%	111.3 ± 169.5	n.a.	2.7 ± 0.8	60.9 ± 41.7	n.a.	2.9 ± 0.7
Nanba K, et al (2019, U.S.) (118)	Ret-Sin	69	Cyp11b2-g	34.2%†‡§	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
De Sousa K, et al (2020, France) (102)	Ret-Sin	48	Cyp11b2-g	43.8%†‡§‖	116.2 (92.6–142.0)	1.0 (1.0–3.2)*	3.1 (2.6–3.2)	93.3 (53.2–150.8)	1.0 (1.0–3.2)*	2.7 (2.1–3.1)
Guo Z, et al (2020, Australia) (115)	Ret-Sin	40	Cyp11b2-g	35.0%†‡§‖	99.1 (49.4–167.5)	2.2 (0.9–2.8)*	2.8 (2.6–3.0)	n.a.	n.a.	n.a.
Nanba K, et al (2020, Japan) (107)	Ret-Sin	115	Cyp11b2-g	72.6%†‡§	44.4 (29.6–61.7)	0.2 (0.1–0.4)	n.a.	33.2 (21.8–41.9)	0.2 (0.1–0.3)	n.a.
Meyer LS, et al (2021, Germany) (116)	Pro-Sin	41	Cyp11b2-g	56.1%	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

The prevalence of *KCNJ5* mutation in aldosterone-producing adenoma (APA) is summarized. Akerstrom T et al. included *CACNA1D* (n = 5), *ATP1A1* (n = 10), and *ATP2B3* (n = 5) mutated APAs in *KCNJ5* wild APAs. Guo Z et al. excluded *CACNA1H* and *CLCN2* mutated APAs from *KCNJ5*-mutated APAs.

Abbreviations are described in the same way as in Table 2. The others are following. APA; aldosterone-producing adenoma, Conv; conventional approach, Cyp11b2-g; CYP11B2 guided sequencing, * Plasma renin concentration (mIU/L); *CACNA1D* (†), *ATP1A1* (‡), *ATP2B3* (§), *CTNNB1* (‖) mutated APA excluded from *KCNJ5* wild-type APAs

10.3389/fendo.2024.1372683

Natural history of primary aldosteronism

The identification of aldosterone driver mutations in the adrenal glands of normotensive participants also raises the question of whether aldosterone secretion abnormalities occur before the onset of hypertension. The answer to this question will clarify the natural history of PA, allowing us to reconsider when and how screening tests should be performed. A study in 2017 examined 210 normotensive participants with a PRA below 1.0 ng/mL/h, of which 14% were subsequently diagnosed with PA (128). Although no significant difference was observed in the ARR between confirmed PA cases and controls, aldosterone levels were significantly higher in the PA group. Furthermore, even among suspected and unconfirmed PA cases, 20% of them received a confirmed PA diagnosis over 5 years, with one-third of cases showing a unilateral subtype. These results indicated that the pathogenesis of PA is continuous and progressive.

Another finding from these studies is that the ARR may not always accurately reflect the pathogenesis of PA. A recent meta-analysis evaluating the sensitivity and specificity of ARR to detect patients with PA demonstrated a wide variation in sensitivity from 10% to 100% and specificity from 70% to 100% (133). Of note, 3 of 10 studies reported ARR sensitivity of less than 50%, suggesting a limited ability of ARR to adequately identify patients with PA. A recent study used the amount of aldosterone excreted daily in the urine instead of the ARR to detect PA. Using 24-h urinary aldosterone excretion can address diurnal aldosterone variations in a screening test. As salt intake is a major factor in diagnosing PA (89), this study confirmed the salt intake and analyzed cases of renin suppression (32). The results showed that 22% of patients with resistant hypertension and 11% of normotensives had PA. The sensitivity of ARR in this study was less than 30%. Furthermore, a continuum of aldosterone levels and biomarkers of MR activity, such as urinary sodium–potassium ratio, was observed from normotension to hypertension resistance. This finding has been confirmed in a recent elegant study (134). This human physiological study demonstrates a continuum of dysregulated aldosterone production in the low-renin phenotype. Based on a series of studies, we speculated that in patients with PA, dysregulated aldosterone secretion in response to salt leads to renin suppression and demonstrates a continuous and progressive pathophysiology. In the natural history of PA, blood pressure is determined by individual sensitivity, and hypertension occurs when an aldosterone hypersecretion reaches a certain threshold. Suppressed renin seems to be an early biomarker for the detecting PA.

Emerging evidence on the association of low renin with cardiovascular complications

Whether high aldosterone levels per se cause cardiovascular diseases should be investigated. Extraordinarily high aldosterone levels due to chronic sodium deficiency never induce high blood pressure but rather low or normal blood pressure or any cardiovascular or renal damage (135). Thus, inappropriate aldosterone secretion—inappropriate for salt intake (136)—should be a key player in excessive vascular risk. Renin, receiving a feedback inhibition by aldosterone, may serve as a valuable biomarker for identifying dysregulated aldosterone secretion (137, 138).

A well-designed study has added new evidence regarding the association between suppressed renin, high aldosterone levels, and cardiovascular disease (139). The authors demonstrated an association between serum aldosterone concentration and coronary artery calcium (CAC) scores, a marker of subclinical atherosclerosis, in a multiethnic population without antihypertensive medication. The striking result of their study was that a marked association between aldosterone levels and CAC score and an increased risk of all-cause mortality were observed only among individuals with low renin levels. They also showed that the association between elevated aldosterone levels and subclinical atherosclerosis was only partially mediated by blood pressure, indicating the direct cardiovascular damage of aldosterone independently of hypertension. More recently, one study clarified whether renin-independent aldosteronism (i.e., subclinical PA), which fails to diagnose PA using current diagnostic criteria, is involved in cardiovascular disease. Elevated ARR, independent of brachial blood pressure, was associated with greater arterial stiffness and adverse cardiac remodeling, which was also observed in normotensive participants (140). Therefore, a low renin phenotype seems to be necessary to predict cardiovascular complications due to dysregulated aldosterone secretion.

In contrast, whether reversal of renin suppression ameliorates the excess risk of cardiovascular complications due to dysregulated aldosterone secretion should be investigated. Several studies have demonstrated that in patients with PA, adrenalectomy and MRA can ameliorate the unfavorable effects of excess aldosterone to achieve similar mortality rates in patients with essential hypertension (141, 142). Additionally, a recent large retrospective cohort study demonstrated that patients with APA that undergo surgical adrenalectomy had a significantly lower risk for cardiovascular events than patients with essential hypertension by 40% (6). In medically treated PA patients, the same investigators demonstrated different outcomes between the two subpopulations with unsuppressed or suppressed PRA. Surprisingly, the former showed an identical risk profile to that of essential hypertensives, whereas the latter showed an almost three times higher risk. A similar association was observed in the occurrence of atrial fibrillation (143). Therefore, we may need to start a renin check to estimate future cardiovascular risk due to dysregulated aldosterone secretion, which is also useful for monitoring surgical or medical treatment efficacy in patients with PA.

Optimal screening strategy for PA

As observed in other endocrine disorders (e.g., hyperthyroidism and hyperparathyroidism), the dysregulated hormones are not always beyond the normal range, and the hormone-receiving feedback loop is more sensitive in reflecting the disease. This may also be true for patients with PA. PA disrupts the homeostatic

feedback loop between aldosterone and salt status (136). As we have overviewed, PA is a disease with a spectrum, and inappropriate aldosterone secretion increases gradually. Furthermore, aldosterone secretion is affected by diurnal variations and salt sensitivity, which vary widely between individuals. Additionally, aldosterone driver mutations, such as *KCNJ5* and *CACNA1D* mutations, significantly affect aldosterone secretion, with large sex and ethnic differences. Therefore, rather than setting a certain threshold for aldosterone levels to detect PA, using renin suppression as a feedback loop for inappropriate aldosterone secretion early in its natural history is reasonable. However, establishing a clear threshold for PRA suppression is challenging. Therefore, we should begin with the values used in the current guidelines (PRA <1.0 ng/mL/h) as a standard to accumulate further knowledge.

We propose that individual hormone levels of renin and aldosterone can help diagnose PA (Figure 1A). We demonstrated the prevalence of PA at a general outpatient clinic in 2004, where endocrine markers of secondary hypertension, such as renovascular hypertension, Cushing's syndrome, and pheochromocytoma, were evaluated (22). We used PAC_{RIA} (>12 ng/dL) and PRA (<1.0 ng/mL/h) individually for screening in this study, finding a prevalence of PA of 6.0%, which is consistent with a recent report in a primary care setting (30). For individuals exhibiting a low-renin phenotype, a PAC_{CLEIA} exceeding 10 ng/dL (or equivalently, a PAC_{RIA} greater than 20 ng/dL) or the presence of hypokalemia (serum potassium less than 3.5 mEq/L) may be sufficient to diagnose PA without the need for further confirmatory testing (9). Notably, a few patients with PA show high plasma renin levels due to comorbidity (144) (such as excess cortisol secretion, chronic kidney disease, nephrotic syndrome, liver dysfunction, and chronic heart failure).

In settings in which proper evaluation of reninemia is not feasible, MRA is a useful strategy for ex Juvantibus diagnosis (Figure 1B). Renin is more sensitive than the ARR for detecting PA (8, 20, 145). A low-renin phenotype indicates extracellular fluid volume expansion or an MR-activated state (146–149). In patients with hypertension but without a PA diagnosis, those with suppressed renin levels experience a greater blood pressure reduction from MRA treatment, particularly if they have higher plasma aldosterone levels within the normal range (150, 151). This suggests that such patients may represent a wider spectrum of

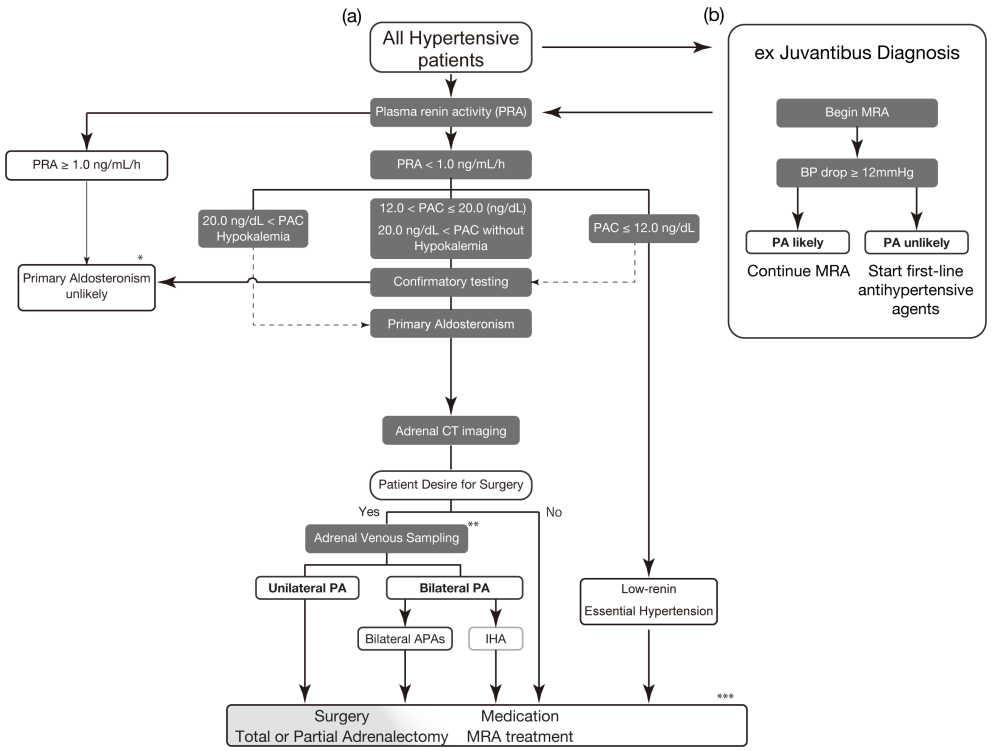


FIGURE 1
Proposed treatment strategy for primary aldosteronism **(A)** The screening for PA in all hypertensive patients should begin with PRA evaluation. Withdrawal of interfering antihypertensive drugs is preferable. However, many medications and conditions do not significantly hinder the detection of typical PA. RIA is used for PAC values in this figure. Low-renin indicates PRA <1.0 ng/mL/h. Physicians should perform confirmatory testing for cases with PAC of less than 12.0 ng/dL if PA is clinically suspected. Confirmatory testing options include the oral sodium loading test, captopril challenge test, saline infusion test, and 24-h urinary aldosterone excretion (>12 μg/24 h) after sodium intake correction (9). **(B)** If the proper evaluation of renin is difficult due to interfering factors, proceed with ex juvantibus diagnosis. Begin administration of MRA (e.g., spironolactone 25 mg/day) for 4 weeks to see whether there is a drop in blood pressure. A drop of 12 mmHg or more is suspected of a high likelihood of PA.
* Reconsider the diagnosis if the case has the factors increasing renin (e.g., excess cortisol secretion, chronic kidney disease, nephrotic syndrome, liver dysfunction, and chronic heart failure) ** Consider segment-selective AVS if the clinical diagnosis and conventional AVS diagnosis are inconsistent. *** Partial adrenalectomy is a treatment option for cases with bilateral APAs. PAC, plasma aldosterone concentration; PRA, plasma renin activity; CT, computed tomography; PA, primary aldosteronism; APA, aldosterone-producing adenoma; IHA, idiopathic hyperaldosteronism; MRA, mineral corticoid receptor antagonist.

potential patients with PA (6, 141, 142, 152–154). We referred to a recent Commentary from Dr. Funder (155), who proposed to begin the administration of spironolactone 25 mg/day for 4 weeks and measure the blood pressure response. In hypertensives, a drop of less than 10 mmHg indicated a low probability of PA, whereas a drop of 12 mmHg or more suggested a high likelihood of PA. The same applies to newly developed hypertension, where spironolactone is prescribed 25 mg/day for 4 weeks. If blood pressure falls within the normal range, continue; otherwise, prescribe first-line antihypertensive agents. These steps are an effective strategy to ensure that as many patients with PA as possible receive the necessary medical treatment, regardless of the medical environment. Treatment with MRA carries certain risks, such as hyperkalemia and a decline in glomerular filtration rate. Occasionally, MRA may not effectively reverse renin suppression. In such situations, or if the patients wish to explore the possibility of curative treatment, they should be referred to an appropriate specialized center for reevaluation of the diagnosis of PA.

The perceptions of primary care physicians who see patients with PA are also critical for lowering the hurdles for PA screening. Actions are needed to increase knowledge of PA among these physicians, including its high prevalence and minor presentation of hypokalemia. The rapid immunoassay for plasma aldosterone and renin may lessen the hurdle for their measurement and encourage screening procedures (69). This will contribute to an increase in the population diagnosed with PA by more than >1% (156).

Perspectives

To design a better screening method, we addressed the following questions: 1) Is early intervention for normotensive renin-independent aldosteronism beneficial for the patient's prognosis? 2) What is the cutoff for PRA and DRC to stratify the population according to excess cardiovascular risk due to hyper-aldosteronism? 3) What are the most cost-effective screening methods? 4) What is the clinically helpful definition of renin-independent aldosteronism and essential hypertension and vice versa? These answers will help us design a better screening algorithm for PA. Additionally, we need evidence that the algorithm can identify all cases that benefit from PA treatment at an early stage. Finally, we emphasize that evidence using the PAC value by CLEIA is warranted. Accumulated clinical data from larger samples will facilitate the development of a new screening strategy for PA.

Author contributions

TK: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. YR: Writing – review & editing. HK: Writing – review & editing. KI: Writing – review & editing. YM: Writing – review & editing. MO: Writing – review & editing. KN: Writing – review & editing. YT: Writing – review & editing. JS: Writing – review & editing. KK: Writing – review & editing. KY: Writing – review & editing. TN: Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by a grant from the Ministry of Health, Labor, and Welfare, Japan (23FC1041 to TN and JS), JSPS KAKENHI Grant Numbers JP22K16422 (TK).

Acknowledgments

We thank all the medical staff in the endocrinology department of Yokohama Rosai Hospital and Dr. Kenichi Sakamoto (Rutgers Robert Wood Johnson Medical School) for the fruitful discussions.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Conn JW. Primary aldosteronism. *J Lab Clin Med.* (1955) 45:661–4.

2. Conn JW, Cohen EL, Rovner DR, Nesbit RM. Normokalemic primary aldosteronism. A detectable cause of curable "Essential" Hypertension. *JAMA.* (1965) 193:200–6. doi: 10.1001/jama.1965.03090030022005

3. Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *J Am Coll Cardiol.* (2006) 48:2293–300. doi: 10.1016/j.jacc.2006.07.059

4. Catena C, Colussi G, Nadalini E, Chiuch A, Baroselli S, Lapenna R, et al. Cardiovascular outcomes in patients with primary aldosteronism after treatment. *Arch Internal Med.* (2008) 168:80–5.

5. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol.* (2005) 45:1243–8. doi: 10.1016/j.jacc.2005.01.015

6. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. *Lancet Diabetes Endocrinol.* (2018) 6:51–9. doi: 10.1016/S2213-8587(17)30367-4

7. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol Jan.* (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4

8. Hiramatsu K, Yamada T, Yukimura Y, Komiya I, Ichikawa K, Ishihara M, et al. A screening test to identify aldosterone-producing adenoma by measuring plasma renin activity: results in hypertensive patients. *Arch Internal Med.* (1981) 141:1589–93. doi: 10.1001/archinte.1981.00340130033011

9. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* (2016) 101:1889–916. doi: 10.1210/jc.2015-4061

10. Wu VC, Hu YH, Er LK, Yen RF, Chang CH, Chang YL, et al. Case detection and diagnosis of primary aldosteronism - The consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc.* (2017) 20. doi: 10.1016/j.jfma.2017.06.004

11. Whelton Paul K, Carey Robert M, Aronow Wilbert S, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, et al. ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: A report of the american college of cardiology/american heart association task force on clinical practice guidelines. *Hypertension.* (2017) 71:e13–e115. doi: 10.1161/HYP.0000000000000065

12. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur Heart J.* (2018) 39:3021–104. doi: 10.1093/eurheartj/ehy339

13. Mulatero P, Monticone S, Deinum J, Amar L, Prejbisz A, Zennaro MC, et al. Genetics, prevalence, screening and confirmation of primary aldosteronism: a position statement and consensus of the Working Group on Endocrine Hypertension of The European Society of Hypertension∗. *J Hypertension.* (2020) 38.

14. Rossi GP, Bisogni V, Bacca AV, Belfiore A, Cesari M, Concistre A, et al. The 2020 Italian Society of Arterial Hypertension (SIIA) practical guidelines for the management of primary aldosteronism. *Int J Cardiol Hypertension.* (2020) 5:100029. doi: 10.1016/j.ijchy.2020.100029

15. Naruse M, Katabami T, Shibata H, Sone M, Takahashi K, Tanabe A, et al. Japan Endocrine Society clinical practice guideline for the diagnosis and management of primary aldosteronism 2021. *Endocrine J.* (2022) 69:327–59. doi: 10.1507/endocrj.ej21-0508

16. Lim PO, Dow E, Brennan G, Jung RT, MacDonald TM. High prevalence of primary aldosteronism in the Tayside hypertension clinic population. *J Hum Hypertens May.* (2000) 14:311–5. doi: 10.1038/sj.jhh.1001013

17. Loh KC, Koay ES, Khaw MC, Emmanuel SC, Young WF. Prevalence of primary aldosteronism among Asian hypertensive patients in Singapore. Article. *J Clin Endocrinol Metab.* (2000) 85:2854–9. doi: 10.1210/jc.85.8.2854

18. Rossi E, Regolisti G, Negro A, Sani C, Davoli S, Perazzoli F. High prevalence of primary aldosteronism using postcaptopril plasma aldosterone to renin ratio as a screening test among Italian hypertensives. *Am J Hypertens.* (2002) 15:896–902. doi: 10.1016/s0895-7061(02)02969-2

19. Mosso L, Carvajal C, Gonzalez A, Barraza A, Avila F, Montero J, et al. Primary aldosteronism and hypertensive disease. *Hypertension. Aug.* (2003) 42:161–5. doi: 10.1161/01.HYP.0000079505.25750.11

20. Stowasser M, Gordon RD, Gunasekera TG, Cowley DC, Ward G, Archibald C, et al. High rate of detection of primary aldosteronism, including surgically treatable forms, after 'non-selective' screening of hypertensive patients. *J Hypertens.* (2003) 21:2149–57. doi: 10.1097/00004872-200311000-00025

21. Strauch B, Zelinka T, Hampf M, Bernhardt R, Widimsky J Jr. Prevalence of primary hyperaldosteronism in moderate to severe hypertension in the Central Europe region. *J Hum Hypertens.* (2003) 17:349–52. doi: 10.1038/sj.jhh.1001554

22. Omura M, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T. Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan. *Hypertens Res.* (2004) 27:193–202. doi: 10.1291/hypres.27.193

23. Nishizaka MK, Pratt-Ubunama M, Zaman MA, Cofield S, Calhoun DA. Validity of plasma aldosterone-to-renin activity ratio in African American and white subjects with resistant hypertension. *Am J Hypertens.* (2005) 18:805–12. doi: 10.1016/j.amjhyper.2005.01.002

24. Fogari R, Preti P, Zoppi A, Rinaldi A, Fogari E, Mugellini A. Prevalence of primary aldosteronism among unselected hypertensive patients: a prospective study based on the use of an aldosterone/renin ratio above 25 as a screening test. *Hypertens Res Feb.* (2007) 30:111–7. doi: 10.1291/hypres.30.111

25. Douma S, Petidis K, Doumas M, Papaefthimiou P, Triantafyllou A, Kartali N, et al. Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. *Lancet Jun 7.* (2008) 371:1921–6. doi: 10.1016/S0140-6736(08)60834-X

26. Westerdahl C, Bergenfelz A, Isaksson A, Nerbrand C, Valdemarsson S. Primary aldosteronism among newly diagnosed and untreated hypertensive patients in a Swedish primary care area. *Scand J Prim Health Care.* (2011) 29:57–62. doi: 10.3109/02813432.2011.554015

27. Sigurjonsdottir HA, Gronowitz M, Andersson O, Andersson O, Eggertsen R, Herlitz H, Sakinis A, et al. Unilateral adrenal hyperplasia is a usual cause of primary hyperaldosteronism. *Results Swedish screening study. BMC Endocr Disord.* (2012) 12:17. doi: 10.1186/1472-6823-12-17

28. Sang X, Jiang Y, Wang W, Yan L, Zhao J, Peng Y, et al. Prevalence of and risk factors for primary aldosteronism among patients with resistant hypertension in China. *J Hypertens.* (2013) 31:1465–71. doi: 10.1097/HJH.0b013e328360ddf6

29. Galati SJ, Cheesman KC, Springer-Miller R, Hopkins SM, Krakoff L, Bagiella E, et al. Prevelence of primary aldosteronism in an urban hypertensive population. *Endocr Pract Nov.* (2016) 22:1296–302. doi: 10.4158/E161332.OR

30. Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, et al. Prevalence and clinical manifestations of primary aldosteronism encountered in primary care practice. *J Am Coll Cardiol.* (2017) 69:1811–20. doi: 10.1016/j.jacc.2017.01.052

31. Kayser SC, Deinum J, de Grauw WJ, Schalk BW, Bor HJ, Lenders JW, et al. Prevalence of primary aldosteronism in primary care: a cross-sectional study. *Br J Gen Pract.* (2018) 68:e114–22. doi: 10.3399/bjgp18X694589

32. Brown JM, Siddiqui M, Calhoun DA, Carey RM, Hopkins PN, Williams GH, et al. The unrecognized prevalence of primary aldosteronism: A cross-sectional study. *Ann Intern Med.* (2020) 173:10–20. doi: 10.7326/M20-0065

33. Xu Z, Yang J, Hu J, Song Y, He W, Luo T, et al. Primary aldosteronism in patients in China with recently detected hypertension. *J Am Coll Cardiol.* (2020) 75:1913–22. doi: 10.1016/j.jacc.2020.02.052

34. Xu F, Gao Z, Wang G, Gao Y, Guo Y, Guo Y, et al. Prevalence, subtype classification, and outcomes of treatment of primary aldosteronism: A prospective study in China. *Endocr Pract.* (2021) 27:478–83. doi: 10.1016/j.eprac.2020.10.007

35. Asbach E, Kellnar A, Bekeran M, Schelling J, Bidlingmaier M, Reincke M. Prevalence of primary aldosteronism in newly diagnosed hypertensive patients in primary care. *Exp Clin Endocrinol Diabetes.* (2022) 130:801–5. doi: 10.1055/a-1938-4242

36. Yoon M, Hong N, Ha J, Lee CJ, Ku CR, Rhee Y, et al. Prevalence and clinical characteristics of primary aldosteronism in a tertiary-care center in Korea. *Hypertens Res.* (2022) 45:1418–29. doi: 10.1038/s41440-022-00948-7

37. Hannemann A, Wallaschofski H. Prevalence of primary aldosteronism in patient's cohorts and in population-based studies–a review of the current literature. *Horm Metab Res.* (2012) 44:157–62.

38. Lubitz CC, Economopoulos KP, Sy S, Johanson C, Kunzel HE, Reincke M, et al. Cost-effectiveness of screening for primary aldosteronism and subtype diagnosis in the resistant hypertensive patients. *Circulation: Cardiovasc Qual Outcomes.* (2015) 8:621–30. doi: 10.1161/circoutcomes.115.002002

39. Sato M, Morimoto R, Seiji K, Iwakura Y, Ono Y, Kudo M, et al. Cost-effectiveness analysis of the diagnosis and treatment of primary aldosteronism in Japan. *Horm Metab Res.* (2015) 47:826–32. doi: 10.1055/s-0035-1559645

40. Woode ME, Wong K, Reid CM, Stowasser M, Russell G, Gwini S, et al. Cost-effectiveness of screening for primary aldosteronism in hypertensive patients in Australia: a Markov modelling analysis. *J Hypertension.* (2023) 41:1615–25. doi: 10.1097/hjh.0000000000003513

41. Jaffe G, Gray Z, Krishnan G, Stedman M, Zheng Y, Han J, et al. Screening rates for primary aldosteronism in resistant hypertension. *Hypertension.* (2020) 75:650–9. doi: 10.1161/hypertensionaha.119.14359

42. Cohen JB, Cohen DL, Herman DS, Leppert JT, Byrd JB, Bhalla V. Testing for primary aldosteronism and mineralocorticoid receptor antagonist use among US veterans: a retrospective cohort study. *Ann Internal Med.* (2021) 174:289–97.

43. Liu Y-Y, King J, Kline GA, Padwal RS, Pasieka JL, Chen G, et al. Outcomes of a specialized clinic on rates of investigation and treatment of primary aldosteronism. *JAMA Surgery.* (2021) 156:541. doi: 10.1001/jamasurg.2021.0254

44. Hundemer GL, Imsirovic H, Vaidya A, Yozamp N, Goupil R, Madore F, et al. Screening rates for primary aldosteronism among individuals with hypertension plus hypokalemia: A population-based retrospective cohort study. *Hypertension.* (2022) 79:178–86. doi: 10.1161/hypertensionaha.121.18118

45. Graudal NA, Hubeck-Graudal T, Jurgens G. Effects of low sodium diet versus high sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride. *Cochrane Database Syst Rev.* (2020) 12:CD004022. doi: 10.1002/14651858.CD004022.pub5

46. Newton MA, Laragh JH. Effects of glucocorticoid administration on aldosterone excretion and plasma renin in normal subjects, in essential hypertension and in primary aldosteronism. *J Clin Endocrinol Metab.* (1968) 28:1014–22. doi: 10.1210/jcem-28-7-1014

47. Oelkers WK. Effects of estrogens and progestogens on the renin-aldosterone system and blood pressure. *Steroids.* (1996) 61:166–71. doi: 10.1016/0039-128x(96)00007-4

48. Ahmed AH, Calvird M, Gordon RD, Taylor PJ, Ward G, Pimenta E, et al. Effects of two selective serotonin reuptake inhibitor antidepressants, sertraline and escitalopram, on aldosterone/renin ratio in normotensive depressed male patients. *J Clin Endocrinol Metab.* (2011) 96:1039–45. doi: 10.1210/jc.2010-2603

49. Young WF, Calhoun DA, Lenders JWM, Stowasser M, Textor SC. Screening for endocrine hypertension: an endocrine society scientific statement. *Endocrine Rev.* (2017) 38:103–22. doi: 10.1210/er.2017-00054

50. Reincke M, Bancos I, Mulatero P, Scholl UI, Stowasser M, Williams TA. Diagnosis and treatment of primary aldosteronism. *Lancet Diabetes Endocrinol.* (2021) 9:876–92. doi: 10.1016/S2213-8587(21)00210-2

51. Rehan M, Raizman JE, Cavalier E, Don-Wauchope AC, Holmes DT. Laboratory challenges in primary aldosteronism screening and diagnosis. *Clin Biochem.* (2015) 48:377–87. doi: 10.1016/j.clinbiochem.2015.01.003

52. Thuzar M, Young K, Ahmed AH, Ward G, Wolley M, Guo Z, et al. Diagnosis of primary aldosteronism by seated saline suppression test—Variability between immunoassay and HPLC-MS/MS. *J Clin Endocrinol Metab*. (2019) 105:e477–83. doi: 10.1210/clinem/dgz150

53. Tamura N, Watanabe E, Shirakawa R, Nakatani E, Yamada K, Hatakeyama H, et al. Comparisons of plasma aldosterone and renin data between an automated chemiluminescent immunoanalyzer and conventional radioimmunoassays in the screening and diagnosis of primary aldosteronism. *PloS One*. (2021) 16:e0253807.

54. Nishikawa T, Omura M, Kawaguchi M, Takatsu A, Satoh F, Ito S, et al. Calibration and evaluation of routine methods by serum certified reference material for aldosterone measurement in blood. *Endocrine J*. (2016) 63:1065–80. doi: 10.1507/endocrj.ej16-0304

55. Nishikawa T, Satoh F, Takashi Y, Yanase T, Itoh H, Kurihara I, et al. Comparison and commutability study between standardized liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) and chemiluminescent enzyme immunoassay for aldosterone measurement in blood. *Endocrine J*. (2022) 69:45–54. doi: 10.1507/endocrj.ej21-0278

56. Fries CM, Bae YJ, Rayes N, Sandner B, Isermann B, Stumvoll M, et al. Prospective evaluation of aldosterone LC-MS/MS-specific cutoffs for the saline infusion test. *Eur J Endocrinology*. (2020) 183:191–201. doi: 10.1530/eje-20-0030

57. Guo Z, Poglitsch M, McWhinney BC, Ungerer JPJ, Ahmed AH, Gordon RD, et al. Aldosterone LC-MS/MS assay-specific threshold values in screening and confirmatory testing for primary aldosteronism. *J Clin Endocrinol Metab*. (2018) 103:3965–73. doi: 10.1210/jc.2018-01041

58. Sealey JE, Gordon RD, Mantero F. Plasma renin and aldosterone measurements in low renin hypertensive states. *Trends Endocrinol Metab*. (2005) 16:86–91. doi: 10.1016/j.tem.2005.02.006

59. Brossaud J, Corcuff JB. Pre-analytical and analytical considerations for the determination of plasma renin activity. *Clin Chim Acta*. (2009) 410:90–2. doi: 10.1016/j.cca.2009.09.018

60. Derkx FH, de Bruin RJ, van Gool JM, van den Hoek MJ, Beerendonk CC, Rosmalen F, et al. Clinical validation of renin monoclonal antibody-based sandwich assays of renin and prorenin, and use of renin inhibitor to enhance prorenin immunoreactivity. *Clin Chem Jul*. (1996) 42:1051–63.

61. Deinum J, Derkx FH, Schalekamp MA. Improved immunoradiometric assay for plasma renin. *Clin Chem*. (1999) 45:847–54. doi: 10.1093/clinchem/45.6.847

62. Leung AA, Orton DJ, Chin A, Sadrzadeh H, Kline GA. Novel approach to establishing an aldosterone: renin ratio cutoff for primary aldosteronism. *Hypertension*. (2017) 69:450–6. doi: 10.1161/hypertensionaha.116.08407

63. Unger N, Lopez Schmidt I, Pitt C, Walz M, Philipp T, Mann K, et al. Comparison of active renin concentration and plasma renin activity for the diagnosis of primary hyperaldosteronism in patients with an adrenal mass. *Eur J Endocrinology*. (2004) 150:517–23. doi: 10.1530/eje.0.1500517

64. Dorrian CA, Toole BJ, Alvarez-Madrazo S, Kelly A, Connell JMC, Wallace AM. A screening procedure for primary aldosteronism based on the Diasorin Liaison® automated chemiluminescent immunoassay for direct renin. *Ann Clin Biochemistry: Int J Lab Med*. (2010) 47:195–9. doi: 10.1258/acb.2010.009230

65. Morganti A, obotEsgftvoDldr a. A comparative study on inter and intralaboratory reproducibility of renin measurement with a conventional enzymatic method and a new chemiluminescent assay of immunoreactive renin. *J Hypertension*. (2010) 28:1307–12. doi: 10.1097/HJH.0b013e32833857ad

66. Gruson D, Maisin D, Lison P, Maiter D, Persu A. Two-site automated chemiluminescent assay for measurement of immunoreactive renin. *Biomarkers*. (2011) 16:605–9. doi: 10.3109/1354750X.2011.614015

67. Li X, Goswami R, Yang S, Li Q. Aldosterone/direct renin concentration ratio as a screening test for primary aldosteronism: A meta-analysis. *J Renin Angiotensin Aldosterone Syst*. (2016) 17:1470320316657450. doi: 10.1177/1470320316657450

68. Shanik MH, Romao IJ, Velayati S. Aldosterone/direct renin concentration ratio versus aldosterone/plasma renin activity for diagnosis of primary hyperaldosteronism: case presentation. *J Endocrine Society*. (2021) 5:A113–4. doi: 10.1210/jendso/bvab048.228

69. Morimoto R, Ono Y, Tezuka Y, Kudo M, Yamamoto S, Arai T, et al. Rapid screening of primary aldosteronism by a novel chemiluminescent immunoassay. *Hypertension*. (2017) 70:334–41. doi: 10.1161/HYPERTENSIONAHA.117.09078

70. Heinrich DA, Adolf C, Rump LC, Quack I, Quinkler M, Hahner S, et al. Primary aldosteronism: key characteristics at diagnosis: a trend toward milder forms. *Eur J Endocrinology*. (2018) 178:605–11. doi: 10.1530/eje-17-0978

71. Savard S, Amar L, Plouin P-F, Steichen O. Cardiovascular complications associated with primary aldosteronism. *Hypertension*. (2013) 62:331–6.

72. Ohno Y, Sone M, Inagaki N, Yamasaki T, Ogawa O, Takeda Y, et al. Prevalence of cardiovascular disease and its risk factors in primary aldosteronism. *Hypertension*. (2018) 71:530–7. doi: 10.1161/hypertensionaha.117.10263

73. Huang KH, Yu CC, Hu YH, Chang CC, Chan CK, Liao SC, et al. Targeted treatment of primary aldosteronism - The consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc*. (2018) 2. doi: 10.1016/j.jfma.2018.01.006

74. Stowasser M, Gordon RD. Primary aldosteronism—careful investigation is essential and rewarding. *Mol Cell Endocrinology*. (2004) 217:33–9. doi: 10.1016/j.mce.2003.10.006

75. Murase K, Nagaishi R, Takenoshita H, Nomiyama T, Akehi Y, Yanase T. Prevalence and clinical characteristics of primary aldosteronism in Japanese patients with type 2 diabetes mellitus and hypertension. *Endocrine J*. (2013) 60:967–76. doi: 10.1507/endocrj.ej13-0060

76. Tiu S-C, Choi C-H, Shek C-C, Ng YW, Chan FK, Ng CM, et al. The use of aldosterone-renin ratio as a diagnostic test for primary hyperaldosteronism and its test characteristics under different conditions of blood sampling. *J Clin Endocrinol Metab*. (2005) 90:72–8. doi: 10.1210/jc.2004-1149

77. Mulatero P, Rabbia F, Milan A, Paglieri C, Morello F, Chiandussi L, et al. Drug effects on aldosterone/plasma renin activity ratio in primary aldosteronism. *Hypertension*. (2002) 40:897–902. doi: 10.1161/01.hyp.0000038478.59760.41

78. Seifarth C, Trenkel S, Schobel H, Hahn EG, Hensen J. Influence of antihypertensive medication on aldosterone and renin concentration in the differential diagnosis of essential hypertension and primary aldosteronism. *Clin Endocrinology*. (2002) 57:457–65. doi: 10.1046/j.1365-2265.2002.01613.x

79. Nagasawa M, Yamamoto K, Rakugi H, Takeda M, Akasaka H, Umakoshi H, et al. Influence of antihypertensive drugs in the subtype diagnosis of primary aldosteronism by adrenal venous sampling. *J Hypertension*. (2019) 37.

80. Calhoun DA, Nishizaka MK, Zaman MA, Thakkar RB, Weissmann P. Hyperaldosteronism among black and white subjects with resistant hypertension. *Hypertension*. (2002) 40:892–6. doi: 10.1161/01.hyp.0000040261.30455.b6

81. Burrello J, Monticone S, Losano I, Cavaglià G, Buffolo F, Tetti M, et al. Prevalence of hypokalemia and primary aldosteronism in 5100 patients referred to a tertiary hypertension unit. *Hypertension*. (2020) 75:1025–33. doi: 10.1161/hypertensionaha.119.14063

82. Parasiliti-Caprino M, Lopez C, Prencipe N, Lucatello B, Settanni F, Giraudo G, et al. Prevalence of primary aldosteronism and association with cardiovascular complications in patients with resistant and refractory hypertension. *J Hypertension*. (2020) ;38.

83. Rossi GP, Seccia TM, Palumbo G, Belfiore A, Bernini G, Caridi G, et al. Within-patient reproducibility of the aldosterone: renin ratio in primary aldosteronism. *Hypertension*. (2010) 55:83–9. doi: 10.1161/hypertensionaha.109.139832

84. Vieweg WV, Veldhuis JD, Carey RM. Temporal pattern of renin and aldosterone secretion in men: effects of sodium balance. *Am J Physiol*. (1992) 262:F871–7. doi: 10.1152/ajprenal.1992.262.5.F871

85. Siragy HM, Vieweg WV, Pincus S, Veldhuis JD. Increased disorderliness and amplified basal and pulsatile aldosterone secretion in patients with primary aldosteronism. *J Clin Endocrinol Metab*. (1995) 80:28–33. doi: 10.1210/jcem.80.1.7829626

86. Tanabe A, Naruse M, Takagi S, Tsuchiya K, Imaki T, Takano K. Variability in the renin/aldosterone profile under random and standardized sampling conditions in primary aldosteronism. *J Clin Endocrinol Metab*. (2003) 88:2489–94. doi: 10.1210/jc.2002-021476

87. Kline GA, Darras P, Leung AA, So B, Chin A, Holmes DT. Surprisingly low aldosterone levels in peripheral veins following intravenous sedation during adrenal vein sampling: implications for the concept of nonsuppressibility in primary aldosteronism. *J Hypertens*. (2019) 37:596–602. doi: 10.1097/HJH.0000000000001905

88. Yozamp N, Hundemer GL, Moussa M, Underhill J, Fudim T, Sacks B, et al. Variability of aldosterone measurements during adrenal venous sampling for primary aldosteronism. *Am J Hypertens*. (2021) 34:34–45. doi: 10.1093/ajh/hpaa151

89. Baudrand R, Guarda FJ, Torrey J, Williams G, Vaidya A. Dietary sodium restriction increases the risk of misinterpreting mild cases of primary aldosteronism. *J Clin Endocrinol Metab*. (2016) 101:3989–96. doi: 10.1210/jc.2016-1963

90. Spence JD, Rayner BL. Hypertension in blacks. *Hypertension*. (2018) 72:263–9. doi: 10.1161/hypertensionaha.118.11064

91. Yozamp N, Hundemer GL, Moussa M, Underhill J, Fudim T, Sacks B, et al. Intraindividual variability of aldosterone concentrations in primary aldosteronism. *Hypertension*. (2021) 77:891–9. doi: 10.1161/hypertensionaha.120.16429

92. Azizan EA, Murthy M, Stowasser M, Gordon R, Kowalski B, Xu S, et al. Somatic mutations affecting the selectivity filter of KCNJ5 are frequent in 2 large unselected collections of adrenal aldosteronomas. *Hypertension. Mar*. (2012) 59:587–91. doi: 10.1161/HYPERTENSIONAHA.111.186239

93. Boulkroun S, Beuschlein F, Rossi GP, Golib-Dzib JF, Fischer E, Amar L, et al. Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism. *Hypertension*. (2012) 59:592–8. doi: 10.1161/HYPERTENSIONAHA.111.186478

94. Williams TA, Monticone S, Schack VR, Stindl J, Burrello J, Buffolo F, et al. Somatic ATP1A1, ATP2B3, and KCNJ5 mutations in aldosterone-producing adenomas. *Hypertension*. (2014) 63:188–95. doi: 10.1161/HYPERTENSIONAHA.113.01733

95. Wu VC, Huang KH, Peng KY, Tsai YC, Wu CH, Wang SM, et al. Prevalence and clinical correlates of somatic mutation in aldosterone producing adenoma-Taiwanese population. *Sci Rep*. (2015) 5:11396. doi: 10.1038/srep11396

96. Zheng FF, Zhu LM, Nie AF, Li XY, Lin JR, Zhang K, et al. Clinical characteristics of somatic mutations in Chinese patients with aldosterone-producing adenoma. *Hypertension. Mar*. (2015) 65:622–8. doi: 10.1161/HYPERTENSIONAHA.114.03346

97. Kitamoto T, Suematsu S, Yamazaki Y, Nakamura Y, Sasano H, Matsuzawa Y, et al. Clinical and steroidogenic characteristics of aldosterone-producing adenomas with ATPase or CACNA1D gene mutations. *J Clin Endocrinol Metab*. (2016) 101:494–503. doi: 10.1210/jc.2015-3284

98. Nanba K, Rainey WE. GENETICS IN ENDOCRINOLOGY: Impact of race and sex on genetic causes of aldosterone-producing adenomas. *Eur J Endocrinology.* (2021) 185:R1–R11. doi: 10.1530/eje-21-0031

99. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science.* (2011) 331:768–72. doi: 10.1126/science.1198785

100. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet.* (2013) 45:440–4, 444e1-2. doi: 10.1038/ng.2550

101. Scholl UI, Goh G, Stolting G, de Oliveira RC, Choi M, Overton JD, et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet.* (2013) 45:1050–4. doi: 10.1038/ng.2695

102. De Sousa K, Boulkroun S, Baron S, Nanba K, Wack M, Rainey WE, et al. Genetic, cellular, and molecular heterogeneity in adrenals with aldosterone-producing adenoma. *Hypertension.* (2020) 75:1034–44. doi: 10.1161/hypertensionaha.119.14177

103. Cheng CJ, Sung CC, Wu ST, Lin YC, Sytwu HK, Huang CL, et al. Novel KCNJ5 mutations in sporadic aldosterone-producing adenoma reduce Kir3. *4 membrane abundance. J Clin Endocrinol Metab.* (2015) 100:E155–63. doi: 10.1210/jc.2014-3009

104. Hong AR, Kim JH, Song YS, Lee KE, Seo SH, Seong MW, et al. Genetics of aldosterone-producing adenoma in korean patients. *PloS One.* (2016) 11:e0147590. doi: 10.1371/journal.pone.0147590

105. Kitamoto T, Suematsu S, Matsuzawa Y, Saito J, Omura M, Nishikawa T. Comparison of cardiovascular complications in patients with and without KCNJ5 gene mutations harboring aldosterone-producing adenomas. *J Atheroscl thrombosis.* (2014) 24.

106. Mohideen SK, Mustangin M, Kamaruddin NA, Muhammad R, Jamal ARA, Sukor N, et al. Prevalence and histopathological characteristics of KCNJ5 mutant aldosterone-producing adenomas in a multi-ethnic Malaysian cohort. Original research. *Front Endocrinol (Lausanne).* (2019) 10:666. doi: 10.3389/fendo.2019.00666

107. Nanba K, Yamazaki Y, Bick N, Onodera K, Tezuka Y, Omata K, et al. Prevalence of somatic mutations in aldosterone-producing adenomas in Japanese patients. *J Clin Endocrinol Metab.* (2020) 105:e4066–73. doi: 10.1210/clinem/dgaa595

108. Taguchi R, Yamada M, Nakajima Y, Satoh T, Hashimoto K, Shibusawa N, et al. Expression and mutations of KCNJ5 mRNA in Japanese patients with aldosterone-producing adenomas. *J Clin Endocrinol Metab.* (2012) 97:1311–9. doi: 10.1210/jc.2011-2885

109. Wang B, Li X, Zhang X, Ma X, Chen L, Zhang Y, et al. Prevalence and characterization of somatic mutations in chinese aldosterone-producing adenoma patients. *Medicine.* (2015) 94:e708. doi: 10.1097/md.0000000000000708

110. Warachit W, Atikankul T, Houngngam N, Sunthornyothin S. Prevalence of somatic KCNJ5 mutations in thai patients with aldosterone-producing adrenal adenomas. *J Endocrine Society.* (2018) 2:1137–46. doi: 10.1210/js.2018-00097

111. Akerstrom T, Crona J, Delgado Verdugo A, Starker LF, Cupisti K, Willenberg HS, et al. Comprehensive re-sequencing of adrenal aldosterone producing lesions reveal three somatic mutations near the KCNJ5 potassium channel selectivity filter. *PloS One.* (2012) 7:e41926. doi: 10.1371/journal.pone.0041926

112. Akerstrom T, Willenberg HS, Cupisti K, Ip J, Backman S, Moser A, et al. Novel somatic mutations and distinct molecular signature in aldosterone-producing adenomas. *Endocr Relat Cancer.* (2015) 22:735–44. doi: 10.1530/ERC-15-0321

113. Arnesen T, Glomnes N, Strømsøy S, Knappskog S, Heie A, Akslen LA, et al. Outcome after surgery for primary hyperaldosteronism may depend on KCNJ5 tumor mutation status: a population-based study from Western Norway. *Langenbeck's Arch Surgery.* (2013) 398:869–74. doi: 10.1007/s00423-013-1093-2

114. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension.* (2014) 64:354–61. doi: 10.1161/hypertensionaha.114.03419

115. Guo Z, Nanba K, Udager A, McWhinney BC, Ungerer JPJ, Wolley M, et al. Biochemical, histopathological, and genetic characterization of posture-responsive and unresponsive APAs. *J Clin Endocrinol Metab.* (2020) 105:e3224–35. doi: 10.1210/clinem/dgaa367

116. Meyer LS, Handgriff L, Lim JS, Udager AM, Kinker IS, Ladurner R, et al. Single-center prospective cohort study on the histopathology, genotype, and postsurgical outcomes of patients with primary aldosteronism. *Hypertension.* (2021) 78:738–46. doi: 10.1161/HYPERTENSIONAHA.121.17348

117. Nanba K, Omata K, Else T, Beck PCC, Nanba AT, Turcu AF, et al. Targeted molecular characterization of aldosterone-producing adenomas in white americans. *J Clin Endocrinol Metab.* (2018) 103:3869–76. doi: 10.1210/jc.2018-01004

118. Nanba K, Omata K, Gomez-Sanchez Celso E, et al. Genetic characteristics of aldosterone-producing adenomas in blacks. *Hypertension.* (2019) 73:885–92. doi: 10.1161/HYPERTENSIONAHA.118.12070

119. Scholl UI, Healy JM, Thiel A, Fonseca AL, Brown TC, Kunstman JW, et al. Novel somatic mutations in primary hyperaldosteronism are related to the clinical, radiological and pathological phenotype. *Clin Endocrinology.* (2015) 83:779–89. doi: 10.1111/cen.12873

120. Kitamoto T, Nishikawa T. Clinical translationality of KCNJ5 mutation in aldosterone producing adenoma. *Int J Mol Sci.* (2022) 23:9042. doi: 10.3390/ijms23169042

121. Nishimoto K, Nakagawa K, Li D, Kosaka T, Oya M, Mikami S, et al. Adrenocortical zonation in humans under normal and pathological conditions. *J Clin Endocrinol Metab.* (2010) 95:2296–305. doi: 10.1210/jc.2009-2010

122. Nishimoto K, Tomlins SA, Kuick R, Cani AK, Giordano TJ, Hovelson DH, et al. Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands. *Proc Natl Acad Sci U S A.* (2015) 112:E4591–9. doi: 10.1073/pnas.1505529112

123. Omata K, Anand SK, Hovelson DH, Liu C-J, Yamazaki Y, Nakamura Y, et al. Aldosterone-producing cell clusters frequently harbor somatic mutations and accumulate with age in normal adrenals. *J Endocrine Society.* (2017) 1:787–99. doi: 10.1210/js.2017-00134

124. Omata K, Satoh F, Morimoto R, Ito S, Yamazaki Y, Nakamura Y, et al. Cellular and genetic causes of idiopathic hyperaldosteronism. *Hypertension. Oct.* (2018) 72:874–80. doi: 10.1161/HYPERTENSIONAHA.118.11086

125. Omura M, Sasano H, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T. Clinical characteristics of aldosterone-producing microadenoma, macroadenoma, and idiopathic hyperaldosteronism in 93 patients with primary aldosteronism. *Hypertens Res.* (2006) 29:883–9.

126. Nanba K, Vaidya A, Williams GH, Zheng I, Else T, Rainey WE. Age-related autonomous aldosteronism. *Circulation.* (2017) 136:347–55. doi: 10.1161/circulationaha.117.028201

127. Nanba K, Vaidya A, Rainey WE. Aging and adrenal aldosterone production. *Hypertension.* (2018) 71:218–23. doi: 10.1161/hypertensionaha.117.10391

128. Baudrand R, Guarda FJ, Fardella C, Hundemer G, Brown J, Williams G, et al. Continuum of renin-independent aldosteronism in normotension. *Hypertension.* (2017) 69:950–6. doi: 10.1161/hypertensionaha.116.08952

129. Le Floch E, Cosentino T, Larsen CK, Beuschlein F, Reincke M, Amar L, et al. Identification of risk loci for primary aldosteronism in genome-wide association studies. *Nat Commun.* (2022) 13:5198. doi: 10.1038/s41467-022-32896-8

130. Williams TA, Peitzsch M, Dietz AS, Dekkers T, Bidlingmaier M, Riester A, et al. Genotype-specific steroid profiles associated with aldosterone-producing adenomas. *Hypertension. Jan.* (2016) 67:139–45. doi: 10.1161/HYPERTENSIONAHA.115.06186

131. Inoue K, Yamazaki Y, Kitamoto T, Hirose R, Saito J, Omura M, et al. Aldosterone suppression by dexamethasone in patients with KCNJ5-mutated aldosterone-producing adenoma. *J Clin Endocrinol Metab.* (2018) 103:3477–85. doi: 10.1210/jc.2018-00738

132. Tezuka Y, Yamazaki Y, Kitada M, Morimoto R, Kudo M, Seiji K, et al. 18-oxocortisol synthesis in aldosterone-producing adrenocortical adenoma and significance of KCNJ5 mutation status. *Hypertension.* (2019). doi: 10.1161/HYPERTENSIONAHA.118.12064

133. Hung A, Ahmed S, Gupta A, Davis A, Kline GA, Leung AA, et al. Performance of the aldosterone to renin ratio as a screening test for primary aldosteronism. *J Clin Endocrinol Metab.* (2021) 106:2423–35. doi: 10.1210/clinem/dgab348

134. Parksook WW, Brown JM, Omata K, Tezuka Y, Ono Y, Satoh F, et al. The spectrum of dysregulated aldosterone production: an international human physiology study. *J Clin Endocrinol Metab.* (2024). doi: 10.1210/clinem/dgae145

135. Brilla CG, Weber KT. Mineralocorticoid excess, dietary sodium, and myocardial fibrosis. *J Lab Clin Med.* (1992) 120:893–901.

136. Funder JW. Primary aldosteronism and salt. *Pflugers Arch.* (2015) 467:587–94. doi: 10.1007/s00424-014-1658-0

137. Ueda T, Tsurutani Y, Osada J, Inoue K, Hoshino Y, Ono M, et al. Comparison of echocardiographic changes between surgery and medication treatment in patients with primary aldosteronism. *J Am Heart Assoc.* (2022) 11:e023813. doi: 10.1161/JAHA.121.023813

138. Katsuragawa S, Goto A, Shinoda S, Inoue K, Nakai K, Saito J, et al. Association of reversal of renin suppression with long-term renal outcome in medically treated primary aldosteronism. *Hypertension.* (2023) 80:1909–20. doi: 10.1161/HYPERTENSIONAHA.123.21096

139. Inoue K, Goldwater D, Allison M, Seeman T, Kestenbaum BR, Watson KE. Serum aldosterone concentration, blood pressure, and coronary artery calcium: the multi-ethnic study of atherosclerosis. *Hypertension.* (2020). doi: 10.1161/HYPERTENSIONAHA.120.15006

140. Hundemer GL, Agharazii M, Madore F, Vaidya A, Brown JM, Leung AA, et al. Subclinical primary aldosteronism and cardiovascular health: A population-based cohort study. *Circulation.* (2024) 149:124–34. doi: 10.1161/CIRCULATIONAHA.123.066389

141. Reincke M, Fischer E, Gerum S, Merkle K, Schulz S, Pallauf A, et al. Observational study mortality in treated primary aldosteronism: the German Conn's registry. *Hypertension. Sep.* (2012) 60:618–24. doi: 10.1161/HYPERTENSIONAHA.112.197111

142. Marzano L, Colussi G, Sechi LA, Catena C. Adrenalectomy is comparable with medical treatment for reduction of left ventricular mass in primary aldosteronism: meta-analysis of long-term studies. *Am J Hypertension.* (2015) 28:312–8. doi: 10.1093/ajh/hpu154

143. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Incidence of atrial fibrillation and mineralocorticoid receptor activity in patients with medically and surgically treated primary aldosteronism. *JAMA Cardiol.* (2018) 3:768–74. doi: 10.1001/jamacardio.2018.2003

144. Araujo-Castro M, Ruiz-Sánchez JG, Ramírez PP, Martín Rojas-Marcos P, Aguilera-Saborido A, Gómez Cerezo JF, et al. Practical consensus for the treatment

and follow-up of primary aldosteronism: a multidisciplinary consensus document. *Endocrine*. (2024) 85:532–44. doi: 10.1007/s12020-024-03773-9

145. McKenna TJ, Sequeira SJ, Heffernan A, Chambers J, Cunningham S. Diagnosis under random conditions of all disorders of the renin-angiotensin-aldosterone axis, including primary hyperaldosteronism. *J Clin Endocrinol Metab*. (1991) 73:952–7. doi: 10.1210/jcem-73-5-952

146. Jose A, Crout JR, Kaplan NM. Suppressed plasma renin activity in essential hypertension. *Roles plasma volume Blood pressure sympathetic nervous system. Ann Intern Med*. (1970) 72:9–16. doi: 10.7326/0003-4819-72-1-9

147. Carey RM, Douglas JG, Schweikert JR, Liddle GW. The syndrome of essential hypertension and suppressed plasma renin activity. *Normalization Blood Pressure spironolactone. Arch Intern Med*. (1972) 130:849–54. doi: 10.1001/archinte.1972.03650060041007

148. Laragh JH, Letcher RL, Pickering TG. Renin profiling for diagnosis and treatment of hypertension. *JAMA*. (1979) 241:151–6. doi: 10.1001/jama.1979.03290280031022

149. Baudrand R, Vaidya A. The low-renin hypertension phenotype: genetics and the role of the mineralocorticoid receptor. *Int J Mol Sci*. (2018) 19. doi: 10.3390/ijms19020546

150. Williams B, Macdonald TM, Morant S, Webb DJ, Sever P, McInnes G, et al. Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): a randomised, double-blind, crossover trial. *Lancet*. (2015) 386:2059–68. doi: 10.1016/s0140-6736(15)00257-3

151. Williams B, Macdonald TM, Morant SV, Webb DJ, Sever P, McInnes G, et al. Endocrine and haemodynamic changes in resistant hypertension, and blood pressure responses to spironolactone or amiloride: the PATHWAY-2 mechanisms substudies. *Lancet Diabetes Endocrinology*. (2018) 6:464–75. doi: 10.1016/s2213-8587(18)30071-8

152. Fischer E, Beuschlein F, Degenhart C, Jung P, Bidlingmaier M, Reincke M. Spontaneous remission of idiopathic aldosteronism after long-term treatment with spironolactone: results from the German Conn's Registry. *Clin endocrinology*. (2012) 76:473–7.

153. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Renal outcomes in medically and surgically treated primary aldosteronism. *Hypertension*. (2018). doi: 10.1161/HYPERTENSIONAHA.118.11568

154. Satoh M, Maruhashi T, Yoshida Y, Shibata H. Systematic review of the clinical outcomes of mineralocorticoid receptor antagonist treatment versus adrenalectomy in patients with primary aldosteronism. *Hypertens Res*. (2019). doi: 10.1038/s41440-019-0244-4

155. Funder JW. Who and how should we screen for primary aldosteronism? *Hypertension*. (2023) 80:2495–500. doi: 10.1161/HYPERTENSIONAHA.123.20536

156. Funder JW. Primary aldosteronism as a public health issue. *Lancet Diabetes Endocrinology*. (2016) 4:972–3. doi: 10.1016/s2213-8587(16)30272-8

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 17 January 2025
DOI 10.3389/fendo.2024.1497787

OPEN ACCESS

EDITED BY
Fabrizio Buffolo,
University of Turin, Italy

REVIEWED BY
Ivana Kraljevic,
University of Zagreb, Croatia
Nirupa Sachithanandan,
St Vincent's Health, Australia

*CORRESPONDENCE
Michiel F. Nijhoff
m.f.nijhoff@lumc.nl

†These authors have contributed equally to this work and share first authorship

RECEIVED 17 September 2024
ACCEPTED 16 December 2024
PUBLISHED 17 January 2025

CITATION
ter Haar SNM, van Goor SJ, Corssmit EPM, van Erkel AR, Ballieux BEPB, Dekkers OM and Nijhoff MF (2025) A clinical decision model for failed adrenal vein sampling in primary aldosteronism.
Front. Endocrinol. 15:1497787.
doi: 10.3389/fendo.2024.1497787

A clinical decision model for failed adrenal vein sampling in primary aldosteronism

Sophie N. M. ter Haar [1†], Sofie J. van Goor [1†], Eleonora P. M. Corssmit [1], Arian R. van Erkel [2], Bartholomeus E. P. B. Ballieux [3], Olaf M. Dekkers [1,4] and Michiel F. Nijhoff [1*]

[1]Department of Medicine, Division of Endocrinology and Metabolism, Leiden University Medical Center, Leiden, Netherlands, [2]Department of Radiology, Leiden University Medical Center, Leiden, Netherlands, [3]Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, Netherlands, [4]Department of Clinical Epidemiology, Leiden University Medical Centre, Leiden, Netherlands

Objective: Primary aldosteronism (PA) is a common cause of secondary hypertension with unilateral and bilateral subtypes requiring different treatments. Adrenal vein sampling (AVS) is the gold standard for subtype differentiation but can be unsuccessful by challenging right adrenal vein anatomy. This study aimed to develop a clinical decision model using only measurements from the left adrenal vein (LAV) and peripheral blood (IVC) to differentiate between PA subtypes.

Methods: The retrospective cohort study included 54 PA patients who underwent bilaterally successful AVS. The main objective was to determine optimal cut-off values for the LAV/IVC index, using ROC analysis for subtype prediction. The predictive value of this index was assessed with the Area Under the Curve (AUC). The Youden index calculated cut-off values, targeting a specificity >90% for PA subtype differentiation.

Results: The cohort, averaging 48.5 ± 9.5 years in age, comprised 21 women and 33 men, among whom 26 presented with unilateral and 28 with bilateral disease. LAV/IVC values <1.2 indicated unilateral right-sided disease (specificity 91%, sensitivity 96%, AUC 0.98, 95% confidence interval (CI) 0.95-1.0), values 1.2-2.4 suggested bilateral disease (sensitivity 93%, specificity 64%, AUC 0.85, CI 0.73-0.96), whereas values ≥4.4 predicted unilateral left-sided disease (specificity 93%, sensitivity 60%, AUC 0.85, CI 0.73-0.96). Published literature aligns with our results on cut-off values.

Conclusions: Utilizing the LAV/IVC index, over 70% of unsuccessful AVS procedures due to failed right adrenal cannulation could be interpreted with over 90% certainty regarding the PA subtype, preventing unnecessary resampling and aiding in determining the preferred treatment.

KEYWORDS

primary aldosteronism, adrenal vein sampling, LAV/IVC index, disease subtype, failed right cannulation, adrenalectomy

Introduction

Primary aldosteronism (PA) is a common cause of secondary hypertension and is associated with a higher risk of severe cardiovascular and renal complications as compared to essential hypertension (1–3). Autonomous aldosterone secretion can result from a unilateral aldosterone-producing adenoma or bilateral adrenal hyperplasia. Differentiating between these subtypes is crucial as preferential treatment and associated outcomes differ (4, 5). The preferred treatment for unilateral disease is adrenalectomy, aiming to cure PA. Curation is associated with better overall and long-term outcomes, including improved disease control, quality of life and mortality (5–10). Patients with bilateral disease are generally treated with mineralocorticoid receptor antagonists (MRA). MRA treatment is associated with poorer cardiovascular outcomes, substantial side effects, and reduced quality of life as compared to adrenalectomy (1, 4, 11). Therefore, identifying PA patients that will benefit from surgery is essential.

The gold standard for differentiating unilateral from bilateral PA is adrenal vein sampling (AVS) (12–14). During AVS, both adrenal veins are cannulated, and cortisol and aldosterone levels are measured and expressed as an aldosterone/cortisol (A/C) ratio (15). These ratios will be compared to determine the disease subtype. However, AVS can fail due to difficult anatomy (16). Unsuccessful sampling is mostly the result of failed cannulation of the right adrenal vein (RAV). Despite increasing expertise and success rates, recent findings show that failure rates range from 5% to 30% (12, 15, 17, 18). In case of failed sampling, repeat AVS is recommended; otherwise, the etiology remains unknown, preventing optimal treatment.

This study aimed to determine whether the A/C ratio of the left adrenal vein (LAV) compared to the inferior vena cava (IVC) could predict unilateral or bilateral disease in patients with failed RAV sampling. Using two validation cohorts with successful and unsuccessful samplings and subsequent treatment outcomes, along with a comparison of reported cut-off values from the literature, we aim to provide a clinically usable decision-making model to help clinicians predict lateralization in case of unsuccessful right-sided AVS.

Methods

Study design and patient population

We conducted a retrospective cohort study of adult patients with PA who underwent AVS between May 2014 and July 2022 at the Leiden University Medical Center (LUMC), a Dutch tertiary referral center for adrenal disease. Patients with confirmed PA and successful bilateral AVS were included.

Patients were excluded if they had other adrenal diseases like Cushing's disease or if there were major deviations in the pre-protocol work-up, potentially leading to unreliable AVS outcomes. We quantified the total amount of antihypertensive drugs, including MRA, as the daily defined dose (DDD), ATC/DDD WHO Index 2023. All patients underwent computed tomography (CT) or magnetic resonance imaging (MRI) of the adrenal glands prior to sampling. This retrospective study was approved by the scientific board of the LUMC, code W2020.058, and patients were given the opportunity to object to the use of their coded clinical data.

Primary aldosteronism diagnosis

The diagnostic work-up for PA starts with the aldosterone-to-renin ratio (ARR) (12). The ARR was calculated as the plasma aldosterone concentration (PAC) in pmol/L divided by the plasma renin concentration (PRC) in mU/L. An ARR >100 pmol/mU along with spontaneous hypokalemia confirmed the diagnosis PA (19). Before May 2015, PAC was measured in nmol/L and the plasma renin activity (PRA) in µg/L/hour; in this scenario an ARR >0.85 nmol/µg/hour and hypokalemia confirmed the diagnosis. If patients did not meet these criteria with an ARR >30 pmol/mU, an additional salt loading test (SLT) was performed, during which two liters of 0.9% sodium chloride (NaCl) were administered in a seated position (20). Plasma aldosterone measurements were taken before and immediately after saline infusion. A consistently elevated PAC >179 pmol/L after SLT confirmed PA (21). During both the ARR and SLT, antihypertensive medication known to interfere with renin and/or aldosterone was substituted with non-interfering alternatives (Appendix S1) (12). Potassium was supplemented to maintain normal range (3.5-5.0 mmol/L).

Hormone measurements

Serum aldosterone and renin were determined using chemiluminescence technology (ImmunoDiagnostic Systems GMBH, Germany), while cortisol levels were measured through electro-chemiluminescence immunoassay (Elecsys Cortisol gen2 ECLIA Roche Diagnostic, Germany). The analytical variation was 4.0%-6.3% for aldosterone, 3.4%-4.3% for renin and 2.5%-4.1% for cortisol. Before May 2015, a DiaSorin Plasma Renin activity RIA (Gammacoat Plasma Renin Activity RIA, CA1553, DiaSorin, Italy) was utilized. The ARR was clinically validated for both aldosterone and renin assays, establishing a cut-off of 31 pmol/mU [equivalent to 1.12 (ng/dl)/(µU/ml)], with a sensitivity of 99% and a specificity of 79% (20).

Adrenal vein sampling

AVS was performed under continuous intravenous stimulation of synthetic adrenocorticotropic hormone (ACTH) Synacthen® at 50 µg/hour. This ACTH infusion enhances the specificity of AVS by minimizing fluctuations in cortisol levels, assuming symmetrical cortisol secretion, as cortisol functions as an internal control (22). Autonomous cortisol secretion was ruled out with a dexamethasone suppression test in case of clinical suspicion for Cushing's syndrome or in case of incidental detected adenomas. The right common femoral

vein was punctured and a 5F sheath was inserted. Selective catheterization of the left and right adrenal vein was performed under fluoroscopic guidance. At least two blood samples were obtained from each adrenal vein, along with two peripheral samples from the sheath with the tip in the inferior vena cava. Aldosterone and cortisol levels were measured in these blood samples, and the A/C ratios of the LAV, RAV and IVC were compared to determine lateralization and/or suppression. The selectivity index (SI), defined as the cortisol ratio between the adrenal veins and the IVC, was used to assess for sampling adequacy. A SI index of 3-fold greater in both adrenal veins indicated successful bilateral sampling (Supplementary Table S1) (23, 24).

Definition of bilateral and unilateral disease

The A/C ratio was determined by calculating the average of the two samples from each site. To distinguish patients with unilateral disease, the study utilized the lateralization index (LI), (A/C ratio of the dominant vein)/(A/C ratio of the non-dominant vein), and contralateral suppression index (CSI), (A/C ratio non-dominant vein)/(A/C ratio IVC) (25). In our center, a LI ≥ 4 was considered indicative of unilateral disease. However, adrenalectomy was offered from LI ≥3 onwards, given the high likelihood of biochemical cure and clinical improvement. Additionally, a CSI <1 was considered consistent with unilateral disease (Supplementary Table S1). Patients failing to meet the criteria for both LI and CSI were categorized as having bilateral disease. The LAV/IVC index was defined as the A/C ratio between the left adrenal vein and the inferior vena cava.

Definition of cure

Post-operative cure was assessed within the first-year post-adrenalectomy. The definition of biochemical cure, according to the Post-Adrenalectomy Surgical Outcomes (PASO) criteria, was used (5). Biochemical cure was defined as the correction of hypokalemia (if present pre-surgery) and normalization of the ARR post-operatively; when ARR was not normalized, the salt loading test was repeated. In addition, we assessed clinical improvement, defined as improved control of hypertension, a reduction in antihypertensive medication use, and symptom resolution.

Data collection of literature

A search strategy (Appendix S2) was developed using different variations of the keywords 'primary aldosteronism, 'adrenal vein sampling' and 'subtyping'. The PubMed database was explored based on titles; 22 abstracts were screened. Full texts of the eligible studies were evaluated, and a total of 8 studies were included for the literature overview, focusing on unsuccessful sampling of the RAV and using the LAV/IVC index to predict lateralization.

Statistical analysis

Baseline characteristics were reported as mean ± standard deviation (SD) or median and interquartile range (IQR) if not normally distributed. Categorical variables were expressed as absolute numbers and percentages. Differences between unilateral and bilateral PA patients were tested using the independent T-test and Mann-Whitney U-test. Additionally, the Kruskall Wallis test was used to compare multiple groups for numerical values, and the Chi-squared test for categorical values (26). Differences between pre- and post-adrenalectomy outcomes in the unsuccessfully sampled group were tested using the Wilcoxon signed-rank test and McNemar test for categorical data. Receiver operating characteristics (ROC) analysis was used to calculate cut-off values for both the LAV/IVC index and A/C ratio, aiming to predict the disease subtype. The predictive value of these indices was measured by the area under the curve (AUC). The ratio with the highest AUC, representing the highest predictive value, was selected for further analyses. The Youden index was used to select the optimal cut-off values, ensuring a specificity over 90%, to limit false positive errors and their clinical consequences associated with misclassifying patients (27). Other optimal cut-off values with different desired specificities (>85% and >95%) were identified and presented in the supplementary. A post-hoc power calculation was conducted. Assuming an α of 0.05 and a power of 0.80, calculations showed that a sample size of at least 26 patients in each group would be necessary to detect a difference between a 90% cure rate in the intervention group and a 50% in the reference group (assuming that half of the patients has unilateral disease). A p-value of ≤0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 29.0.

Results

Patient characteristics

Between 2014 and 2022, 92 patients with PA were identified, of whom 82 underwent AVS. Among these, 54 (66%) samplings were bilaterally successful and included in the study. Of these, 28 patients had bilateral disease, while 26 had unilateral disease. The unilateral disease group exhibited a more severe phenotype of PA with a higher incidence of hypokalemia (85% *vs.* 32%, p<0.001) and a trend towards a higher ARR ratio than those in the bilateral group (353 *vs.* 215, p=0.21). Antihypertensive medication use was higher in the unilateral disease group (4.6 *vs.* 3.6). Both groups showed a substantial prevalence of cardiovascular comorbidities (e.g. chronic kidney disease - defined as reduced eGFR or presence of albuminuria - and left ventricular hypertrophy (LVH) - defined as meeting ECG or ultrasonographic criteria for LVH) at baseline (Table 1A).

Adrenal imaging revealed abnormalities in 58% of the unilateral disease group and 32% of the bilateral disease group (p = 0.01). In patients with unilateral disease, imaging showed both ipsilateral and contralateral abnormalities, including adenomas and hyperplastic

10.3389/fendo.2024.1497787

TABLE 1A Baseline characteristics of patients with bilaterally successful AVS.

	Overall	Bilateral	Unilateral	P-value
	N=54	*N=28*	*N=26*	
Sex, male (%)	33 (61.1)	15 (53.6)	18 (69.2)	0.24[c]
Age (mean ± SD)	48.5 ± 9.5	46.3 ± 9.3	50.9 ± 9.3	0.07[a]
BMI (mean ± SD)	29.9 ± 5.9	30.4 ± 5.9	29.4 ± 5.9	0.57[a]
Number of antihypertensives before treatment (mean ± SD)	4.1 ± 3.1	3.6 ± 3.0	4.6 ± 3.1	0.23[a]
Hypokalemia (%)	31 (57)	9 (32)	22 (85)	<0.001[c]
ARR Renin in mU/L (mean ± SD) Renin activity (median [IQR])	 284.2 (386.4) 1.49 [1.1-2.9]	 215.2 (331.7) 1.39 [1.0-2.4]	 353.1 (430.0) 1.59 [no range]	 0.21[a] 0.66[b]
Aldosterone before SLT (mean ± SD)	761.6 (385.8)	766.6 (211.5)	753.9 (571.0)	0.93[a]
Aldosterone after SLT (mean ± SD)	716.4 (1128.5)	455.2 (171.6)	1106.4 (1705.3)	0.07[a]
Cardiovascular comorbidities Reduced kidney function (%) Left ventricle hypertrophy (%) Presence of albuminuria (%)	 17 (32) 9 (17) 16 (30)	 9 (32) 4 (14) 8 (29)	 8 (31) 5 (20) 8 (31)	 0.91[c] 0.62[c] 0.16[c]
Adequate controlled hypertension, yes (%)	10 (19)	6 (21)	4 (15)	0.57[c]

BMI, body mass index; ARR, aldosterone renin ratio; SLT, salt loading test; SD, standard deviation; IQR, interquartile range. ARR measured in pmol/mU or renin activity in nmol/mcg/hour, number of antihypertensives reported in DDD (daily defined doses). Reported as mean ± SD, median [IQR] or N (%). [a]t-test. [b]Man Whitney U -test, [c]chi square.

TABLE 1B Sampling and treatment outcomes of patients with bilaterally successful AVS.

	Overall	Bilateral	Unilateral	P-value
	N =54	*N=28*	*N=26*	
Outcome AVS (N, %) Bilateral disease Unilateral left-sided disease Unilateral right-sided disease		28 (52)	 15 (28) 11 (20)	
Outcome adrenal imaging (%) Normal adrenal glands Bilateral abnormalities Unilateral abnormalities	 30 (55.6) 7 (13.0) 17 (31.5)	 19 (67.9) 0 (0.0) 9 (32.1)	 11 (42.3) 7 (26.9) 8 (30.8)	0.01[a]
A/C ratio (median [IQR]) Bilateral disease Unilateral left-sided disease Unilateral right-sided disease	3.9 [2.2-10.7]	 3.8 [2.8-9.3]	 10.6 [5.5-15.3] 0.9 [0.4-2.0]	<0.001[b]
LAV/IVC index (median [IQR]) Bilateral disease Unilateral left-sided disease Unilateral right-sided disease	2.1 [1.4-3.7]	 2.1 [1.7-3.2]	 4.8 [3.2-5.9] 0.6 [0.1-1.0]	<0.001[b]
Underwent adrenalectomy with available follow-up data (cured, %) Unilateral left-sided disease (N=14) Unilateral right-sided disease (N=5)			 13 (92.9%) 5 (100%)	
Post-operative biochemical outcomes Hypokalemia (%) ARR (median [IQR])			*N = 19* 0 (0) 6.2 [2.0-16.7]	
Post-operative clinical outcomes Improvement of symptoms, yes (%) Total number of			*N = 19* 10 (53) 1.0 [0.0-2.5]	

(Continued)

TABLE 1B Continued

	Overall	Bilateral	Unilateral	P-value
	N =54	*N=28*	*N=26*	
antihypertensives (median [IQR])				
Reduced number of antihypertensives (median [IQR])			2.8 [1.8-4.5]	
Adequately controlled hypertension, yes (%)			12 (63)	

AVS, adrenal vein sampling; A/C, aldosteron/cortisol; LAV, left adrenal vein; IVC, inferior vena cava; IQR, interquartile range; ARR, aldosterone renin ratio. ARR measured in pmol/mU, number of antihypertensives reported in DDD (daily defined doses). Reported as mean ± SD, median [IQR] or N (%). t-test. Man Whitney U-test, [a]chi-square, [b]Kruskal Wallis (unilateral left *vs.* bilateral *vs.* unilateral right).

adrenal glands. In the bilateral disease group, no abnormalities in both adrenal glands were seen, but unilateral abnormalities were observed in 10 patients on either the left or right side. Full details are provided in Supplementary Table S2.

Adrenalectomy was performed in 22 patients with unilateral disease, surgery was recommended for an additional 3 patients but not (yet) performed. Post-adrenalectomy, 93% of the unilateral left group and 100% of the unilateral right group achieved biochemical cure of PA (Table 1B). Symptom improvement, particularly better cognitive functioning such as improved concentration, was observed in 53% of the patients. Antihypertensive medication use could be reduced in nearly all patients (95%) and fully eliminated in 13% (Table 1B). Follow-up data was not available for three patients: one was lost to follow-up, and two patients no longer had hypertension, antihypertensive medication use, or symptoms, but their clinicians did not verify biochemical cure (Supplementary Figure S1). Regarding histology, the pathology of the adrenal glands revealed adenoma in 14 patients and hyperplasia was found in 14 patients. The observed pathological variants of aldosterone-producing adenomas in our cohort were KCNJ5 (N=5), ATP1A1 (N=5), and CACNA1D (N=4) mutations.

Subtyping of primary aldosteronism

The A/C ratio differed between groups (p<0.001): unilateral right-sided (median: 0.9, IQR [0.4-2.0]), bilateral (median: 3.8, IQR [2.8-9.3]) and left-sided disease (median: 10.6, IQR [5.5-15.3]) (Table 1B). Similar differences were observed in the LAV/IVC index (p<0.001): unilateral right-sided (median: 0.6, IQR [0.1-1.0]), bilateral (median: 2.1, IQR [1.7-3.2]) and unilateral left-sided disease (median: 4.8, IQR [3.2-5.9]). ROC analysis evaluated the accuracy of the A/C ratio and LAV/IVC index in predicting disease subtype (Figure 1). Both ratios showed good predictive ability; however, the A/C ratio had a lower area under the curve. Therefore, this study focused on the LAV/IVC values. For cut-off values off the A/C ratio, see Appendix S3.

Unilateral left-sided disease

Using the LAV/IVC index, a cut-off value of ≥4.4 predicted unilateral left-sided disease with a sensitivity of 60% and specificity of 93% (AUC 0.85, 95% confidence interval (CI) 0.73–0.96), Figure 2A. In other words, when roughly half of the patients have unilateral disease, the LAV/IVC index of ≥4.4 has a positive predictive value of 93% for left-sided disease. However, this cut-off value missed 40% of patients with left-sided disease. Additional cut-off values derived from the same ROC-curve of >4.1 and >5.9 showed a sensitivity of 60% and 27%, with a specificity of 89% and 96%, respectively (Figure 2A).

Unilateral right-sided disease

The optimal cut-off value of the LAV/IVC index for the left adrenal vein was found at <1.2 for predicting right-sided disease

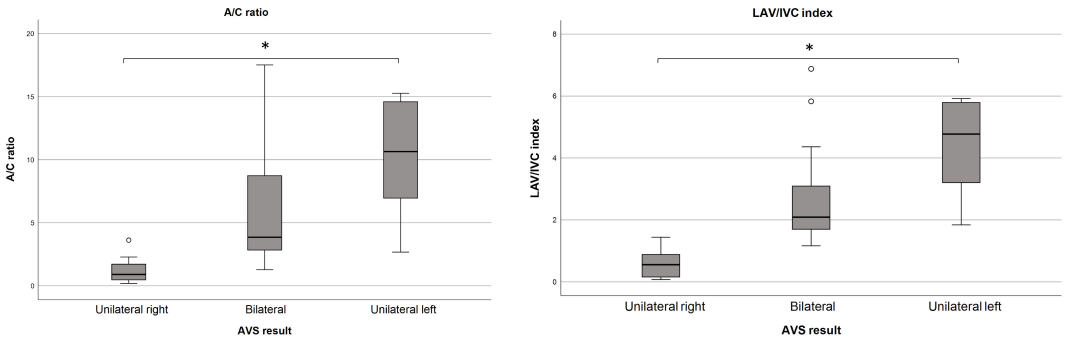


FIGURE 1
Distribution of the A/C ratio and LAV/IVC index. A/C ratio distribution in the LAV according to disease subtype. Outliers from the unilateral left group (35.3, 41.4 and 47.9) are not displayed. Median A/C ratios (p<0.001); unilateral right 0.9, bilateral 3.8, unilateral left 10.6.B. LAV/IVC index, representing the A/C ratio between LAV and IVC, according to disease subtype. Outliers from the unilateral left group (9.7, 10.8 and 25.1) are not displayed. Median LAV/IVC index (p<0.001); unilateral right 0.6, bilateral 2.1, unilateral left 4.8. A/C, aldosterone/cortisol; LAV, left adrenal vein; IVC, inferior vena cava. * P < 0.001.

10.3389/fendo.2024.1497787

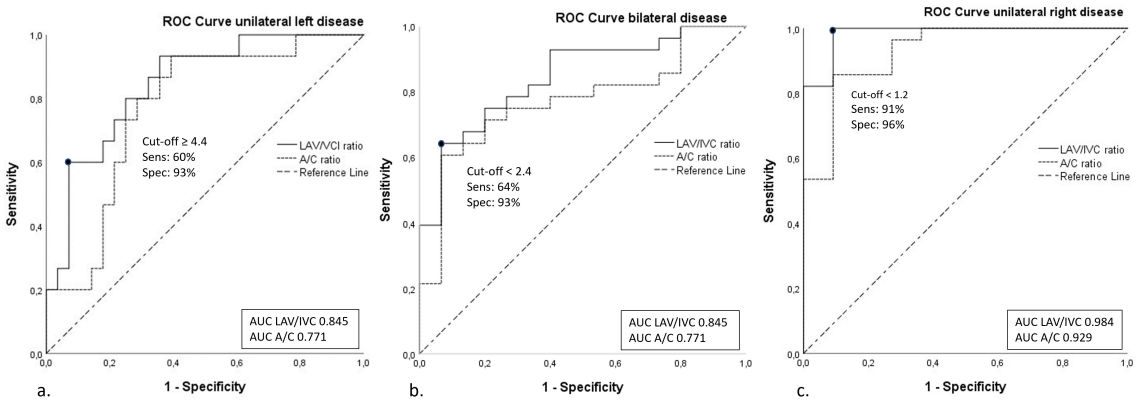


FIGURE 2
ROC curves of the A/C ratio and LAV/IVC index. ROC-curves of A/C and LAV/IVC cut-off values for **(A)**. Unilateral left-sided disease; **(B)**. Bilateral disease; **(C)**. Unilateral right-sided disease. A/C, aldosterone/cortisol; LAV, left adrenal vein; IVC, inferior vena cava; AUC, area under the curve.

with a sensitivity of 96% and specificity of 91% (AUC 0.98, CI 0.95-1.00), Figure 2C. Other cut-off values of <1.1 and <1.5 derived from the same ROC-curve, resulted in a sensitivity of 91% and 100% with a specificity of 100% and 82%, respectively.

Bilateral disease

To predict bilateral disease, a cut-off of value <2.4 was found to have a sensitivity of 64% and a specificity of 93% (AUC 0.85, CI 0.73–0.96). Alternative cut-off values of ≤1.8 and <2.5 derived from the same ROC-curve provided a sensitivity of 39% and 64% with a specificity of 100% and 87%, respectively (Figure 2B).

Clinical decision model

Combining the calculated (optimal) cut-off values, we developed a clinical decision model (Figure 3). Additional cut-off values for different specificities (>85% and >95%) for the clinical decision model are provided in Supplementary Figure S2. Validating this model on our own study cohort of bilaterally successful sampled patients (N=54), we found that the disease subtype could have been predicted for 74% of the patients. Of whom, 20% were diagnosed with right-sided disease, 35% with bilateral disease, and 19% with left-sided disease. To further validate the tool, we extended the analysis with patients who had unsuccessfully right-sided sampling followed by an adrenalectomy (Table 2). During the study period, right-sided sampling failed in 24 patients, of whom 17 were predicted to have unilateral disease by our clinical model. Among those who opted for surgery according to our model's recommendation, 14/14 (100%) achieved biochemical cure post-adrenalectomy. In this group *(male 79%, age 49 [43-60] years, BMI 30.1 ± 6.1 kg/m^2)*, differences were observed postoperatively in both the number of antihypertensive medications (in DDD) and the prevalence of uncontrolled hypertension. Additionally, 36% reported a reduction in symptoms, particularly cognitive improvements (Table 2).

Review of the literature

Various studies have investigated the use of the LAV/IVC index to predict disease subtype. Using the previously described search strategy, 8 articles were identified comparing the LAV/IVC index (28–35). Their cut-off values, including sensitivity, specificity, LI, and SI together with our own data is presented in Table 3.

Discussion

This study provides evidence that left vein AVS data alone effectively classifies PA subtypes in over 70% of cases, reducing the need for resampling or treatment deferral.

Previous studies have shown a nearly 40% discordance between AVS outcomes and anatomical imaging (22). We similarly observed poor concordance in our cohort (Supplementary Table S2), which underscores the necessity of AVS for accurately distinguishing unilateral and bilateral PA. Although AVS is a reliable diagnostic procedure, there are important challenges, such as the risk of unsuccessful right-sided sampling, invasiveness, costs, and the associated major medication adjustments, which can lead to a period of uncontrolled hypertension (11–13, 16, 17, 22). Altogether, these factors highlight the importance of strategies to reduce the reliance on resampling.

Our clinical decision model accurately predicts disease subtypes with a specificity of >90% based on left-sided sampling data alone: LAV/IVC <1.2 predicts unilateral right-sided disease, 1.2 to 2.4 predicts bilateral disease, and ≥4.4 predicts unilateral left-sided disease. Resampling is only recommended for LAV/IVC values between 2.4 and 4.4. Optional cut-off values with higher (>95%) or lower (>85%) specificities can be chosen (Supplementary Figure S2). Furthermore, our model not only reduces unnecessary invasive resamplings, but also optimizes resource use and lowers healthcare costs. At the LUMC, the second-largest AVS expertise center in the Netherlands (performing 25–50 AVS procedures annually with a success rate of 66%), approximately 30% of the AVS procedures require resampling. Using our model, over 70% of failed procedures

10.3389/fendo.2024.1497787

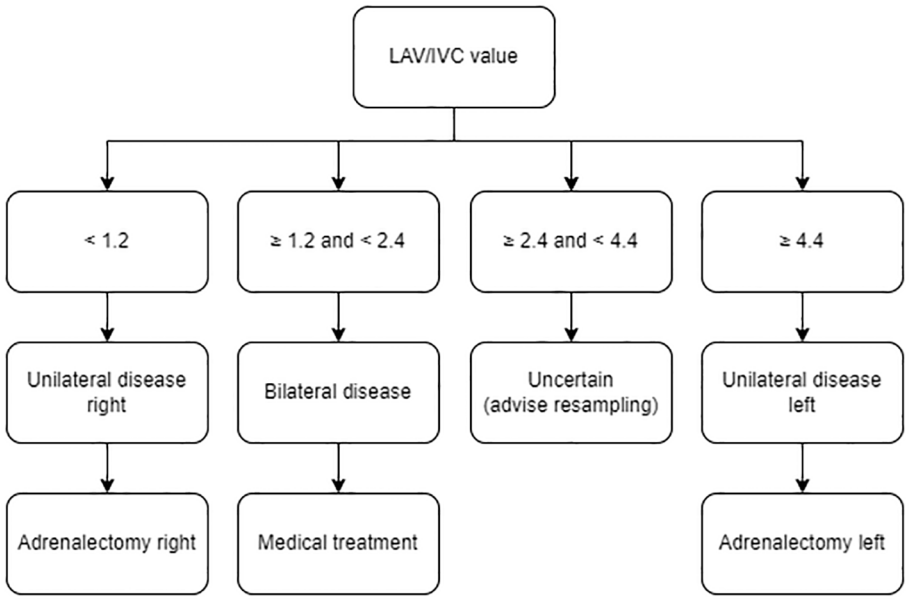


FIGURE 3
Clinical decision model. Treatment algorithm to interpret AVS sampling data of isolated successful left-sided sampling using the LAV/IVC index, based on specificity >90%. AVS, adrenal vein sampling; LAV, left adrenal vein; IVC, inferior vena cava.

can still be interpreted, reducing the resampling rate to 9 per 100 cases, translating to an annual cost saving of approximately €35,000 (36).

Our study is the first to develop a complete clinical model based on cut-off values for predicting lateralization or bilateral disease, incorporating the CSI for right-sided disease. Additionally, it includes a comparison of reported cut-off values from existing literature. In contrast to our study, the clinical tool proposed by Zibar Tomsic et al. proposed lower thresholds (<0.37) for right-sided disease and lower thresholds (0.38-0.68) for bilateral disease (35). Differences stem from their exclusion of the CSI, as it was found to have limited value by Young et al. (37). Recent consensus guidelines however, demonstrated the utility of the CSI for subtyping PA and therefore, it was implemented in our study (12, 15, 18, 38–40). The 100% biochemical cure rate observed in our cohort for right-sided disease - based on the LAV/IVC index - supports the inclusion of the CSI. Furthermore, could their focus on high sensitivity, while we prioritized specificity to avoid misclassification, explain the differences in cut-off values.

While thresholds like LI ≥4 are widely used in the international literature, our model considered LI ≥3 for offering adrenalectomy. This was based on clinical evidence suggesting a high likelihood of cure or significant symptomatic improvement, even at lower thresholds. In our cohort, over 90% of patients with LI ≥3 who

TABLE 2 Pre- and post-operative data of patients with unsuccessful right-sided sampling, N = 14.

	Pre-operative	Post-operative	P-value
Sex, male (%)	11 (79)		
Age (median [IQR])	49 [43-60]		
BMI (mean ± SD)	30.1 ± 6.1		
Number of antihypertensives (median [IQR])	3.3 [1.8-6.5]	0 [0-2.3]	0.03[a]
Hypokalemia (%)	12 (86)	0 (0)	<0.001[b]
ARR Renin in mU/L (median [IQR]) Renin activity (median [IQR])	 136.7 [69.3-494.0] 4.3 [4.3-5.1]	 7.1 [0.9-15] 0.5 [no range]	 0.05[a]
Adequately controlled hypertension (N, %)	2 (14)	12 (86)	0.08[b]
Presence of symptom, yes (%) Decrease Increase Similar	 5 (36) 8 (57) 1 (7)		

BMI, body mass index; ARR, aldosterone renin ratio; SD, standard deviation; IQR, interquartile range. ARR measured in pmol/mU or renin activity in nmol/mcg/hour, number of antihypertensives reported in DDD (daily defined doses). Reported as mean ± SD, median [IQR] or N (%). [a]Wilcoxon Signed Rank – test. [b]Mc Nemar.

10.3389/fendo.2024.1497787

TABLE 3 Comparison of cut-off values with sensitivity/specificity >90%, derived from other literature.

Authors	Number of patients	Cut-off value (LAV/IVC)	Sensitivity %	Specificity %	LI	SI	Stimulated with ACTH
Unilateral left-sided disease							
Wang et al.	222	≥8.6	19%	97%	3:1	3:1	+
Our study	**54**	**≥5.9**	**27%**	**96%**	**3:1**	**3:1**	**+**
Kocjan et al.	168	≥5.9	30%	99%	4:1	5:1	+
Strajina et al.	150	≥5.5	45%	82%	4:1	5:1	+
Kocjan et al.	168	>5.5	32%	97%	4:1	5:1	+
Pasternak et al.	36	>5.5	32%	97%	4:1	unknown	+
Lee et al.	121	≥5.5	34%	100%	4:1	5:1	+
Wang et al.	222	≥5.5	49%	89%	3:1	3:1	+
Our study	**54**	**>5.5**	**33%**	**93%**	**3:1**	**3:1**	**+**
Our study	**54**	**≥4.4**	**60%**	**93%**	**3:1**	**3:1**	**+**
Kocjan et al.	168	≥4.3	51%	95%	4:1	5:1	+
Zibar Tomsic et al.	60	>3.4	5.8%	100%	4:1	5:1	+
Lee et al.	121	≥3.1	74%	82%	4:1	5:1	+
Bilateral disease							
Kocjan et al.	168	<2.5	70%	87%	4:1	5:1	+
Suntornlohanakul et al.	62	<2.4	64%	89%	4:1	5:1	+
Our study	**54**	**<2.4**	**64%**	**93%**	**3:1**	**3:1**	**+**
Our study	**54**	**≤1.8**	**39%**	**100%**	**3:1**	**3:1**	**+**
Unilateral right-sided disease							
Kocjan et al.	168	<1.25	97%	87%	4:1	5:1	+
Our study	**54**	**≤1.2**	**91%**	**93%**	**3:1**	**3:1**	**+**
Our study	**54**	**<1.1**	**91%**	**100%**	**3:1**	**3:1**	**+**
Lee et al.	121	<1.0	98%	93%	4:1	5:1	+
Kocjan et al.	168	≤0.5	57%	95%	4:1	5:1	+
Kocjan et al.	168	≤0.5	47%	95%	4:1	5:1	+
Strajina et al.	150	≤0.5	81%	100%	4:1	5:1	+
Lee et al.	121	<0.5	96%	96%	4:1	5:1	+
Wang et al.	222	<0.5	71%	95%	3:1	3:1	+
Pasternak et al.	36	≤0.5	47%	95%	4:1	unknown	+
Zibar Tomsic et al.	60	≤0.37	97.1%	88.4%	4:1	5:1	+
Wang et al.	222	<0.3	71%	97%	3:1	3:1	+
Lin et al.	111	<0.07	40%	100%	2:1	2:1	–
Suntornlohanakul et al.	62	≤0.08	10%	99%	4:1	5:1	+

LAV, left adrenal vein; IVC, inferior vena cava; LI, lateralization index; SI, sensitivity index; ACTH, adrenocorticotropic hormone.

underwent adrenalectomy achieved biochemical cure, aligning with published cure rates for LI ≥4. Crucially, shared decision-making plays a critical role in cases with intermediate LI values, as the probability of cure progressively increases with higher LI thresholds.

Importantly, the LAV/IVC index values predicting unilateral or bilateral disease in our study align well with findings from most other studies (Table 3). Although previous studies have explored cut-off values for interpreting lateralization using the LAV/IVC index, no

consensus has been reached. Pasternak et al. initially proposed cut-off values (>5.5 for unilateral left-sided disease, <0.5 for unilateral right-sided disease) (31). Subsequent studies have tested and adapted these values (28–35). Pasternak's cut-off value of >5.5 for left-sided disease, showed a sensitivity of 32% and specificity of 97%; our study found comparable values of 33% sensitivity and 93% specificity. Both results are consistent with those found by Kocjan and Wang's et al. (28, 34). Using the Youden's index, the optimal cut-off value with a desired specificity >90% was found, yielding ≥4.4, which demonstrated similar sensitivity and specificity as Kocjan's cut-off value of ≥4.3 (28).

In contrast to many other studies, our cut-off values for bilateral disease were determined at 1.2-2.4 with 93% specificity, while similar studies suggested slightly higher cut-offs with lower specificities (87-89%) (28, 33). For unilateral right-sided disease, our study found a value of <1.2, with a specificity of 91%, aligning with values reported by Lee and Kocjan et al. (28, 29). Discrepancies in reported cut-off values for right-sided disease in other studies may be due to the exclusion of the CSI, potentially missing patients with right-sided disease. Factors such as severe PA in the cohort (Suntornlohanakul et al.) and the use of unstimulated AVS (Lin et al.) could explain their extremely low reported cut-off values. Since our model is developed with ACTH-stimulated AVS, its applicability for centers with unstimulated AVS is questionable. Our results, reflecting similar sensitivities, specificities, and patient demographics as found in existing literature, are representative for the PA population.

Limitations of our study include its retrospective study design and relatively small study group. To address this, we performed an extensive literature analysis that supports our data. Furthermore, subtype determination relied on successful sampling outcomes, whereas other studies used the post-adrenalectomy data to confirm unilateral disease. However, our cure rates >95% post-adrenalectomy, align with published outcomes (5–9), supporting the validity of our findings.

While our high biochemical cure rates highlight the validity of our approach, these reflect biochemical outcomes only, as defined by the PASO criteria. Clinical cure, defined as complete blood pressure normalization without antihypertensive medication, was less applicable to our cohort due to the considerable presence of pre-existing cardiovascular damage (e.g. chronic kidney disease, left ventricular hypertrophy) in our patients at baseline (Table 1A). These comorbidities reduced the likelihood of achieving complete clinical cure, even in patients with normalized ARR and correction of potassium levels postoperatively. Instead, we assessed clinical improvement in patients who underwent adrenalectomy, encompassing better-controlled hypertension, reduced medication use, and symptom resolution (Table 1B). These findings indicate that adrenalectomy not only resolves biochemical hyperaldosteronism but also leads to meaningful clinical benefits, including enhanced quality of life. This aligns with existing literature, such as Venema et al., who reported significant improvements in both physical and mental health domains after treatment for primary aldosteronism (41).

Our prediction model, validated internally and on unsuccessfully sampled patients, demonstrated its reliability. Notably, 74% of patients with successful bilateral sampling had their disease subtype predicted based on left-sided data alone. Furthermore, the clinical decision model accurately predicted lateralization in 14 out of 14 unsuccessfully sampled patients of whom follow-up data was available, as confirmed by biochemical cure post-adrenalectomy.

Our study presents a reliable prediction model, although there is a 40% chance it may not detect patients with left-sided disease due to the model's sensitivity of 60%. This raises concerns about potential missed opportunities for beneficial adrenalectomies and, consequently, the cure of PA. However, the model's specificity exceeding 90% acts as a safeguard, reducing the likelihood of unintended surgeries.

Misclassifying bilateral patients as unilateral often occurs with exceptionally high LAV/IVC values, suggesting significant left adrenal gland overproduction. However, even in cases of misclassification, adrenalectomy in bilateral patients with this high LAV/IVC can still be beneficial by reducing disease severity and improving quality of life, even without complete cure of the disease (25, 42–45). Certain clinical scenarios could alter the reliability of this model. When in doubt, expert consultation, either locally or through a collaboration such as the European Network Reference, is essential. Future research should focus on validating the model in external cohorts, standardized AVS protocols and the reproductivity in cases of unexpectedly failed left-sided sampling.

In conclusion, our study emphasizes the LAV/IVC index's utility as a valuable predictor for primary aldosteronism subtype, when right-sided sampling fails. Our proposed clinical decision model, integrating the CSI, defines thresholds and could potentially reduce the need for resampling in over 70% of cases with failed right-sided cannulation. Overall, this model facilitates a more efficient and precise approach to subtype classification in patients with PA.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The requirement of ethical approval was waived by Scientific Board Internal Medicine - Leiden University Medical Center for the studies involving humans. The studies were conducted in accordance with the local legislation and institutional requirements. The participants were given the opportunity to object to the use of their data.

Author contributions

StH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft,

Writing – review & editing. SvG: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. EC: Conceptualization, Writing – review & editing. AvE: Conceptualization, Writing – review & editing. BB: Conceptualization, Writing – review & editing. OD: Conceptualization, Methodology, Writing – review & editing. MN: Conceptualization, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We acknowledge the patients for using their clinical data for this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1497787/full#supplementary-material

References

1. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. *Lancet Diabetes Endocrinol.* (2018) 6:51–9. doi: 10.1016/S2213-8587(17)30367-4

2. Mulatero P, Monticone S, Bertello C, Viola A, Tizzani D, Iannaccone A, et al. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. *J Clin Endocrinol Metab.* (2013) 98:4826–33. doi: 10.1210/jc.2013-2805

3. Young WF. Primary aldosteronism: renaissance of a syndrome. *Clin Endocrinol (Oxf).* (2007) 66:607–18. doi: 10.1111/j.1365-2265.2007.02775.x

4. Lim PO, Young WF, MacDonald TM. A review of the medical treatment of primary aldosteronism. *J Hypertens.* (2001) 19:353–61. doi: 10.1097/00004872-200103000-00001

5. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, et al. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol.* (2017) 5:689–99. doi: 10.1016/S2213-8587(17)30135-3

6. Hannon MJ, Sze WC, Carpenter R, Parvanta L, Matson M, Sahdev A, et al. Clinical outcomes following unilateral adrenalectomy in patients with primary aldosteronism. *Qjm.* (2017) 110:277–81. doi: 10.1093/qjmed/hcw194

7. Katabami T, Fukuda H, Tsukiyama H, Tanaka Y, Takeda Y, Kurihara I, et al. Clinical and biochemical outcomes after adrenalectomy and medical treatment in patients with unilateral primary aldosteronism. *J Hypertens.* (2019) 37:1513–20. doi: 10.1097/HJH.0000000000002070

8. Sukor N, Kogovsek C, Gordon RD, Robson D, Stowasser M. Improved quality of life, blood pressure, and biochemical status following laparoscopic adrenalectomy for unilateral primary aldosteronism. *J Clin Endocrinol Metab.* (2010) 95:1360–4. doi: 10.1210/jc.2009-1763

9. Ishihara Y, Umakoshi H, Kaneko H, Nanba K, Tsuiki M, Kusakabe T, et al. Prediction of long-term biochemical cure in patients with unilateral primary hyperaldosteronism treated surgically based on the early post-operative plasma aldosterone value. *Endocr J.* (2022) 69:407–15. doi: 10.1507/endocrj.EJ21-0430

10. Chen SY, Chen JY, Huang WC, Puar THK, Chin Kek P, Chueh JS, et al. Cardiovascular outcomes and all-cause mortality in primary aldosteronism after adrenalectomy or mineralocorticoid receptor antagonist treatment: a meta-analysis. *Eur J Endocrinol.* (2022) 187:S47–s58. doi: 10.1530/EJE-22-0375

11. Ghose RP, Hall PM, Bravo EL. Medical management of aldosterone-producing adenomas. *Ann Intern Med.* (1999) 131:105–8. doi: 10.7326/0003-4819-131-2-199907200-00005

12. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* (2016) 101:1889–916. doi: 10.1210/jc.2015-4061

13. Kocjan T, Jensterle M, Vidmar G, Vrckovnik R, Berden P, Stankovic M. Adrenal vein sampling for primary aldosteronism: a 15-year national referral center experience. *Radiol Oncol.* (2020) 54:409–18. doi: 10.2478/raon-2020-0052

14. Stowasser M. Adrenal venous sampling for differentiating unilateral from bilateral primary aldosteronism: still the best, but could be better. *Hypertension.* (2015) 65:704–6. doi: 10.1161/HYPERTENSIONAHA.115.04930

15. Quencer KB. Adrenal vein sampling: technique and protocol, a systematic review. *CVIR Endovasc.* (2021) 4:38. doi: 10.1186/s42155-021-00220-y

16. Araki T, Imaizumi A, Okada H, Onishi H. Unusual anatomical variants of the left adrenal vein via the renal capsular vein preventing successful adrenal vein sampling. *Cardiovasc Intervent Radiol.* (2017) 40:1296–8. doi: 10.1007/s00270-017-1652-y

17. Stewart PM, Allolio B. Adrenal vein sampling for Primary Aldosteronism: time for a reality check. *Clin Endocrinol (Oxf).* (2010) 72:146–8. doi: 10.1111/j.1365-2265.2009.03714.x

18. Wolley MJ, Gordon RD, Ahmed AH, Stowasser M. Does contralateral suppression at adrenal venous sampling predict outcome following unilateral adrenalectomy for primary aldosteronism? A retrospective study. *J Clin Endocrinol Metab.* (2015) 100:1477–84. doi: 10.1210/jc.2014-3676

19. Jansen PM, van den Born BJ, Frenkel WJ, de Bruijne EL, Deinum J, Kerstens MN, et al. Test characteristics of the aldosterone-to-renin ratio as a screening test for primary aldosteronism. *J Hypertens.* (2014) 32:115–26. doi: 10.1097/HJH.0b013e3283656b54

20. Manolopoulou J, Fischer E, Dietz A, Diederich S, Holmes D, Junnila R, et al. Clinical validation for the aldosterone-to-renin ratio and aldosterone suppression testing using simultaneous fully automated chemiluminescence immunoassays. *J Hypertens.* (2015) 33:2500–11. doi: 10.1097/HJH.0000000000000727

21. Rossi GP, Belfiore A, Bernini G, Desideri G, Fabris B, Ferri C, et al. Prospective evaluation of the saline infusion test for excluding primary aldosteronism due to aldosterone-producing adenoma. *J Hypertens.* (2007) 25:1433–42. doi: 10.1097/HJH.0b013e328126856e

22. Kempers MJ, Lenders JW, van Outheusden L, van der Wilt GJ, Schultze Kool LJ, Hermus AR, et al. Systematic review: diagnostic procedures to differentiate unilateral from bilateral adrenal abnormality in primary aldosteronism. *Ann Intern Med.* (2009) 151:329–37. doi: 10.7326/0003-4819-151-5-200909010-00007

23. Rossi GP, Maiolino G, Seccia TM. Adrenal venous sampling: where do we stand? *Endocrinol Metab Clin North Am.* (2019) 48:843–58. doi: 10.1016/j.ecl.2019.08.012

24. Rossi GP, Auchus RJ, Brown M, Lenders JW, Naruse M, Plouin PF, et al. An expert consensus statement on use of adrenal vein sampling for the subtyping of

primary aldosteronism. *Hypertension*. (2014) 63:151–60. doi: 10.1161/HYPERTENSIONAHA.113.02097

25. Prins MLM, Ballieux B, Meijer OC, Pereira AM, Nijhoff MF. Adrenal vein sampling in a patient with primary hyperaldosteronism and severe contrast allergy. *J Endocr Soc*. (2021) 5:bvab122. doi: 10.1210/jendso/bvab122

26. le Cessie S, Goeman JJ, Dekkers OM. Who is afraid of non-normal data? Choosing between parametric and non-parametric tests. *Eur J Endocrinol*. (2020) 182: E1–e3. doi: 10.1530/EJE-19-0922

27. Youden WJ. Index for rating diagnostic tests. *Cancer*. (1950) 3:32–5. doi: 10.1002/1097-0142(1950)3:1<32::AID-CNCR2820030106>3.0.CO;2-3

28. Kocjan T, Vidmar G, Vrckovnik R, Stankovic M. Limited diagnostic utility of partially successful adrenal vein sampling for primary aldosteronism subtyping. *Endokrynol Pol*. (2021) 72:293–300. doi: 10.5603/EP.a2021.0030

29. Lee SE, Park SW, Choi MS, Kim G, Yoo JH, Ahn J, et al. Primary aldosteronism subtyping in the setting of partially successful adrenal vein sampling. *Ther Adv Endocrinol Metab*. (2021) 12:2042018821989239. doi: 10.1177/2042018821989239

30. Lin L, Zhou L, Guo Y, Liu Z, Chen T, Liu Z, et al. Can incomplete adrenal venous sampling data be used in predicting the subtype of primary aldosteronism? *Ann Endocrinol (Paris)*. (2019) 80:301–7. doi: 10.1016/j.ando.2019.10.001

31. Pasternak JD, Epelboym I, Seiser N, Wingo M, Herman M, Cowan V, et al. Diagnostic utility of data from adrenal venous sampling for primary aldosteronism despite failed cannulation of the right adrenal vein. *Surgery*. (2016) 159:267–73. doi: 10.1016/j.surg.2015.06.048

32. Strajina V, Al-Hilli Z, Andrews JC, Bancos I, Thompson GB, Farley DR, et al. Primary aldosteronism: making sense of partial data sets from failed adrenal venous sampling-suppression of adrenal aldosterone production can be used in clinical decision making. *Surgery*. (2018) 163:801–6. doi: 10.1016/j.surg.2017.10.012

33. Suntornlohanakul O, Soonthornpun S, Srisintorn W, Murray RD, Kietsiriroje N. Performance of the unilateral AV/IVC index in primary hyperaldosteronism subtype prediction: A validation study in a single tertiary centre. *Clin Endocrinol (Oxf)*. (2020) 93:111–8. doi: 10.1111/cen.14210

34. Wang TS, Kline G, Yen TW, Yin Z, Liu Y, Rilling W, et al. A multi-institutional comparison of adrenal venous sampling in patients with primary aldosteronism: caution advised if successful bilateral adrenal vein sampling is not achieved. *World J Surg*. (2018) 42:466–72. doi: 10.1007/s00268-017-4327-6

35. Zibar Tomsic K, Dusek T, Alduk A, Knezevic N, Molnar V, Kraljevic I, et al. Subtyping primary aldosteronism by inconclusive adrenal vein sampling: A derivation and validation study in a tertiary centre. *Clin Endocrinol (Oxf)*. (2022) 97:849–59. doi: 10.1111/cen.14794

36. Rossi GP, Barisa M, Allolio B, Auchus RJ, Amar L, Cohen D, et al. The Adrenal Vein Sampling International Study (AVIS) for identifying the major subtypes of primary aldosteronism. *J Clin Endocrinol Metab*. (2012) 97:1606–14. doi: 10.1210/jc.2011-2830

37. Young WF, Stanson AW, Thompson GB, Grant CS, Farley DR, van Heerden JA. Role for adrenal venous sampling in primary aldosteronism. *Surgery*. (2004) 136:1227–35. doi: 10.1016/j.surg.2004.06.051

38. Lee J, Kang B, Ha J, Kim MH, Choi B, Hong TH, et al. Clinical outcomes of primary aldosteronism based on lateralization index and contralateral suppression index after adrenal venous sampling in real-world practice: a retrospective cohort study. *BMC Endocr Disord*. (2020) 20:114. doi: 10.1186/s12902-020-00591-8

39. Umakoshi H, Tanase-Nakao K, Wada N, Ichijo T, Sone M, Inagaki N, et al. Importance of contralateral aldosterone suppression during adrenal vein sampling in the subtype evaluation of primary aldosteronism. *Clin Endocrinol (Oxf)*. (2015) 83:462–7. doi: 10.1111/cen.2015.83.issue-4

40. Burrello J, Burrello A, Pieroni J, Sconfienza E, Forestiero V, Amongero M, et al. Prediction of hyperaldosteronism subtypes when adrenal vein sampling is unilaterally successful. *Eur J Endocrinol*. (2020) 183:657–67. doi: 10.1530/EJE-20-0656

41. Velema M, Dekkers T, Hermus A, Timmers H, Lenders J, Groenewoud H, et al. Quality of life in primary aldosteronism: A comparative effectiveness study of adrenalectomy and medical treatment. *J Clin Endocrinol Metab*. (2018) 103:16–24. doi: 10.1210/jc.2017-01442

42. Hundemer GL, Vaidya A. MANAGEMENT OF ENDOCRINE DISEASE: The role of surgical adrenalectomy in primary aldosteronism. *Eur J Endocrinol*. (2020) 183: R185–r96. doi: 10.1530/EJE-20-0863

43. Szabo Yamashita T, Shariq OA, Foster TR, Lyden ML, Dy BM, Young WF Jr., et al. Unilateral adrenalectomy for primary aldosteronism due to bilateral adrenal disease can result in resolution of hypokalemia and amelioration of hypertension. *World J Surg*. (2023) 47:314–8. doi: 10.1007/s00268-022-06780-x

44. Williams TA, Gong S, Tsurutani Y, Tezuka Y, Thuzar M, Burrello J, et al. Adrenal surgery for bilateral primary aldosteronism: an international retrospective cohort study. *Lancet Diabetes Endocrinol*. (2022) 10:769–71. doi: 10.1016/S2213-8587(22)00253-4

45. Mulatero P, Sechi LA, Williams TA, Lenders JWM, Reincke M, Satoh F, et al. Subtype diagnosis, treatment, complications and outcomes of primary aldosteronism and future direction of research: a position statement and consensus of the Working Group on Endocrine Hypertension of the European Society of Hypertension. *J Hypertens*. (2020) 38:1929–36. doi: 10.1097/HJH.0000000000002520

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 14 April 2025
DOI 10.3389/fendo.2025.1555572

OPEN ACCESS

EDITED BY
Mirko Parasiliti-Caprino,
University of Turin, Italy

REVIEWED BY
Piotr Glinicki,
Centre of Postgraduate Medical Education, Poland
Chiara Lopez,
University of Turin, Italy

*CORRESPONDENCE
Nikita Makhnov
nikita.makhnov@uu.se

RECEIVED 04 January 2025
ACCEPTED 10 March 2025
PUBLISHED 14 April 2025

CITATION
Makhnov N, Skov J, Åkerström T, Axling F, Andernord D, Bergenheim M, Waldén M and Hellman P (2025) Screening for primary aldosteronism in 1,181 Swedish primary care patients with hypertension.
Front. Endocrinol. 16:1555572.
doi: 10.3389/fendo.2025.1555572

Screening for primary aldosteronism in 1,181 Swedish primary care patients with hypertension

Nikita Makhnov[1,2,3]*, Jakob Skov[4,5], Tobias Åkerström[1,6], Fredrik Axling[1], Daniel Andernord[3], Mikael Bergenheim[2,7], Mauritz Waldén[2] and Per Hellman[1,6]

[1]Department of Surgical Sciences, Uppsala University, Uppsala, Sweden, [2]Department of Surgery, Karlstad Central Hospital, Karlstad, Sweden, [3]Center for Clinical Research and Education, Karlstad, Sweden, [4]Department of Medicine, Karlstad Central Hospital, Karlstad, Sweden, [5]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, [6]Department of Surgery, Uppsala University Hospital, Uppsala, Sweden, [7]Department of Health Sciences, Karlstad University, Karlstad, Sweden

Objective: Primary aldosteronism (PA) is a common cause of hypertension. It entails elevated morbidity and mortality that do not sufficiently improve with conventional antihypertensive therapy. Screening for PA by plasma aldosterone–renin ratio (ARR) enables discovery and specific treatment of affected patients. By screening primary care patients with hypertension and evaluating them further according to the Endocrine Society guidelines, we aimed to assess the prevalence of PA, the factors affecting biochemical diagnostics, and the outcome of lateralization studies and of specific treatment of the discovered PA cases.

Design, patients, and methods: Prospective evaluation of screening for PA was conducted in 1,181 patients. Screening by ARR was performed under current therapy, but without mineralocorticoid receptor antagonists (MRA), under normokalemia, and confirmed by the intravenous saline suppression test, SST#1. Those with results in a defined gray zone underwent therapy adjustment and then completed SST#2. Plasma aldosterone and ARR were compared under different stages of the diagnostic process. All patients with PA were offered adrenal venous sampling, or, in certain cases, adrenocortical-specific positron emission tomography. Lateralizing cases were offered laparoscopic adrenalectomy. Patients with bilateral disease were treated with MRA. Treatment results were assessed after a minimum of 6 months.

Results: A total of 53 discovered cases of (mostly mild) PA corresponded to its prevalence of 4.5%. Initial seated ARR was higher than recumbent ARR before SST#1. At SST#2, initial ARR and final aldosterone were higher than at SST#1. Localizing studies (accepted by 45 patients) found 14 lateralized cases. Of the 11 operated cases, 4 had aldosterone-producing adenoma, and the remainder had micro- and macronodular histopathology. A total of 31 patients had bilateral PA. Both surgical and conservative treatments were well tolerated and led to improved blood pressure and higher renin, indicating risk amelioration.

10.3389/fendo.2025.1555572

Conclusions: PA is prevalent among primary care patients with hypertension and can be screened for under current antihypertensive therapy. Aldosterone-producing adenoma was rare in this cohort. The study results support active screening of primary care patients with hypertension for PA in order to offer appropriate treatment options.

KEYWORDS

primary aldosteronism, screening, hypertension, outpatients, aldosterone, renin, therapeutics

1 Introduction

Primary aldosteronism (PA), manifested by excessive autonomous production of aldosterone in the adrenal cortex, is the most common cause of secondary hypertension (1). Arterial hypertension globally affects over 30% of adults 30–79 years old (2), and a considerable part of these patients have documented PA (3–5). In particular, high prevalence of PA is shown in resistant or refractory hypertension (6). Moreover, PA comprises a continuum of subclinical to clinical conditions where the risk of cardiovascular, metabolic, and renal disorders rises along with the degree of hyperaldosteronism—unrelated to blood pressure control *per se* (7–11). Accumulating evidence supports the existence of subclinical forms of PA—preceding the development of hypertension (7–9, 12). The public health aspect of PA is underlined by the fact that, compared with primary hypertension, PA considerably worsens cardiovascular morbidity and mortality, which cannot be effectively controlled by traditional antihypertensive treatment (13–15), at the same time as specific surgical and medical treatment strategies exist (16–19). To be able to offer the patients the best possible treatment, it is necessary to evaluate if the pathologic aldosterone production takes place in only one or in both adrenals, which requires adrenal vein sampling (AVS), and lately may also be evaluated with the introduction of adrenocortical positron emission tomography/computed tomography (PET/CT, 20, 21). PA often presents no pathognomonic symptoms, which is why biochemical screening—most often by aldosterone–renin ratio (ARR)—has hitherto been necessary for the identification of suspicious cases, requiring further confirmatory diagnostic workup (20). In some instances of much elevated ARR combined with hypokalemia, the confirmatory testing might be superfluous (20, 22).

The Endocrine Society guidelines (20) describe a PA prevalence of approximately 5%–13% among patients with hypertension. Previous studies in Sweden involving small numbers of patients showed a prevalence of PA of up to 5.5% in newly diagnosed hypertensives and 8.5% in known hypertensives in a primary care setting (23, 24). The latest meta-analysis of the PA prevalence was hard to interpret, mainly due to the heterogeneity of the various included study designs, diagnostic methods, and criteria (5, 25–27). There exists an intimate connection between assessed prevalence rates of PA and its diagnostic thresholds, which are strikingly different at different centers (28), and with a tendency towards higher prevalence rates in more recent studies (15). At this moment, a PA prevalence of at least 10% of all individuals with hypertension and at least 20% in those with treatment resistant hypertension (15, 29) seems probable, while the prevalence in cohorts considered to have low risk for PA is more uncertain (8). Today, less than 2% of patients with risk of PA are screened within routine clinical practice (15).

Pathophysiologic research into the cellular changes in the affected adrenals in PA has recently resulted in the HISTALDO consensus on histological forms of the disease (30). Introduction of immunohistochemical (IHC) analysis of the enzyme aldosterone synthase, encoded by the gene *CYP11B2*, has made it possible to discern aldosterone production in removed adrenal specimens, thus defining previously unseen aldosterone-producing micronodules [APMs, earlier named aldosterone-producing cell clusters (APCCs)], as well as aldosterone-producing nodules (APNs) and aldosterone-producing adenomas (APAs). Contrary to previous understanding, it now seems that the majority of unilateral PA is represented by such nodular forms and to a lesser extent by APAs, whereas diffuse CYP11B2-positive hyperplasia is unusual (31, 32). Unilateral PA is definitely best treated by surgery (33).

Using ARR, we have performed a large screening in primary care individuals with hypertension and evaluated them further according to the Endocrine Society guidelines. The aims were to estimate the prevalence of PA in an unselected cohort of primary care patients with hypertension in Sweden; to subclassify the found patients; to describe the practical complexity of the guidelines-recommended approach; and to report the results of diagnostic evaluation, as well as the outcome of offered treatment.

2 Materials and methods

2.1 Study population and recruitment

Figures 1 and 2 summarize the study protocol. A regional healthcare database was used to access postal addresses of primary care patients who had a registered diagnosis code for

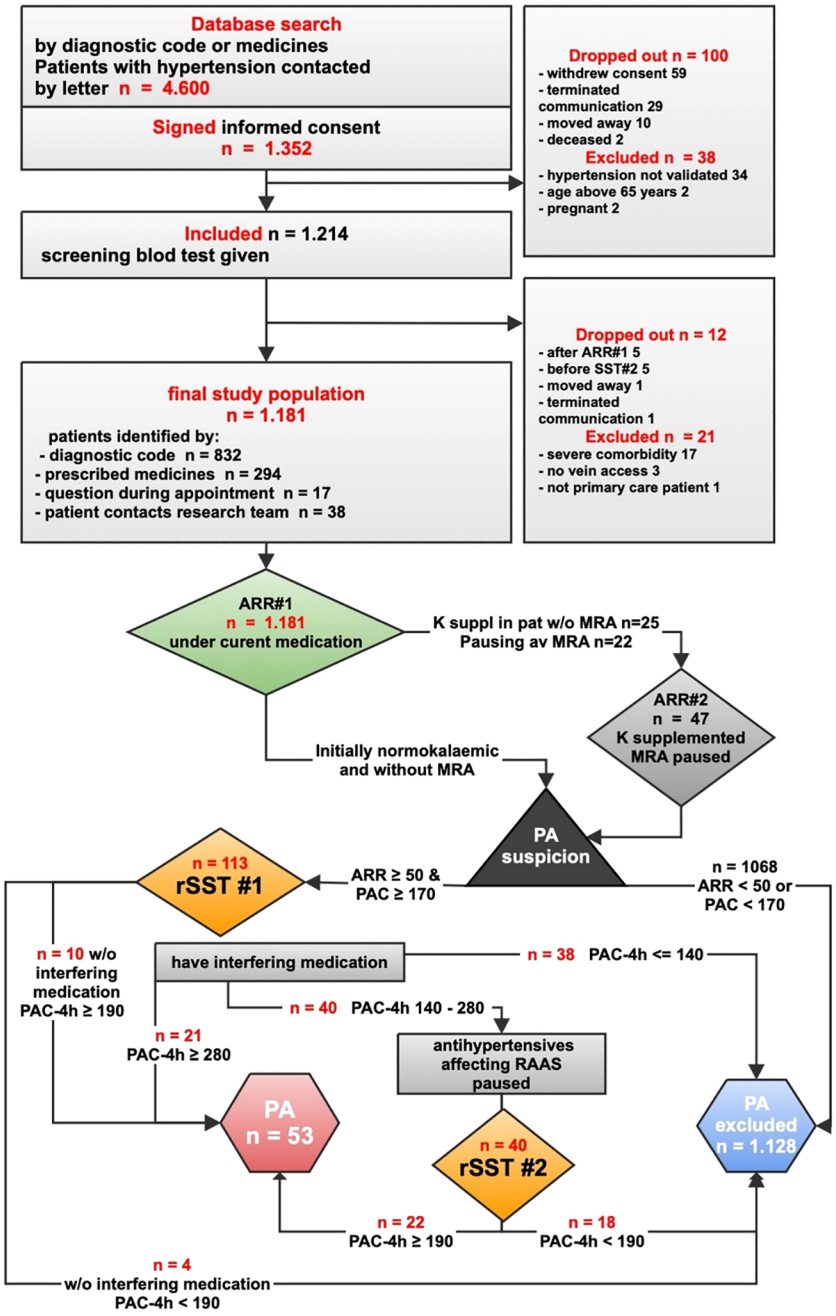


FIGURE 1
Study flowchart 1—screening for and diagnosing primary aldosteronism. ARR, aldosterone–renin ratio (pmol/mIU); MRA, mineralocorticoid receptor antagonists; K, potassium; K-suppl in pat w/o MRA, potassium supplementation in patients without MRA; PAC, plasma aldosterone concentration (pmol/L); rSST, recumbent saline suppression test; PAC4h, plasma aldosterone concentration at the end of rSST; RAAS, renin–angiotensin–aldosterone system; PA, primary aldosteronism.

hypertension and/or were taking antihypertensive medicines. The search was done for patients 18–65 years old, pertaining to the participating outpatient clinics located in and around Karlstad (the central city of the Swedish region of Värmland). The patients were then contacted by letter containing study information, a health questionnaire, and a written consent form that, if signed, gave the study personnel access to the relevant medical chart data. Information posters were displayed at the participating primary care centers, giving patients with hypertension a possibility to contact the study personnel and receive the same letter. For individuals who consented to participation, diagnosis of arterial hypertension was validated through medical charts by either presence of a diagnosis code for hypertension or at least three instances of blood pressure ≥ 140/90 mmHg at rest. Inclusion and exclusion criteria are described in Table 1. Self-reported data, as the age at which hypertension was discovered, cardiovascular diseases,

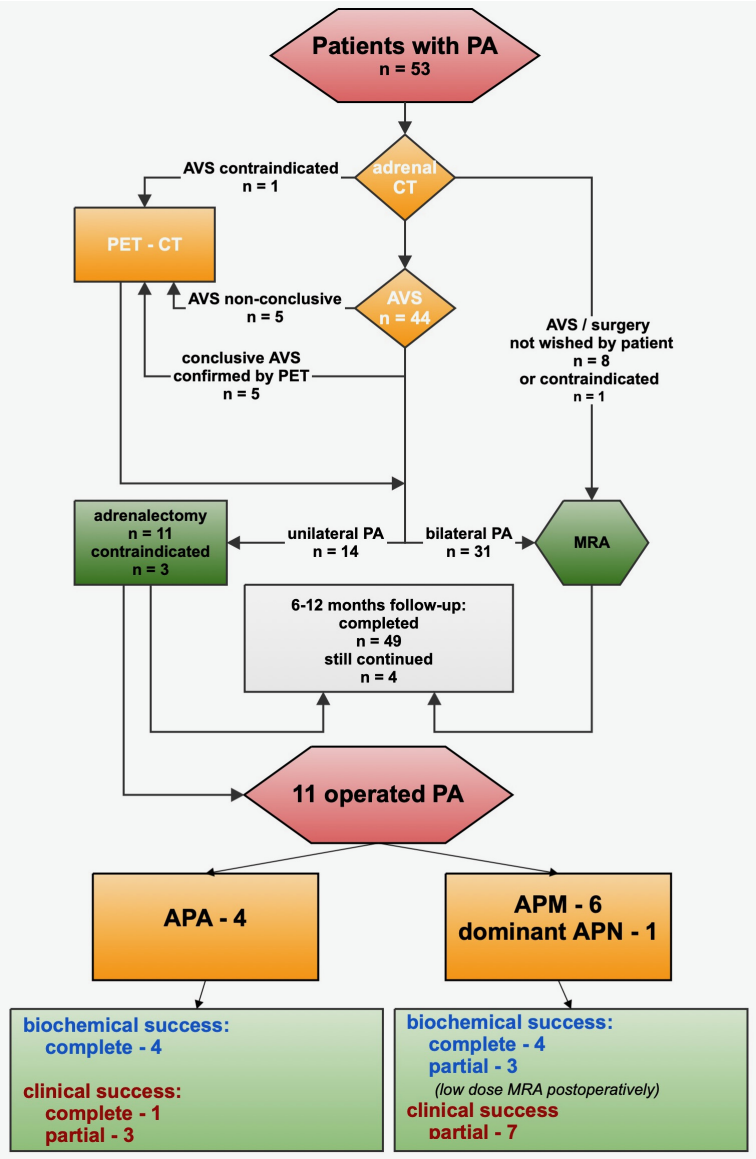


FIGURE 2
Workup and treatment of 53 cases of primary aldosteronism; pathological, biochemical, and clinical outcomes of operated cases. PA, primary aldosteronism; CT, computed tomography; dexa-cortisol, dexamethasone suppressed cortisol; AVS, adrenal venous sampling; PET-CT, positron emission tomography with computed tomography; MRA, mineralocorticoid receptor antagonists.

and current medication, were validated through the patients' medical charts. Tobacco and licorice use were self-reported.

Calculations assuming a prevalence of 5% of PA within the screened population, a type 1 error of maximum 0.05, and a power of 80% resulted in a necessary number of screened individuals to be 1,168. Altogether, 1,214 patients who signed the informed consent and actually gave the first screening blood test were included in the study, and 1,181 of them completed the study. Two of the patients who signed the informed consent had already been diagnosed with PA before through routine clinical investigation corresponding to the study protocol. They were included but not reinvestigated. Inclusion in the study took place between April 2017 and June 2022, with a remarkable delay of the screening and diagnostic procedures during the COVID-19 pandemic.

2.2 Overview of the screening and diagnostic protocol

In an attempt to allow greater sensitivity and simplicity in screening, the initial ARR (ARR#1) was taken in all included subjects and under current medication. Otherwise, the screening, confirmation of the suspected PA cases (requiring adjustment of medication in some patients), and evaluation for lateralization of the disease in potential surgical candidates were performed according to the Endocrine Society guidelines. Calculation of ARR was done by dividing the plasma aldosterone concentration (PAC) by direct renin concentration (DRC). Even later in the study, renin was assessed as DRC. When the DRC was below the lower

10.3389/fendo.2025.1555572

TABLE 1 Inclusion and exclusion criteria.

Inclusion criteria:	
Validated diagnosis of arterial hypertension	
Age	18–65 years
Possession of Swedish personal number	Permits elective medical care
Exclusion criteria:	
Pregnancy	
Patients followed for PA or hypertension within specialized centers and not within primary care	
Conditions rendering diagnostic protocol (rSST#1[a], pausing of MRA or other medicines before ARR#2[b] or rSST#2[c]) a high-risk procedure (as described on the right):[d]	Hypertension with blood pressure levels > 180/110 in spite of rigorous within-study efforts to optimize treatment
	Chronic heart failure, New York Heart Association stage > 2
	Severe respiratory, hepatic, or renal failure

[a]rSST#1, recumbent saline suppression test number 1 (see study protocol in Figure 1).
[b]ARR#2, aldosterone–renin ratio number 2 (see Figure 1).
[c]rSST#2, recumbent saline suppression test number 2 (see Figure 1).
[d]Patients who were deemed not fit for rSST or for pausing of medication when necessary were excluded from further study participation and advised to be initiated/continued on a clinically adjusted dose of mineralocorticoid receptor antagonists (MRA) through their primary care physician.

limit of 1.6 mIU/L reported by the laboratory, then ARR was calculated using a DRC value of 1.6 mIU/L.

2.2.1 Screening procedures

Before screening, the patients were advised a 2-week run-in period with liberal salt intake and abstinence from licorice and chewing tobacco. Blood samples were taken at 8–10 a.m., ideally 2 h after awakening and being in upright position. A period of seated rest of 5–15 min was followed by measuring blood pressure and then obtaining blood samples: plasma sodium, potassium, creatinine, and ARR (denoted ARR#1, thus taken under current antihypertensive medication and, at this moment, regardless of the current MRA use or potassium level). See Figure 1 for more details.

Normokalemic patients without MRA, with PAC < 170 pmol/L, or with ARR < 50 pmol/mIU were considered not to have PA and thus finished their study participation. In case of PAC ≥ 170 and ARR ≥ 50, PA was suspected.

Individuals with plasma potassium <3.5 mmol/L received oral potassium substitution (continued throughout the diagnostic assessment) and were evaluated later under normokalemia. If initial PAC (PAC#1) or ARR#1 at the time of hypokalemia was below the diagnostic threshold, another ARR (ARR#2) was taken under normokalemia.

All patients taking MRA were advised a period of MRA discontinuation, which was effectuated under close clinical supervision and control of plasma potassium. If successfully paused, MRA was discontinued during the remaining diagnostic procedures. At least 6 weeks after MRA discontinuation, ARR#2 was taken. An ARR#2 ≥ 50 pmol/mIU in combination with PAC#2 ≥ 170 pmol/L motivated confirmation testing. Patients with ARR ≥ 50 pmol/mIU and PAC < 170 pmol/L were considered as not being eligible for confirmation testing.

2.2.2 Confirmation of PA

Confirmation of PA was performed using the recumbent intravenous saline suppression test (rSST). At the time of initiating the study, rSST was the gold standard, which today has changed to seated SST. The effort was made to maintain normokalemia prior to rSST and not to introduce new antihypertensive preparations with known effect on the renin–angiotensin–aldosterone system (RAAS).

The rSST was initiated at 8–10 a.m. Infusion of 2 L of physiologic sodium chloride solution was given during 4 h under repetitive control of blood pressure, pulse, and wellbeing. Thirteen patients whose blood pressure was above 165/105 received doses of doxazosin, verapamil, or amlodipine. Most patients (n = 106 of 113) needed no additional medication under rSST#1, and no patient experienced any discomfort.

The results of the rSST#1 were interpreted according to the guidelines (20) (Figure 1). If final aldosterone (PAC-4h) was ≤140 pmol/L, PA was deemed not present, and the study participation was discontinued. If PAC-4h was ≥280 pmol/L, the diagnosis of PA was confirmed. Aldosterone within the "gray zone" of 140–280 pmol/L was denoted "possible PA".

The patients with "possible PA" who took no antihypertensive preparations besides long-acting calcium blockers and/or doxazosin were not diagnosed with PA if PAC-4h after rSST#1 was below 190 pmol/L or diagnosed with PA if PAC-4h was ≥190 pmol/L, as suggested by the guidelines (20).

Patients with "possible PA" taking any other antihypertensive drugs were advised to temporarily and gradually discontinue those and substitute those with long-acting calcium blockers and/or doxazosin, under close supervision of a research physician. Six weeks after complete substitution as above, the patients underwent rSST#2. In 12 cases, the complete discontinuation and substitution was deemed unsafe, and rSST#2 was performed under a maximal clinically acceptable change of medication. In some cases, rSST#2 was done 2–4 weeks after maximally optimized medication due to reduced clinical tolerance of the medication change. All rSST#2 procedures were well tolerated, with no need for extra hypotensive medication.

During rSST#2, according to the guidelines (20), a PAC-4h of ≥ 190 pmol/L was considered as proof of PA. If PAC-4h was <190 pmol/L, then the patients were not diagnosed with PA and their study participation was terminated. One patient who could not discontinue nonsteroidal anti-inflammatory drugs (NSAIDs) had clinically probable PA, which was confirmed in spite of a PAC-4h of 180 pmol/L after rSST#2. Good subsequent blood pressure response to MRA served as an additional proof of PA in that case.

Our intention was to assure normokalemia before proceeding to both rSST#1 and rSST#2. Nevertheless, in some cases, hypokalemia

occurred anyway, but if PAC-4h of rSST#1 was below 140 pmol/L, then rSST#2 was completed after optimized potassium substitution. Subjects not diagnosed with PA were referred back to their primary care physician.

2.2.3 Further workup of PA cases, radiology, and localization

See Figure 2 for the study protocol concerning the diagnosed cases of PA. CT of adrenals was performed without contrast. Exclusion of cortisol and catecholamine overproduction was assured with regular biochemical screening. Patients not objecting to eventual surgical treatment (n = 45) were referred to Uppsala University Hospital for AVS, which was performed without further adjustment of ongoing medication (MRA already being paused in all patients referred for AVS).

In 52 of the 53 cases with PA, dexamethasone suppression test (DST) was carried out when PA was diagnosed, before proceeding to eventual AVS or PET. In eight patients, DST resulted in serum cortisol slightly above the upper reference level of 50 nmol/L. The highest value was 71 nmol/L (that patient had normal 24-h urinary cortisol). After thorough evaluation, none of these patients was considered to have mild autonomous cortisol secretion, and the cortisol values under AVS did not affect the interpretation of the results.

AVS was performed consecutively, without Synacthen stimulation, and was deemed successful if selectivity index (SI) on each side (plasma cortisol in the respective adrenal vein divided by plasma cortisol in the vena cava inferior) was ≥2.0. Aldosterone production was defined as dominant on one side if the lateralization index (LI, the greater ratio of plasma aldosterone to plasma cortisol in one adrenal vein divided by the smaller plasma aldosterone to plasma cortisol ratio in the other adrenal vein) was ≥4.0. If LI was <4, then the PA was defined as bilateral, except in one case where the clinical decision was taken to consider an LI of 3.2 as a sign of lateralization, and the patient later underwent surgery.

Adrenocortical PET/CT was performed in 11 patients: in 5 patients where AVS was (sometimes repeatedly) unsuccessful, in 5 patients to confirm the result of successful AVS, and in 1 patient without prior AVS. Of these 11 PET/CT procedures, 9 were performed with 11C-metomidate (MTO), which has been used at Uppsala University Hospital for approximately 20 years (34), while the 2 most recent PET/CT procedures were performed with the newly adapted and somewhat more specific tracer para-chloro-2-[^{18}F]fluoroethyletomidate (18F-CETO) (35).

The ratio allowing the diagnosis of lateralized PA based on MTO- or CETO-PET/CT was 1.25 between the (higher) standardized uptake value (SUV) in one adrenal divided by the (lower) SUV in the other adrenal. The ratio of 1.25 was chosen after initial studies on MTO-PET/CT performed at the Uppsala University Hospital and Cambridge University (36), which, in retrospect, also served as a basis for the later published MATCH-study (21). On clinical and pragmatic grounds concerning non-inferior sensitivity and specificity of CETO compared to MTO, the same ratio of 1.25 was applied to cases subjected to CETO-PET/CT. However, the optimal ratio for 18F-CETO-PET is under ongoing investigation.

2.3 Treatment options

The patients with lateralized aldosterone production were offered laparoscopic unilateral adrenalectomy. The patients not wishing or not suitable for surgical management and those with bilateral PA by AVS or by adrenocortical PET/CT were started on slowly and progressively optimized doses of MRA (eplerenone or spironolactone) under repetitive control of blood pressure, plasma creatinine, sodium, and potassium. The MRA doses were titrated with the intention of having potassium within the higher normal range and DRC (taken at least 2 months after MRA start) no longer suppressed (at least above 8 mIU/L). Other antihypertensive preparations (both in surgically and in medically treated patients with PA) were adjusted to achieve as normalized blood pressure as possible. Clinical follow-up after adrenalectomy or after MRA initiation continued within the study for at least 6–12 months.

2.4 Histopathology

Operated specimens underwent histopathologic examination at the Clinical Pathology Department at Uppsala University Hospital using a clinically validated protocol. Both hematoxylin–eosin staining and immunohistochemistry for CYP11B2 were carried out.

2.5 Ethics statement

The study was approved by the Swedish Ethical Review Authority and registered on ClinicalTrials.gov (NCT03105531). Data protection laws were adhered to.

2.6 Clinical, diagnostic, and laboratory sites

The screening, diagnostic workup, and medical and surgical treatment of diagnosed PA cases were performed at Karlstad Central Hospital. The screening tests were mostly taken by the research nurse. A number of patients (especially during the COVID pandemic) took the screening ARR through the laboratory service of their respective primary care facilities, with the results later analyzed by the research physician. All of the patients who needed to adjust medication within the study did so in direct clinical contact with the research physician (within the Surgical Department of the Karlstad Central Hospital).

Saline suppression testing was done in part by the specialist nurse at the Outpatient Endocrinology Department of Karlstad Central Hospital, and in part by the research nurse at the Surgical Department of the Karlstad Central Hospital.

Aldosterone and renin analyses, AVS, PET/CT, and postoperative histopathology were conducted at Uppsala

University Hospital. PAC and DRC were measured by routine clinical analysis (Chemiluminescence Immunoassay, LIAISON Analyzer, DiaSorin Inc., Saluggia, Italy). According to the manufacturer, the LIAISON® Aldosterone analysis had a limit of quantitation of 40.2 pmol/L and reliably measured aldosterone concentration up to 2,770 pmol/L. The functional sensitivity of the LIAISON® Renin analysis was 1.6 mIU/L, with the reliably detected concentration up to 500 mIU/L. There was linearity in measurement of these substances within the named ranges.

2.7 Statistical analysis

Variables were expressed as mean (standard deviation, SD) where parametric methods were employed, or as median [interquartile range (IQR)] if nonparametric methods were used. Categorical variables were expressed as absolute numbers and percentages. Differences between groups with nonparametric testing were analyzed by the Mann–Whitney *U* test or the Wilcoxon signed-rank test. When appropriate, the Student's paired *t*-test or (if equality of variances was not obviously present) the Welch *t*-test was used. Categorical data were assessed by chi-square or Fisher's exact test. All the statistical tests used were two-sided. p 0.05 was considered significant. IBM SPSS Statistics version 26 software was used for statistical analysis.

3 Results

Baseline clinical characteristics of the *total* study population including current medication are presented in Tables 2 and 3. Screening of 1,181 persons resulted in 53 found cases of PA, amounting to a prevalence of 4.5%. See Tables 2 and 4 concerning the clinical characteristics and medication for the groups of PA and primary (essential) hypertension HT.

Subjects not diagnosed with PA were clinically assessed on an individual basis for other possible causes of secondary hypertension. No cases that would benefit from other relevant specific treatment were found, and these patients continued to be taken care of by their primary care physician with the diagnosis of HT.

Most baseline clinical characteristics were comparable between the groups of HT and PA. However, the patients with PA had significantly higher blood pressure, which also was discovered at an earlier age than in the HT group. The PA group had initially slightly but significantly higher sodium and lower potassium in plasma (Table 2). The number of initially used antihypertensive medicines was slightly but significantly higher for patients with PA than for those with HT (Table 4).

The ARR (without MRA and under normokalemia) in cases diagnosed with PA ranged between 50 and 500 pmol/mIU (Figure 3). In total, 20 patients (38%) had ARR 50–100 pmol/mIU and 13 (25%) had ARR 100–150 pmol/mIU. Considering the initial clinical parameters and the medical history of each patient with PA found in the study, only approximately 60% of them would have been included in the risk groups to be screened according to the latest guidelines (20).

Of all excluded patients, 15 were excluded directly after inclusion due to perceived high risk of diagnostic workup with regard to comorbidities (n = 10, 5 of whom with heart failure), risk of MRA discontinuation (n = 3), or difficult vein access (n = 1). One patient who was followed by both a primary care and a specialized hypertension clinic was excluded.

In patients who underwent ARR#2 due to hypokalemia and not due to initial use of MRA (n = 25), potassium at the time of ARR#1 [median 3.3 (IQR 3.2–3.4)] was lower than at ARR#2 [3.8 (3.6–3.9)], Wilcoxon signed-rank test $p < 0.001$. In spite of that, the difference between ARR#1 [median 6.3 (IQR 1.8–16.3)] and ARR#2 [10.4 (3.1–28.9)] was not significant (Wilcoxon signed-rank test $p = 0.143$).

Eight patients of those initially on MRA (n = 22) were diagnosed with PA. In six of these, renin was below normal at ARR#1, and in another two patients, it was in the middle of the reference range. Remarkably, all of these eight patients had ARR#1 > 50, in spite of the ongoing use of MRA.

3.1 Confirmation testing

Therapy adjustment between rSST#1 and rSST#2 was well-tolerated. One patient was excluded due to anticipated high risk from therapy adjustment, and another patient was excluded due to worsening of blood pressure and heart failure symptoms concomitant with medication changes before rSST#2.

Of all patients lost to follow-up, five discontinued participation in the study during the process of therapy adjustment before rSST#2, without giving the study personnel any particular reason. None of the 40 patients who completed rSST#2 displayed any related complications.

Final aldosterone levels after the diagnostic SST in the group with lateralized PA were significantly higher than those in the group with bilateral PA, 320 (270–464) pmol/L vs. 246 (203–300) pmol/L, Mann–Whitney *U* test $p = 0.009$.

3.2 Aldosterone, renin, and ARR at different stages

Aldosterone, renin, and ARR were compared during different stages of the diagnostic process. ARR taken just at the start of rSST#1 (n = 113; "first ARR under rSST#1") was compared with the last ARR taken before that ("last ARR before rSST#1"). Paired *t*-test was possible and showed a significantly lower level of the "first ARR under rSST#1" in comparison with the "last ARR before rSST#1." Mean difference (95% confidence interval, CI) was 28 pmol/mIU (CI 15-41), $p < 0.001$ (because 14 outliers did not significantly affect the results after their exclusion, they were left in place for the final calculation of the paired *t*-test).

Comparison of PAC-4h after rSST#1 and after SST#2 showed significantly higher aldosterone after rSST#2, after excluding one

10.3389/fendo.2025.1555572

TABLE 2 Demographic and clinical characteristics of the groups within the study [values are expressed as median (interquartile range, IQR) or *n* (%)].

		Total study population *n* = 1,181	Primary aldosteronism (PA) *n* = 53	Primary hypertension (HT) *n* = 1,128	Difference between PA and HT, *p*[a]
Male		580 (49%)	27 (51%)	553 (49%)	0.785
Female		601 (51%)	26 (49%)	575 (51%)	
Age (years)		58 (53–62)	57 (53–61)	58 (53–62)	0.30
BMI		28.4 (25.6–31.6)	29.4 (25.9–33.1)	28.3 (25.6–31.5)	0.12
Blood pressure at screening	Systolic	138 (15)	145 (16)	138 (14)	**0.005***[c]
	Diastolic	87 (9)	91 (10)	87 (9)	**0.003***[c]
Plasma creatinine, µmol/L		69 (61–80)	73 (63–80)	69 (61–80)	0.34
eGFR, mL/min[b]		83 (76–90)	82 (75–90)	83 (76–90)	0.79
Plasma sodium [mean (SD)]		139.9 (2.37)	140.6 (1.83)	139.9 (2.39)	**0.015***[c]
Plasma potassium [mean (SD)]		3.9 (0.29)	3.7 (0.32)	4.0 (0.28)	**<0.001***[c]
Smoking now		84 (7%)	3 (6%)	81 (7%)	0.674
Former smoking		537 (46%)	21 (40%)	516 (46%)	0.382
Use of chewing tobacco (Swedish "snus")		175 (15%)	7 (13%)	168 (15%)	0.922
Former use of chewing tobacco ("snus")		317 (27%)	12 (23%)	305 (27%)	0.752
Regular consumption of licorice		120 (10%)	3 (6%)	117 (10%)	0.846
Age (years) when hypertension was discovered		50 (40–55)	44.5 (38–53)	50 (40–55)	**0.013***
Angina pectoris/myocardial infarction/ percutaneous coronary intervention in the anamnesis		90 (7.6%)	7 (13%)	83 (7.4%)	0.467
Ischemic cerebrovascular lesion/transitory ischemic attack in the anamnesis		53 (4.7%)	0	53 (4.7%)	0.438
Hemorrhagic stroke in the anamnesis		7 (0.6%)	0	7 (0.6%)	0.828
Peripheral atherosclerosis		9 (0.8%)	0	9 (0.8%)	0.789
Chronic heart failure		5 (0.4%)	0	5 (0.4%)	0.868
Diabetes mellitus or glucose intolerance		188 (15.9%)	8 (15.1%)	180 (16.0%)	0.963
Chronic obstructive lung disease		19 (1.6%)	0	19 (1.7%)	0.620
Chronic renal failure		6 (0.5%)	1 (2%)	5 (0.4%)	0.344
Atrial fibrillation/flutter/other significant acquired chronic arrythmia		50 (4.2%)	2 (4%)	48 (4.3%)	0.962
Hyperlipidemia[d]		370 (31.3%)	15 (28%)	355 (31.5%)	0.866
History of pulmonary embolism		21 (1.8%)	0	21 (1.9%)	0.591
Osteoporosis		19 (1.6%)	0	19 (1.7%)	0.576
Sleep apnea syndrome		137 (11.6%)	7 (13%)	130 (11.5%)	0.973

[a] *p* according to Mann–Whitney *U* test or, respectively, chi-square or Fisher's exact test.
[b] eGFR (mL/min), estimated glomerular filtration rate, MDRD formula (Modification of Diet in Renal Disease Study Group).
[c] *p* according to Welch's *t*-test.
[d] Hyperlipidemia was defined as chronic use of statins of other prescribed lipid-lowering medication.
* and data in bold denote statistically significant difference.

TABLE 3 Current medication for the total study population at the time of screening [*n* = 1,181 (%)].

Number of antihypertensive medicines	0	*n* = 57 (4.8%)
	1	*n* = 501 (42.4%)
	2	*n* = 382 (32.3%)
	3	*n* = 192 (16.3%)
	4	*n* = 42 (3.6%)
	5	*n* = 7 (0.6%)
Antihypertensive medicines		
Angiotensin receptor blockers		50.6%
Dihydropyridine calcium antagonists		40.9%
Angiotensin-converting enzyme inhibitors		27%
Thiazide diuretics		25.1%
Beta blockers		22.4%
Alfa blockers		2%
Mineralocorticoid receptor antagonists		1.9%
Loop diuretics		1.4%
Potassium-sparing diuretics (Amiloride)		1%
Central calcium antagonists (Verapamil)		0.4%
Imidazoline receptor antagonists		0%
Renin inhibitor (Aliskiren)		0%
Other relevant medicines		
Combined estrogen–progesterone preparations		1.4%
Estrogen preparations		6.1%
Selective serotonin reuptake inhibitors		7.6%
Non-steroid anti-inflammatory inhibitor drugs		13.5%

outlier of the data from 40 patients. Mean difference was 28 pmol/L (95%CI 12-44), p=0.001.

The ARR at the initiation of rSST#1 was significantly lower (p = 0.048) than that at the initiation of rSST#2 (n = 40), after excluding four outliers allowing a paired t-test. The mean difference between ARR#1 and ARR#2 was −22.5 pmol/mIU (−0.3 to −44.8).

3.3 Lateralization of PA

Of 53 patients with PA, 44 agreed to and underwent AVS (Figure 2), permitting bilateral PA to be diagnosed in 28 cases and lateralized PA in 11. In two of these patients, AVS was interpretable only when repeated the second time. AVS failed in five cases (and in two of them, twice) due to technical difficulties during catheterization and low SI. These five patients were subjected to PET/CT (showing three symmetrically bilateral and two lateralizing forms of PA). Another patient underwent PET/CT without prior AVS, with the uptake ratio signifying lateralized disease. Thus, in total, 31% (14 out of 45) of all who underwent AVS or PET/CT demonstrated lateralization. There were no complications related to AVS or PET.

3.4 Operated patients

Of the 14 lateralized cases, 11 have been operated with unilateral laparoscopic adrenalectomy (see Figure 2). Postoperative results and histopathology were assessed using the PASO (37) and HISTALDO (30) criteria. Four of the operated patients had an APA, five patients had multiple APM, while in one patient, only one APM was described. In one case, there were multiple APNs including one dominant APN.

Three of the patients with unilateral PA have not been operated. Thus, at least 4 out of 11 (36%) operated, but possibly up to 7 out of 14 lateralized (50%), and 4–7 out of 45 subtype-classified patients with PA (9%–16%) may have had APA.

Consequently, 7–10 out of 14 lateralized cases had some form of nodular disease or true hyperplasia (50%–71%), which supports previous data (31, 38). Assuming that the 31 patients who did not lateralize also had some form of non-APA disease, the total number of patients with non-APA disease approaches 38–41 out of 45 subtype-classified patients with PA (84%–91%). See Figures 4–7 for histological examples of CYP11B2-ICH and its value.

The four cases of adenoma all had complete biochemical success of surgery. In the seven cases of nodular pathology (including the case with a dominant APN), four demonstrated complete postoperative biochemical success; in the other three cases with partial biochemical success (all with APMs), low doses of MRA had to be initiated postoperatively. Complete clinical success (where the patient discontinued all antihypertensive medication postoperatively) was only seen in one case with APA, a 56-year-old female patient with a 25-year history of hypertension.

Of all operated patients, there was one case of postoperative pulmonary embolism in spite of pharmacological anti-thrombotic prophylaxis. Temporary cortisol substitution was needed in two cases—in spite of adequately low preoperative dexamethasone suppressed cortisol (24 and 40 nmol/L, respectively). No other postoperative complications occurred.

3.5 Conservatively treated PA

A total of 42 patients with PA were started on MRA, with the dosage gradually augmented with the intention to reach adequate elevation of plasma renin while keeping serum potassium within the higher normal reference range. These 42 patients were composed of 31 patients with bilateral forms (28 at AVS, 2 at MTO-PET, and 1 at CETO-PET), 8 patients who refrained from AVS, and 3 unilateral patients (2 at AVS and 1 at PET) who finally had to be treated conservatively. Within a median of 13 months (IQR 12–17), 40 of these patients (95.2%) were considered to have reached the optimal possible MRA therapy. The three lateralized patients with PA where conservative treatment was chosen consisted of (i) one patient with a gigantic adrenal cyst and local pressure symptoms demanding

10.3389/fendo.2025.1555572

TABLE 4 Number of groups of antihypertensive medicines in the study groups at the time of screening.

		Total study population, *n* = 1,181	Primary aldosteronism (PA), *n* = 53	Primary hypertension (HT), *n* = 1,128	Difference between PA and HT
Number of groups of antihypertensive medicines	0	57 (4.8%)	4 (7.5%)	53 (4.7%)	Fisher's exact test $p < 0.001$ *
	1	501 (42.4%)	16 (30.2%)	485 (43%)	
	2	382 (32.3%)	9 (17%)	373 (33%)	
	3	192 (16.3%)	17 (32.1%)	175 (15.5%)	
	4	42 (3.6%)	5 (9.4%)	37 (3.3%)	
	5	7 (0.6%)	2 (3.8%)	5 (0.4%)	
Number of groups of antihypertensive medicines	Mean (SD)	1.7 (0.95)	2.2 (1.28)	1.7 (0.93)	Mann–Whitney *U* test $p = 0.006$ *
	Median (IQR)	2 (1-2)	2 (1-3)	2 (1-2)	
Mean rank for Mann–Whitney *U* test			798	585	

*Statistically significant.

adrenalectomy on the opposite side to the aldosterone-overproducing adrenal; (ii) one patient with morbid obesity; and (iii) one patient who declined surgery and already was treated successfully with MRA. The three cases with partial biochemical success postoperatively (all with non-APA forms of aldosterone overproduction) were started on low doses of MRA and responded with an adequate rise in renin.

An adequate rise of DRC was achieved at the latest follow-up in 51 out of 53 patients who completed the follow-up, and was >8.0 mIU/L in 44 of these patients (86%). A DRC value of 8 mIU/L corresponds to a plasma renin activity (PRA) value of 1 μg/L per hour—shown to be the minimal protective plasma renin value for conservatively treated patients (18, 19). DRC below 8 mIU/L was still present in two patients with complete biochemical success after adrenalectomy as shown by low ARR (23 and 17 pmol/mIU, respectively) and low-normal PAC (122 and 75 pmol/L, respectively). In addition, in five of the conservatively treated patients, final DRC was below 8 mIU/L. In two of them, this was deemed due to the concurrent administration of beta blockers or NSAIDs that could not be paused; one patient with totally

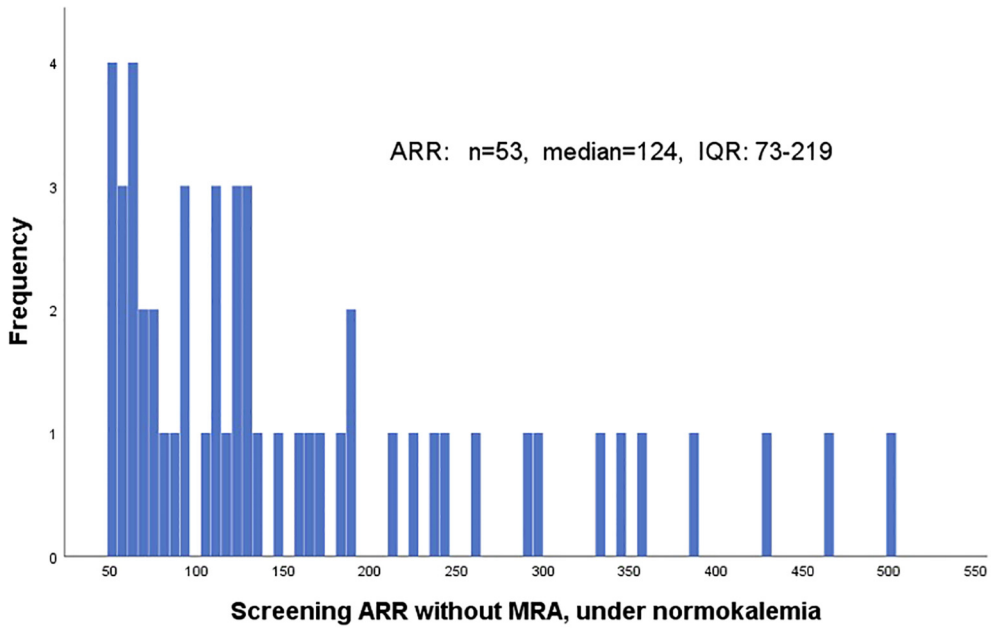


FIGURE 3
Frequency distribution of aldosterone–renin ratio among the diagnosed cases of primary aldosteronism—at the moment of the screening. ARR, aldosterone–renin ratio (pmol/mIU); IQR, interquartile range; MRA, mineralocorticoid receptor antagonists.

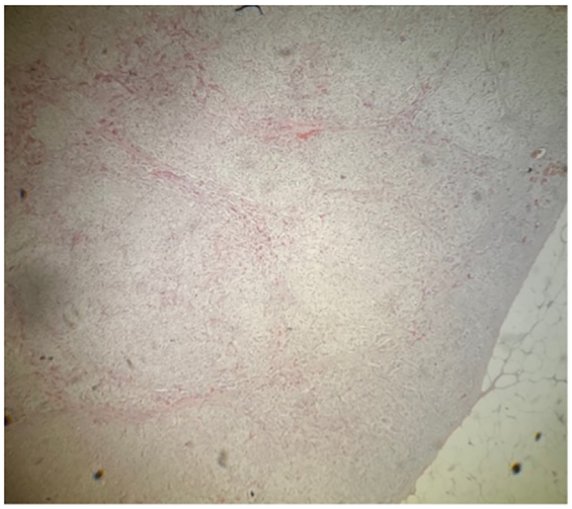


FIGURE 4
Histopathology of specimen from the adrenal gland in a patient with PA. Part of CYP11B2-positive nodule A, hematoxylin–eosin staining. Magnification ×40.

normalized blood pressure on a given dose of MRA wished not to raise the dosage further in spite of low final renin; one patient, treated only with MRA, could not further raise their dosage due to hypotension; and one patient had reported side effects (fatigue and headache) of consecutively tested spironolactone, eplerenone, and amiloride and was thus left on a calcium blocker.

Overall, DRC increased from 4.2 (IQR 2.0–5.7) mIU/L ($n = 51$), measured just before the SST, to 20 (IQR 9.4–41) mIU/L at the latest follow-up (Wilcoxon signed-rank test, $p < 0.001$).

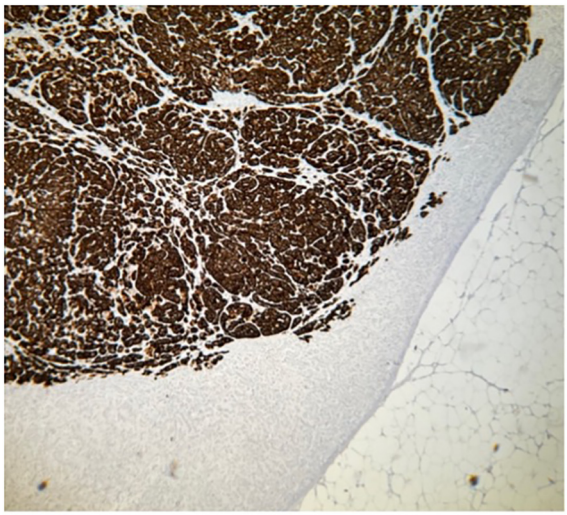


FIGURE 5
Histopathology of specimen from the adrenal gland in a patient with PA. Part of CYP11B2-positive nodule A, immunohistochemistry demonstrating expression of aldosterone synthase, CYP11B2. Magnification ×40.

3.6 Treatment outcome

Of 53 PA cases, 23 had potassium supplementation before ARR screening, and none at follow-up (Fisher's exact test $p < 0.0001$). Potassium levels were significantly higher after adequate treatment [mean 4.26 (SD 0.28) mmol/L] than before the screening [3.73 (0.32)]; $n = 46$, $p < 0.001$.

The mean number of groups of antihypertensive medication at inclusion was 2.2 (median 2, IQR 1–3), while at the latest follow-up, the mean was 2.6 (median 2, IQR 2–4), Wilcoxon signed-rank test $p = 0.020$ ($n = 51$). The aim during the final adjustment of medication was to achieve normal blood pressure if possible, which may well have caused the number of blood pressure medications to rise for the study patients. A positive effect of study-related treatment on blood pressure was noted (see Table 5).

4 Discussion

This study demonstrates that PA is present in 4.5% of a cohort of unselected primary care patients with hypertension, bearing in mind that the screened individuals had predominantly moderate hypertension and were, in most cases, screened during ongoing medication (which was one of the aims of the study). The majority of PA cases found were mild. Adjustment of medication, when necessary, could be carried out safely, but was time-consuming. Several patients with a higher probability of PA, such as those with higher stages of heart failure, could not be subjected to the complete diagnostic process, which underscores the need for optimized diagnostic methods. Moreover, we did not account for any earlier diagnosed cases of PA already followed by a specialist clinic. Several authors underline the uncertainty in the estimation of individual

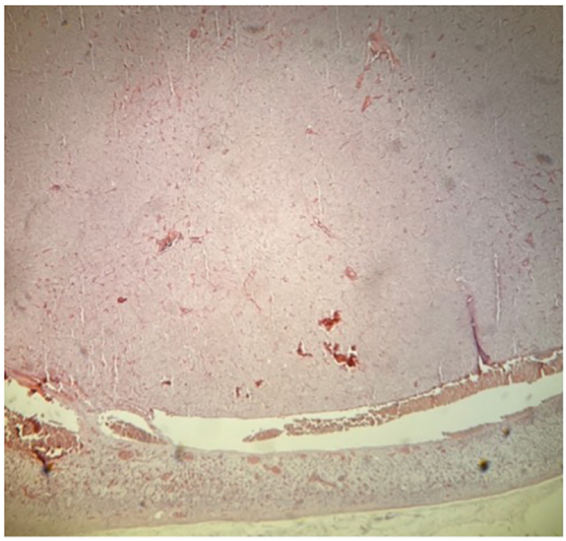


FIGURE 6
Histopathology of specimen from the adrenal gland in a patient with PA. Part of CYP11B2-negative nodule B, hematoxylin–eosin staining. Magnification ×40.

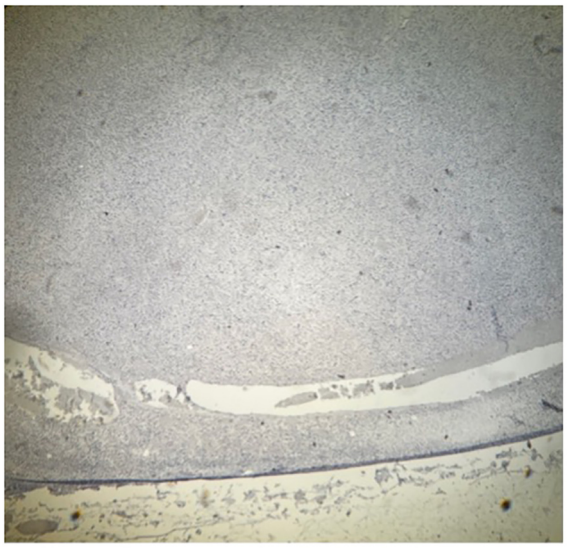


FIGURE 7
Histopathology of specimen from the adrenal gland in a patient with PA. Part of CYP11B2-negative nodule A, immunohistochemistry demonstrating absent expression of aldosterone synthase, CYP11B2. Magnification ×40.

ARR (9, 39–41), which allows possible underdiagnosis of PA when ARR is used for screening, and the search continues for alternative and better biochemical screening for PA (42). Some advocate the use of urinary aldosterone excretion to more reliably find the true prevalence of PA (8, 15, 43). As an illustration for the inherent limitations of existing screening tests, a higher prevalence of PA has been found in studies avoiding the screening test and directly using confirmatory procedures (5). Therefore, the actual prevalence of PA among the total hypertensive population in Sweden is most likely above 4.5%, while this number may well be accurate in the cohort of individuals with milder hypertension followed within primary care in Sweden.

One of the study aims was to investigate whether screening for PA with ARR under ongoing antihypertensive medication, but without MRA (thus simplifying interpretation of the Endocrine Society guidelines), was possible—a hypothesis that our results support. There are some, but not many, studies designed to answer a similar question (44–46). The patients in our project were selected based on the presence of hypertension, and not upon its severity, related symptoms, or the patient's age when hypertension or complications developed, which represents one of the strengths of our study. The guidelines (20) recommend screening for patients in certain risk groups. In our material with mostly mild cases, approximately 40% of patients with diagnosed PA did not belong to such risk groups. Indeed, several research teams recommend screening of all patients with hypertension at least once (7, 15, 47–49).

There is a continuing debate whether screening and confirmation of PA should include preliminary discontinuation of medication known to possibly affect renin and aldosterone (15, 39, 50–54). Even the guidelines are equivocal on the subject (20). The majority of such preparations (besides MRA) can lower aldosterone and (besides betablockers) elevate renin. As each SST implied measurement of "initial" ARR and PAC-4h, we could demonstrate that these values were significantly higher during rSST#2 compared with rSST#1. Thus, our results support that maximal acceptable discontinuation of antihypertensive drugs may contribute to successful evaluation of the final diagnostic procedures.

As mentioned above, at the time of the study's protocol planning, the recumbent SST was a state-of-the-art method for this confirmatory test. Since then, the approach has developed, and it is currently widely accepted that the blood tests during screening and confirmation procedures such as SST should be taken with the patient seated and not recumbent, which further elevates the sensitivity of these procedures (55, 56). The difference between the last ARR before rSST#1 (while sitting) and the initial ARR at rSST#1 (after some minutes lying down) serves as an illustration of this finding.

The role of normokalemia at the time of hormonal evaluation should not be seen as negated by our results. Rather, the results may be seen as indicating only a minor effect of *mild* hypokalemia on ARR.

The majority of scientific publications to date adhere to the longstanding tradition of dividing the anatomical subtypes of PA into "unilateral APA," representing 30%–40% of cases; "bilateral hyperplasia," representing 60%–70% of cases; and other seldom occurring forms of PA, sometimes mentioned as "unilateral hyperplasia." There is, however, growing evidence of the existence of lateralized PA caused by APNs or APMs rather than an APA, which also can be successfully treated with adrenalectomy (30, 31, 38, 57, 58). Unilateral diffuse aldosterone-overproducing hyperplasia is rare (31, 32, 38). Iacobone et al. have noted that up to 74% of AVS-defined unilateral PA cases were represented by non-adenoma lesions (even if IHC was not performed, 38). Our results support this finding, with APA being present in 29%–50% of the patients with lateralized PA. Iacobone et al. also noted that

TABLE 5 Specific treatment of primary aldosteronism: effect on blood pressure (n = 51) evaluated after follow-up of median 12 months (IQR 12–17, range 6–33).

	BP[a] before specific PA treatment (on antihypertensive drugs before diagnosis of PA)		BP after specific PA treatment		Paired t-test
	Systolic	Diastolic	Systolic	Diastolic	
Mean (SD)	145 (16)	91 (10)	126 (9)	79 (5)	$p < 0.001$ *

[a]BP, blood pressure.
*Statistically significant.

unilateral adrenalectomy in these cases was as highly effective at 3 years' follow-up as in those with a clear adenoma in the postoperative specimen (38).

Surgical treatment of unilateral forms of PA is associated with a substantial long-term (mainly cardiovascular) risk reduction (16). In contrast, patients with PA offered specific and continued pharmacological treatment (especially in those cases when it is not sufficient enough to counteract renin suppression) still suffer from significantly greater secondary organ damage and increased mortality (17, 18, 59, 60). Thus, identification of lateralizing cases is important, in order to recommend adrenalectomy in these patients.

Lateralized PA is generally characterized by more pronounced aldosterone overproduction than non-lateralized forms, which may facilitate limiting the number of patients for localizing studies as those are essentially needed just for the potentially operable cases (61, 62). This paradigm is even illustrated in our material where final aldosterone level after diagnostic SST was significantly higher among the lateralized cases compared to the bilateral cases. In contrast, in patients with mild forms of PA, which are predominantly bilateral, as also noted in our cohort, localizing studies could be omitted (10, 63). Conservative treatment in such individuals, controlled by rising renin, may constitute a sound pragmatic alternative. Further development and validation of non-invasive adrenal cortex-specific PET/CT may also simplify the workup. Practically, the results of specific treatment of the PA cases discovered in the current cohort reveal that it is clinically safe and possible to apply the principles of such treatment.

In spite of recent years' attention to PA, the rate of screening, detection, and adequate management of the disease is still very low (43, 64–66). A recent Swedish register-based study of the incidence of PA has documented progressive growth of its diagnostic discovery within the population of a large region during the last years—but still to the degree that corresponds to a far lower number of diagnosed patients with PA than expected (67). Awareness of healthcare professionals—especially primary care practitioners—would be essential in the process of diagnosing the disorder and the risks it implies (13, 66).

The role of screening for PA in hypertensive populations is not merely to improve control of blood pressure. It is also important to adequately reduce the negative effects of high non-physiologic aldosterone levels. We recommend liberal screening by ARR in all hypertensive adults.

5 Limitations

The prevalence of PA found in our study may reasonably be seen through the prism of the factors that could lead to missed cases during screening. Some missed cases might exist among those who never responded to the invitation to participate in the study.

Screening under ongoing antihypertensive treatment (without MRA, but often including preparations potentially affecting RAAS) may have resulted in some number of false-negative individuals. Of course, one has to be careful in the interpretation of biochemical PA screening data obtained under ongoing medication. To reduce this risk, we would have needed to safely adjust the medications in *all* patients before screening, which is cumbersome, excessively time-consuming and labor-intensive, and requires a high degree of motivation from the patients to be able to succeed. These efforts were made among those who underwent rSST#2, thus only a subcohort. Our understanding is that it would not be possible to accomplish that kind of task within available resources for this study, and it was not coherent with our intentions to simplify the diagnostic protocol.

During rSST#1, both recumbency and ongoing medical treatment could have led to false-negative results. We used recumbent SST in the screening, but more recently, sitting SST has been proven superior. Traditionally, the older scientific publications and textbooks presented an axioma that abnormal autonomous aldosterone production should necessarily be unrelated to angiotensin-2 stimulation. The "rule" was shown to be erroneous by a number of studies (55, 56, 68) that illustrate that the majority of cases with PA are sensitive to that stimulation. Furthermore, some forms of dysregulation imply secretion of excessive amounts of aldosterone under physiologic stimulation—such as by adrenocorticotropic hormone (ACTH) released during stress and, to some extent, also by renin (69). Aldosterone hypersecretion due to stimulation by ACTH has been shown to be more pronounced for unilateral forms of PA (as opposed to bilateral PA) and could be even used to differentiate unilateral from bilateral PA (70).

Another limitation of the study is the modest number of patients found and treated for PA. The fact that they coherently originate from an unselected primary care hypertensive population may, on the other hand, be seen as a strong advantage, thus detailing the profile of PA that may be the one more typical for the general majority of the cases.

6 Conclusion

a. Screening for PA in individuals with hypertension within primary care is safely performed using the Endocrine Society guidelines.

b. PA is common among primary care patients with hypertension and should be actively screened for to avoid premature morbidity and mortality, which is also present in milder forms of PA.

c. We advocate this screening for all patients with hypertension, at least under a certain pragmatically defined age.

d. Screening may be performed with plasma ARR taken while sitting, and during ongoing antihypertensive medication, but without MRA.

e. A significant number of patients diagnosed with PA in this screening had ARR just above 50 pmol/mIU, and this cutoff may be used in clinical practice.

f. Discontinuation of medicines that affect ARR may unmask pathologic ARR and raise aldosterone in suppression testing. Therefore, this should be clinically considered if

suspicion of drug-dependent false-negative outcome of biochemical hormonal testing exists.

g. Mild hypokalemia affects the results of such testing to a lesser extent. Both screening and further workup are in need of more straightforward and sensitive diagnostic tools for PA.

h. It is important to suggest that a patient who has undergone negative screening for PA by ARR should not automatically for life be considered as having primary hypertension. Re-screening should be performed if clinically motivated.

i. Mild PA is less often caused by APA than more clinically florid forms of PA. High prevalence of nodal forms and relatively low prevalence of APA (according to HISTALDO criteria) may be features of the lateralizing PA cases found in a setting of liberal screening within primary care patients with hypertension.

j. We also describe the use of adrenocortical PET/CT among the localizing studies and foresee an increased role for this non-invasive method in the future.

k. Clinical work within the study (including all the aspects of treatment) was carried out in a regional hospital (with AVS and PET done at the University Hospital). It is not improbable that the scope of PA diagnosis in the adult population one day reaches quite considerable volumes. In that case, the study may serve as an example of feasibility of decentralization of practical treatment for PA.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Swedish Ethical Review Authority. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

NM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. JS: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Visualization, Writing – review & editing. TÅ: Data curation, Investigation, Methodology, Resources, Validation, Visualization, Writing – review & editing. FA: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Writing – review & editing. DA: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Visualization, Writing – review & editing. MB: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. MW: Funding acquisition, Investigation, Resources, Supervision, Writing – review & editing. PH: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. The authors have received funding from the Regional Research Council for Uppsala-Örebro, Sweden (grant numbers 651241, 842171, and 930708) as well as from the Center for Clinical Research and Education, Värmland Region, Karlstad, Sweden (grant numbers 637541, 741191, 840631, 930327, 939850, 967684, 980171, and 993231).

Acknowledgments

The skillful and dedicated assistance of the project nurse Maria Nordström is greatly appreciated. The authors also express their gratitude to all the clinical and administrative staff who helped and supported the project.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

References

1. Sudano I, Suter P, Beuschlein F. Secondary hypertension as a cause of treatment resistance. *Blood Press*. (2023) 32:2224898. doi: 10.1080/08037051.2023.2224898

2. Zhou B, Carrillo-Larco RM, Danaei G, Riley LM, Paciorek CJ, Stevens GA, et al. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. *Lancet*. (2021) 398:957–80. doi: 10.1016/S0140-6736(21)01330-1

3. Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, et al. Prevalence and clinical manifestations of primary aldosteronism encountered in primary care practice. *J Am Coll Cardiol*. (2017) 69:1811–20. doi: 10.1016/j.jacc.2017.01.052

4. Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *J Am Coll Cardiol*. (2006) 48:2293–300. doi: 10.1016/j.jacc.2006.07.059

5. Käyser SC, Dekkers T, Groenewoud HJ, van der Wilt GJ, Carel Bakx J, van der Wel MC, et al. Study heterogeneity and estimation of prevalence of primary aldosteronism: A systematic review and meta-regression analysis. *J Clin Endocrinol Metab*. (2016) 101:2826–35. doi: 10.1210/jc.2016-1472

6. Parasiliti-Caprino M, Lopez C, Prencipe N, Lucatello B, Settanni F, Giraudo G, et al. Prevalence of primary aldosteronism and association with cardiovascular complications in patients with resistant and refractory hypertension. *J Hypertens*. (2020) 38:1841–8. doi: 10.1097/HJH.0000000000002441

7. Brown JM, Robinson-Cohen C, Luque-Fernandez MA, Allison MA, Baudrand R, Ix JH, et al. The spectrum of subclinical primary aldosteronism and incident hypertension: A cohort study. *Ann Intern Med*. (2017) 167:630. doi: 10.7326/M17-0882

8. Brown JM, Siddiqui M, Calhoun DA, Carey RM, Hopkins PN, Williams GH, et al. The unrecognized prevalence of primary aldosteronism: A cross-sectional study. *Ann Intern Med*. (2020) 173:10–20. doi: 10.7326/M20-0065

9. Baudrand R, Guarda FJ, Fardella C, Hundemer G, Brown J, Williams G, et al. Continuum of renin-independent aldosteronism in normotension. *Hypertension*. (2017) 69:950–6. doi: 10.1161/HYPERTENSIONAHA.116.08952

10. Vaidya A, Mulatero P, Baudrand R, Adler GK. The expanding spectrum of primary aldosteronism: implications for diagnosis, pathogenesis, and treatment. *Endocr Rev*. (2018) 39:1057–88. doi: 10.1210/er.2018-00139

11. Verma A, Vaidya A, Subudhi S, Waikar SS. Aldosterone in chronic kidney disease and renal outcomes. *Eur Heart J*. (2022) 43:3781–91. doi: 10.1093/eurheartj/ehac352

12. Markou A, Pappa T, Kaltsas G, Gouli A, Mitsakis K, Tsounas P, et al. Evidence of primary aldosteronism in a predominantly female cohort of normotensive individuals: A very high odds ratio for progression into arterial hypertension. *J Clin Endocrinol Metab*. (2013) 98:1409–16. doi: 10.1210/jc.2012-3353

13. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol*. (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4

14. Wu X, Yu J, Tian H. Cardiovascular risk in primary aldosteronism: A systematic review and meta-analysis. *Med (Baltimore)*. (2019) 98:e15985. doi: 10.1097/MD.0000000000015985

15. Vaidya A, Hundemer GL, Nanba K, Parksook WW, Brown JM. Primary aldosteronism: state-of-the-art review. *Am J Hypertens*. (2022) 35:967–88. doi: 10.1093/ajh/hpac079

16. Wu VC, Wang SM, Huang KH, Tsai YC, Chan CK, Yang SY, et al. Long-term mortality and cardiovascular events in patients with unilateral primary aldosteronism after targeted treatments. *Eur J Endocrinol*. (2022) 186:195–205. doi: 10.1530/EJE-21-0836

17. Chen SY, Chen JY, Huang WC, Puar THK, Chin Kek P, Chueh JS, et al. Cardiovascular outcomes and all-cause mortality in primary aldosteronism after adrenalectomy or mineralocorticoid receptor antagonist treatment: a meta-analysis. *Eur J Endocrinol*. (2022) 187:S47–58. doi: 10.1530/EJE-22-0375

18. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. *Lancet Diabetes Endocrinol*. (2018) 6:51–9. doi: 10.1016/S2213-8587(17)30367-4

19. Hundemer GL, Leung AA, Kline GA, Brown JM, Turcu AF, Vaidya A. Biomarkers to guide medical therapy in primary aldosteronism. *Endocr Rev*. (2024) 45:69–94. doi: 10.1210/endrev/bnad024

20. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. (2016) 101:1889–916. doi: 10.1210/jc.2015-4061

21. Wu X, Senanayake R, Goodchild E, Bashari WA, Salsbury J, Cabrera CP, et al. 11C]metomidate PET-CT versus adrenal vein sampling for diagnosing surgically curable primary aldosteronism: a prospective, within-patient trial. *Nat Med*. (2023) 29:190–202. doi: 10.1038/s41591-022-02114-5

22. Bioletto F, Lopez C, Bollati M, Arata S, Procopio M, Ponzetto F, et al. Predictive performance of aldosterone-to-renin ratio in the diagnosis of primary aldosteronism in patients with resistant hypertension. *Front Endocrinol*. (2023) 14:1145186. doi: 10.3389/fendo.2023.1145186

23. Westerdahl C, Bergenfelz A, Isaksson A, Wihl A, Nerbrand C, Valdemarsson S. High frequency of primary hyperaldosteronism among hypertensive patients from a primary care area in Sweden. *Scand J Prim Health Care*. (2006) 24:154–9. doi: 10.1080/02813430600830931

24. Westerdahl C, Bergenfelz A, Isaksson A, Nerbrand C, Valdemarsson S. Primary aldosteronism among newly diagnosed and untreated hypertensive patients in a Swedish primary care area. *Scand J Prim Health Care*. (2011) 29:57–62. doi: 10.3109/02813432.2011.554015

25. Käyser SC, Deinum J, De Grauw WJ, Schalk BW, Bor HJ, Lenders JW, et al. Prevalence of primary aldosteronism in primary care: a cross-sectional study. *Br J Gen Pract*. (2018) 68:e114–22. doi: 10.3399/bjgp18X694589

26. Asbach E, Kellnar A, Bekeran M, Schelling J, Bidlingmaier M, Reincke M. Prevalence of primary aldosteronism in newly diagnosed hypertensive patients in primary care. *Exp Clin Endocrinol Diabetes*. (2022) 130:801–5. doi: 10.1055/a-1938-4242

27. Libianto R, Russell GM, Stowasser M, Gwini SM, Nuttall P, Shen J, et al. Detecting primary aldosteronism in Australian primary care: a prospective study. *Med J Aust*. (2022) 216:408–12. doi: 10.5694/mja2.v216.8

28. Naruse M, Murakami M, Katabami T, Kocjan T, Parasiliti-Caprino M, Quinkler M, et al. International multicenter survey on screening and confirmatory testing in primary aldosteronism. *Eur J Endocrinol*. (2023) 188:125–34. doi: 10.1093/ejendo/lvac002

29. Naruse M, Katabami T, Shibata H, Sone M, Takahashi K, Tanabe A, et al. Japan Endocrine Society clinical practice guideline for the diagnosis and management of primary aldosteronism 2021. *Endocr J*. (2022) 69:327–59. doi: 10.1507/endocrj.EJ21-0508

30. Williams TA, Gomez-Sanchez CE, Rainey WE, Giordano TJ, Lam AK, Marker A, et al. International histopathology consensus for unilateral primary aldosteronism. *J Clin Endocrinol Metab*. (2021) 106:42–54. doi: 10.1210/clinem/dgaa484

31. Gunnarsdottir H, Agnarsson BA, Jonasdottir S, Gudmundsson J, Birgisson G, Sigurjonsdottir HA. Immunohistochemical staining seems mandatory for individualizing and shortening follow-up in unilateral primary aldosteronism. *Clin Endocrinol (Oxf)*. (2023) 99:441–8. doi: 10.1111/cen.14958

32. Meyer LS, Handgriff L, Lim JS, Udager AM, Kinker IS, Ladurner R, et al. Single-center prospective cohort study on the histopathology, genotype, and postsurgical outcomes of patients with primary aldosteronism. *Hypertension*. (2021) 78:738–46. doi: 10.1161/HYPERTENSIONAHA.121.17348

33. Reincke M, Williams TA. True unilateral primary aldosteronism exists, and unilateral adrenalectomy saves lives. *Eur J Endocrinol*. (2022) 186:C5–7. doi: 10.1530/EJE-22-0123

34. Hennings J, Lindhe Ö, Bergström M, Långström B, Sundin A, Hellman P. ^{11} C] Metomidate positron emission tomography of adrenocortical tumors in correlation with histopathological findings. *J Clin Endocrinol Metab*. (2006) 91:1410–4. doi: 10.1210/jc.2005-2273

35. Silins I, Sundin A, Lubberink M, O'Sullivan L, Gurnell M, Aigbirhio F, et al. First-in-human evaluation of [18F]CETO: a novel tracer for adrenocortical tumours. *Eur J Nucl Med Mol Imaging*. (2023) 50:398–409. doi: 10.1007/s00259-022-05957-9

36. Burton TJ, Mackenzie IS, Balan K, Koo B, Bird N, Soloviev DV, et al. Evaluation of the sensitivity and specificity of ^{11} C-metomidate positron emission tomography (PET)-CT for lateralizing aldosterone secretion by conn's adenomas. *J Clin Endocrinol Metab*. (2012) 97:100–9. doi: 10.1210/jc.2011-1537

37. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, et al. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol*. (2017) 5:689–99. doi: 10.1016/S2213-8587(17)30135-3

38. Iacobone M, Citton M, Viel G, Boetto R, Bonadio I, Tropea S, et al. Unilateral adrenal hyperplasia: A novel cause of surgically correctable primary hyperaldosteronism. *Surgery*. (2012) 152:1248–55. doi: 10.1016/j.surg.2012.08.042

39. Jansen PM, Van Den Born BJH, Frenkel WJ, De Bruijne ELE, Deinum J, Kerstens MN, et al. Test characteristics of the aldosterone-to-renin ratio as a screening test for primary aldosteronism. *J Hypertens*. (2014) 32:115–26. doi: 10.1097/HJH.0b013e3283656b54

40. Yozamp N, Hundemer GL, Moussa M, Underhill J, Fudim T, Sacks B, et al. Intraindividual variability of aldosterone concentrations in primary aldosteronism: implications for case detection. *Hypertension*. (2021) 77:891–9. doi: 10.1161/HYPERTENSIONAHA.120.16429

41. St-Jean M, Bourdeau I, Martin M, Lacroix A. Aldosterone is aberrantly regulated by various stimuli in a high proportion of patients with primary aldosteronism. *J Clin Endocrinol Metab*. (2021) 106:e45–60. doi: 10.1210/clinem/dgaa703

42. Burrello J, Buffolo F, Domenig O, Tetti M, Pecori A, Monticone S, et al. Renin-angiotensin-aldosterone system triple-A analysis for the screening of primary aldosteronism. *Hypertension*. (2020) 75:163–72. doi: 10.1161/HYPERTENSIONAHA.119.13772

43. Funder J. Primary aldosteronism. *Trends Cardiovasc Med*. (2022) 32:228–33. doi: 10.1016/j.tcm.2021.03.005

44. Li X, Liang J, Hu J, Ma L, Yang J, Zhang A, et al. Screening for primary aldosteronism on and off interfering medications. *Endocrine*. (2023) 83:178–87. doi: 10.1007/s12020-023-03520-6

45. Liu X, Hao S, Bian J, Lou Y, Zhang H, Wu H, et al. Performance of aldosterone-to-renin ratio before washout of antihypertensive drugs in screening of primary aldosteronism. *J Clin Endocrinol Metab*. (2024) 109:e2302–8. doi: 10.1210/clinem/dgae094

46. Ruiz-Sánchez JG, Fernández Sánchez Á, Meneses D. Feasibility of primary aldosteronism diagnosis in initial evaluation without medication withdrawal or confirmatory tests. *Endocrine*. (2024) 85:906–15. doi: 10.1007/s12020-024-03798-0

47. Young WF. Diagnosis and treatment of primary aldosteronism: practical clinical perspectives. *J Intern Med*. (2019) 285:126–48. doi: 10.1111/joim.12831

48. Nishikawa T, Omura M, Satoh F, Shibata H, Takahashi K, Tamura N, et al. Guidelines for the diagnosis and treatment of primary aldosteronism -The Japan Endocrine Society 2009-. *Endocr J*. (2011) 58:711–21. doi: 10.1507/endocrj.EJ11-0133

49. Maiolino G, Calò LA, Rossi GP. The time has come for systematic screening for primary aldosteronism in all hypertensives ∗. *J Am Coll Cardiol*. (2017) 69:1821–3. doi: 10.1016/j.jacc.2017.02.041

50. Schwartz GL, Turner ST. Screening for primary aldosteronism in essential hypertension: diagnostic accuracy of the ratio of plasma aldosterone concentration to plasma renin activity. *Clin Chem*. (2005) 51:386–94. doi: 10.1373/clinchem.2004.041780

51. Schilbach K, Junnila R, Bidlingmaier M. Aldosterone to renin ratio as screening tool in primary aldosteronism. *Exp Clin Endocrinol Diabetes*. (2019) 127:84–92. doi: 10.1055/a-0672-0836

52. Li X, Goswami R, Yang S, Li Q. Aldosterone/direct renin concentration ratio as a screening test for primary aldosteronism: A meta-analysis. *J Renin Angiotensin Aldosterone Syst*. (2016) 17:147032031665745. doi: 10.1177/1470320316657450

53. Funder J. Primary aldosteronism: Treatment of the disease, and new therapeutic approaches. *Best Pract Res Clin Endocrinol Metab*. (2020) 34:101368. doi: 10.1016/j.beem.2019.101368

54. Vaidya A, Carey RM. Evolution of the primary aldosteronism syndrome: updating the approach. *J Clin Endocrinol Metab*. (2020) 105:3771–83. doi: 10.1210/clinem/dgaa606

55. Stowasser M, Ahmed AH, Cowley D, Wolley M, Guo Z, McWhinney BC, et al. Comparison of seated with recumbent saline suppression testing for the diagnosis of primary aldosteronism. *J Clin Endocrinol Metab*. (2018) 103:4113–24. doi: 10.1210/jc.2018-01394

56. Cornu E, Steichen O, Nogueira-Silva L, Küpers E, Pagny JY, Grataloup C, et al. Suppression of aldosterone secretion after recumbent saline infusion does not exclude lateralized primary aldosteronism. *Hypertension*. (2016) 68:989–94. doi: 10.1161/HYPERTENSIONAHA.116.07214

57. Omura M, Sasano H, Fujiwara T, Yamaguchi K, Nishikawa T. Unique cases of unilateral hyperaldosteronemia due to multiple adrenocortical micronodules, which can only be detected by selective adrenal venous sampling. *Metabolism*. (2002) 51:350–5. doi: 10.1053/meta.2002.30498

58. Goh BKP, Tan YH, Chang KTE, Eng PHK, Yip SKH, Cheng CWS. Primary hyperaldosteronism secondary to unilateral adrenal hyperplasia: an unusual cause of surgically correctable hypertension. A review of 30 cases. *World J Surg*. (2007) 31:72–9. doi: 10.1007/s00268-005-0594-8

59. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Renal outcomes in medically and surgically treated primary aldosteronism. *Hypertension*. (2018) 72:658–66. doi: 10.1161/HYPERTENSIONAHA.118.11568

60. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Incidence of atrial fibrillation and mineralocorticoid receptor activity in patients with medically and surgically treated primary aldosteronism. *JAMA Cardiol*. (2018) 3(8):768–74. doi: 10.1001/jamacardio.2018.2003

61. Maiolino G, Rossitto G, Bisogni V, Cesari M, Seccia TM, Plebani M, et al. Quantitative value of aldosterone-renin ratio for detection of aldosterone-producing adenoma: the aldosterone-renin ratio for primary aldosteronism (AQUARR) study. *J Am Heart Assoc*. (2017) 6:e005574. doi: 10.1161/JAHA.117.005574

62. Rossi GP, Bisogni V, Bacca AV, Belfiore A, Cesari M, Concistrè A, et al. The 2020 Italian Society of Arterial Hypertension (SIIA) practical guidelines for the management of primary aldosteronism. *Int J Cardiol Hypertens*. (2020) 5:100029. doi: 10.1097/01.hjh.0000745016.79540.85

63. Nanba K, Rainey WE. Pathophysiology of bilateral hyperaldosteronism. *Curr Opin Endocrinol Diabetes Obes*. (2022) 29:233–42. doi: 10.1097/MED.0000000000000729

64. Jaffe G, Gray Z, Krishnan G, Stedman M, Zheng Y, Han J, et al. Screening rates for primary aldosteronism in resistant hypertension: A cohort study. *Hypertension*. (2020) 75:650–9. doi: 10.1161/HYPERTENSIONAHA.119.14359

65. Hundemer GL, Imsirovic H, Vaidya A, Yozamp N, Goupil R, Madore F, et al. Screening rates for primary aldosteronism among individuals with hypertension plus hypokalemia: A population-based retrospective cohort study. *Hypertension*. (2022) 79:178–86. doi: 10.1161/HYPERTENSIONAHA.121.18118

66. Funes Hernandez M, Bhalla V. Underdiagnosis of primary aldosteronism: A review of screening and detection. *Am J Kidney Dis*. (2023) 82:333–46. doi: 10.1053/j.ajkd.2023.01.447

67. Gkaniatsa E, Ekerstad E, Gavric M, Muth A, Trimpou P, Olsson DS, et al. Increasing incidence of primary aldosteronism in western Sweden during 3 decades – yet an underdiagnosed disorder. *J Clin Endocrinol Metab*. (2021) 106:e3603–10. doi: 10.1210/clinem/dgab327

68. Ahmed AH, Cowley D, Wolley M, Gordon RD, Xu S, Taylor PJ, et al. Seated saline suppression testing for the diagnosis of primary aldosteronism: A preliminary study. *J Clin Endocrinol Metab*. (2014) 99:2745–53. doi: 10.1210/jc.2014-1153

69. Markou A, Sertedaki A, Kaltsas G, Androulakis II, Marakaki C, Pappa T, et al. Stress-induced aldosterone hyper-secretion in a substantial subset of patients with essential hypertension. *J Clin Endocrinol Metab*. (2015) 100:2857–64. doi: 10.1210/jc.2015-1268

70. Jiang Y, Zhang C, Wang W, Su T, Zhou W, Jiang L, et al. Diagnostic value of ACTH stimulation test in determining the subtypes of primary aldosteronism. *J Clin Endocrinol Metab*. (2015) 100:1837–44. doi: 10.1210/jc.2014-3551

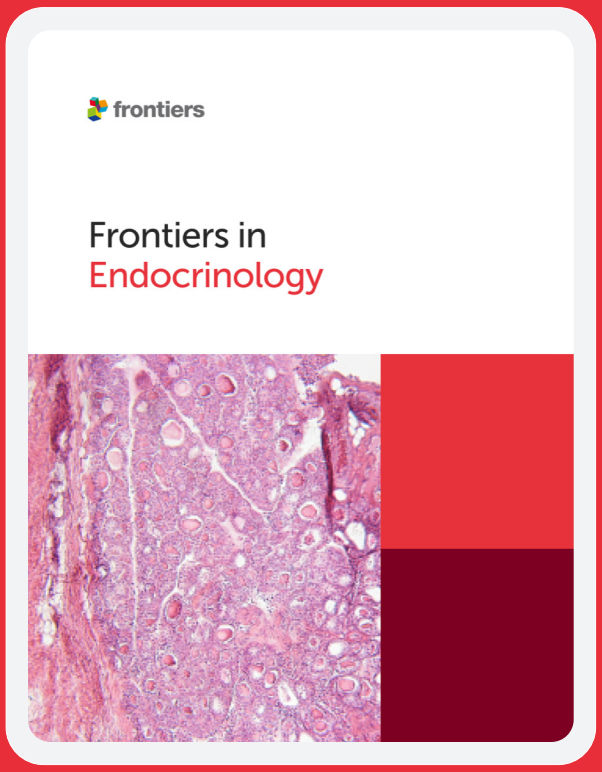
Frontiers in Endocrinology

Explores the endocrine system to find new therapies for key health issues

The second most-cited endocrinology and metabolism journal, which advances our understanding of the endocrine system. It uncovers new therapies for prevalent health issues such as obesity, diabetes, reproduction, and aging.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact