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Screening primary aldosteronism by plasma aldosterone-to-angiotensin II ratio

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Abstract

Objectives: Primary aldosteronism (PA) is a common cause of secondary hypertension. The aldosterone-to-renin ratio (ARR) is the current recommended biomarker for PA screening, but it has limitations. This study evaluates another ratio, the aldosterone-to-angiotensin II ratio (AAIIR), as an alternative screening tool for PA.

Methods: Archived plasma samples for ARR from a group of 152 hypertensive patients undergoing PA screening were retrieved for AAIIR analyses. Both AAIIR and ARR were measured by liquid chromatography-mass spectrometry methods. Correlation analysis, sensitivity, specificity, and receiver operating characteristic (ROC) curve analysis were performed to assess the diagnostic performance of AAIIR relative to ARR.

Results: AAIIR showed a strong positive correlation with ARR (r=0.79, p<0.0001). The area under the ROC curve for AAIIR (0.94, 95 % CI: 0.90–0.98) was satisfactory and not significantly different from ARR (0.94, 95 % CI: 0.90–0.97, p=0.877). The optimal cutoff values were 577 (pmol/L)/(μg/L^{-h}) and 60 for ARR and AAIIR, respectively. The sensitivity of AAIIR was slightly higher than ARR (91 vs. 88 %), while the specificity was comparable (85 vs. 86 %).

Conclusions: AAIIR demonstrates a comparable diagnostic performance to ARR for PA screening, with potential advantages in efficiency and reliability. Further large-scale studies are needed to validate its efficacy and establish its role in routine clinical practice.

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Introduction

Primary aldosteronism (PA) results from autonomous aldosterone production in the adrenal glands. Patients develop hypertension and are at increased risk of subsequent cardiovascular and renal complications [1]. Early and accurate diagnosis of PA is crucial as it is potentially curable. The latest Endocrine Society guideline recommends the measurement of plasma aldosterone-to-renin ratio (ARR) for screening PA [2]. However, analytical challenges for measuring plasma renin activity (PRA) may compromise the screening accuracy of ARR [3].

In clinical laboratories, PRA is assessed by the generation rate of angiotensin I (Ang I). The measurement can be influenced by multiple factors, including cryoactivation of prorenin, pH, and temperature [3]. Additionally, owing to the presence of endogenous Ang I, each sample needs to have two aliquots incubated at different temperatures to accurately estimate the net generation of Ang I. Consequently, the measurement of the PRA to calculate the ARR is extremely laborious and, hence, time-consuming. The reliability of PRA measurement also depends on the concentration of endogenous angiotensinogen. Conditions associated with low concentrations of angiotensinogen (e.g., liver diseases) may potentially lead to underestimation of Ang I production [4].

The plasma renin concentration (PRC) was introduced because of the challenges in PRA measurements. This measurand facilitates inter-laboratory comparisons and enables automation. However, when the plasma angiotensinogen level is abnormally low or high, there is a poor correlation between the PRA and PRC assays [5]. Furthermore, the accuracy of PRC measurement by immunoradiometric assays is suboptimal, especially at low concentrations [6], which is commonly observed in PA patients.

Recently, angiotensin II (Ang II), the bioactive peptide that directly stimulates aldosterone production in the adrenal glands, has been studied as a potential alternative biomarker for PA screening. Its short half-life and low plasma physiological concentration make the measurement

of this analyte challenging. Historically, Ang II has primarily been measured by immunoassays, which could be susceptible to interference by angiotensin peptides involved in the renin-angiotensin-aldosterone system (RAAS) [7]. Nowadays, liquid chromatography-mass spectrometry (LCMS) methods can accurately measure Ang II. Different types of mass spectrometers have been used, including time-of-flight spectrometers [8], triple quadrupoles [9], and ion traps [10]. The sample preparation procedures in these methods are tedious and involve multiple steps, such as solid-phase extraction (SPE) combined with immuno-affinity purification [11]. To overcome the difficulty of measuring the low physiological concentration of Ang II, some studies have proposed measuring equilibrium Ang II (eqAng II), which increases Ang II concentrations by incubation before measurement [12]. Guo et al. also studied the ratio of plasma aldosterone (PALD) to eqAng II for the PA screening [13].

Our laboratory has established an LCMS method for plasma Ang II measurement without incubation [7]. This study aims to evaluate the AAIIR as a screening test for patients with suspected PA.

Materials and methods

Samples collection and selection

The study was approved by the Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee (CRE-2011.601). At the Prince of Wales Hospital, the teaching hospital of the Chinese University of Hong Kong, endocrinologists and other internists utilize the ARR for initial screening of suspected PA. Our endocrinologists would generally request 24-h urine aldosterone (UALD) as an additional PA screening test.

For this study, we selected patients with UALD requested between November 2015 and January 2017. The corresponding ARR results were retrieved from the laboratory information system. The electronic patient record was checked for each sample to review the clinical history and eliminate duplicate requests. A diagnosis of PA or essential hypertension (EH) was established for each case by the endocrinologists based on a combination of biochemical tests (i.e., oral salt loading, saline infusion, and/or postural stimulation tests), imaging findings, and the clinical response to treatment. We excluded patients who were on antihypertensive drugs that are known to significantly affect the RAAS (e.g., Ang II receptor blockers, angiotensin-converting enzyme inhibitors, and beta-blockers), as well as those with non-PA secondary hypertension.

Biochemical tests

Samples storage and handling

In our hospital, EDTA blood samples for PRA and PALD measurements are transported to the laboratory at room temperature to prevent cryoactivation. After receiving the specimens, the EDTA plasma samples are centrifuged at 2,200 *g* for 15 min at room temperature, aliquoted and stored at −80 °C until their scheduled batch analysis. Before the analysis, these frozen samples are placed on a sample mixing roller for 30 min at room temperature to ensure thorough thawing and mixing. Immediate sample processing and extraction are followed. The stability of PRA and PALD under these conditions has been thoroughly validated to ensure reliable quantification.

In this study, archived plasma EDTA samples used for Ang II analysis were also kept at −80 °C before the analysis. Our method validation has confirmed the integrity of Ang II in these archived samples without using protease inhibitors [7]. The stability of Ang II in archived samples (compared to fresh samples) was also validated [7]. Sample handling and processing were similar to PRA and PALD.

Analytical method for PALD

PALD was quantified using electrospray liquid chromatography-tandem mass spectrometry (LC-MS/MS) [14]. This method has been adopted for routine services at our hospital since 2013. The analytical measurement range was 50–5,160 pmol/L. The typical between-batch coefficient of variation (CV) was less than 5 %. Accuracy performance has been monitored regularly through participation in external quality assurance programs (EQAP), for example, the College of American Pathologists (CAP) and Reference Institute for Bioanalytics Hormones Group 1, with confirmation of satisfactory performance.

Analytical method for PRA

The measurement of PRA in EDTA plasma samples was modified from the electrospray LC-MS/MS methods of Bystrom et al. and Carter et al. [15, 16]. A detailed description has been published previously [14]. This method has been adopted for routine services at our hospital since 2013. The analytical measurement range was 0.07–100 $\mu g/L^{-h}$. The typical between-batch CV was less than 8 %. Accuracy performance has been monitored regularly by participating in EQAP, e.g., CAP, with satisfactory results.

Analytical method for Ang II

An offline SPE procedure was used to extract Ang II from EDTA plasma using a Waters MAX µElution plate (Waters Corporation, Milford, MA, USA). Electrospray LC-MS/MS quantitation was performed using the Waters TQS system, as previously described [7]. This method had a measurable range of 3.3–700 pmol/L. The between-batch precision CV was less than 7 % over the Ang II concentrations of 8.6–110 pmol/L.

Statistical analysis

Statistical analysis was performed using MedCalc Statistical Software version 17.0 (MedCalc Software bvba, Ostend, Belgium). Outliers for measured parameters were eliminated by Tukey's method. Correlations among variables were performed by Spearman's rank correlation test. Comparisons for included variables were performed using the Mann-Whitney U test. Receiver operating characteristic (ROC) curve analyses for AAIIR and ARR were performed using the Delong group's method. Youden J statistics determined the cutoffs for AAIIR and ARR. Likelihood ratios were calculated for each ratio based on the ROC curves. The abilities of PA screening by the two ratios were compared by pairwise comparison of ROC curves on the difference between the area under the curve (AUC). Statistical significance was set at $p<0.05$.

Results

Patient samples

Figure 1 shows the algorithm of data extraction. A total of 152 patient samples were included in the final analysis, of which 35 patients were diagnosed with PA. One outlier (Ang II=15.2 pmol/L) was excluded by Tukey's method. Table 1 lists the patient demographics and results of all analytes. PA patients were generally older, with higher UALD, AAIIR, and ARR.

Correlation of PRA and Ang II

Figure 2 shows that PRA and Ang II were significantly correlated independent of the PA status of the patients (Spearman's rank correlation coefficient, r=0.80, p-value <0.0001).

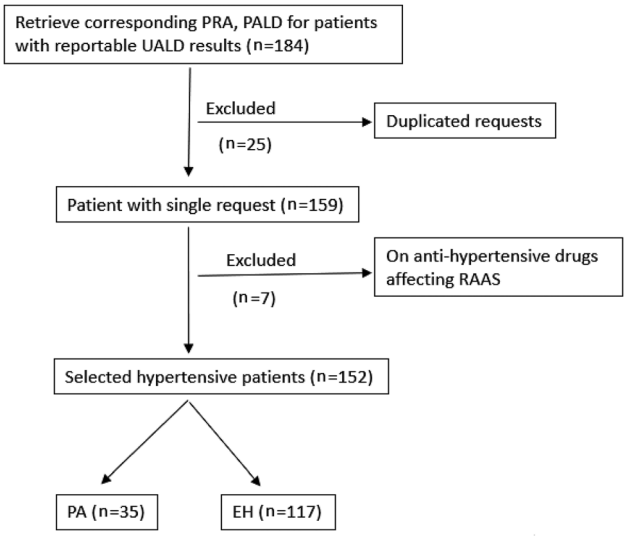

Figure 1: Flowchart showing the selection of patients from the laboratory information system based on request on UALD between December 2015 and January 2017. EH–essential hypertension, PA–primary aldosteronism, PALD–plasma aldosterone, PRA–plasma renin activity, RAAS – renin-angiotensin-aldosterone system, UALD–urinary aldosterone.

Table 1: Comparison of baseline characteristics and analytes in 151 patients (data shown in median with range).

	Patient groups		p-Value
	Essential (n=117)	PA (n=34)	
% Male	53	59	
Age, years	45.0 (15–76)	55.0 (33–69)	<0.0001
UALD, nmol/day	30.0 (1–125)	72.0 (9–266)	<0.0001
24-hour urine sodium, mmol/day	156 (34–904)	170 (87–351)	0.3493
PRA, ng/mL^{-h}	1.27 (0.07–7.69)	0.25 (0.07–2.09)	<0.0001
PALD, pmol/L	203 (49.4–1,227)	465 (125–1,402)	<0.0001
Ang II, pmol/L	6.8 (3.4–25.0)	3.7 (3.4–6.9)	<0.0001
ARR, pmol/L/ng/mL^{-h}	168 (14–2,706)	1,475 (296–10016)	<0.0001
AAIIR	28.0 (7.3–116)	102.7 (37.3–418.5)	<0.0001

UALD, 24-hour aldosterone; PRA, plasma renin activity; PALD, plasma aldosterone; Ang II, plasma angiotensin II; AAIIR, plasma aldosterone-to-angiotensin II ratio; ARR, plasma aldosterone-to-renin ratio.

Correlation of Ang II and PALD

Figure 3 shows that Ang II was correlated with PALD (R=0.35, P-value=0.0001) in patients with EH but not in patients with PA (p-value=0.85).

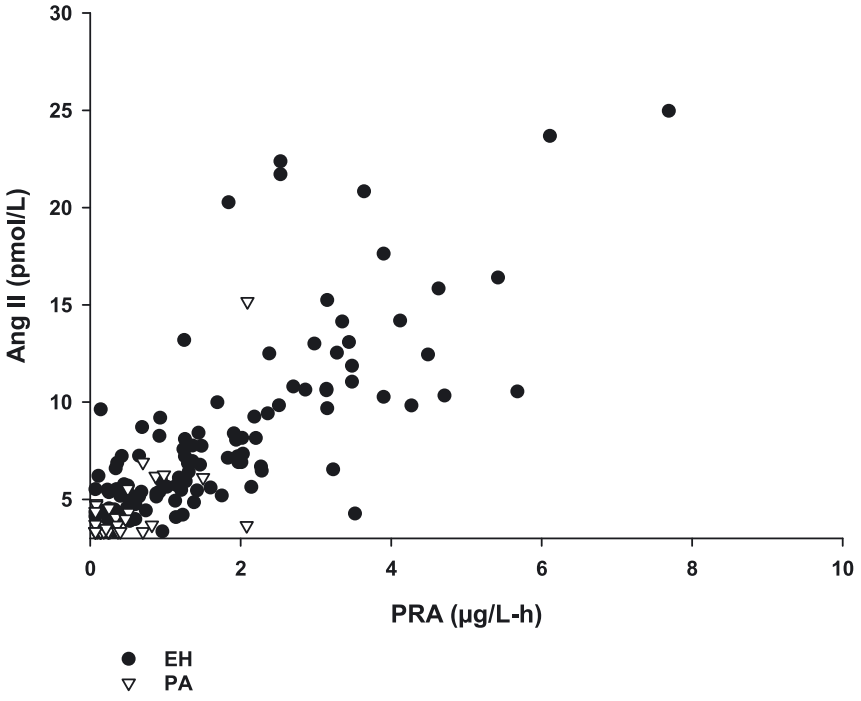

Figure 2: The correlation of Ang II and PRA in patients with EH and PA. Ang II–angiotensin II, EH–essential hypertension, PA–primary aldosteronism, PRA–plasma renin activity.

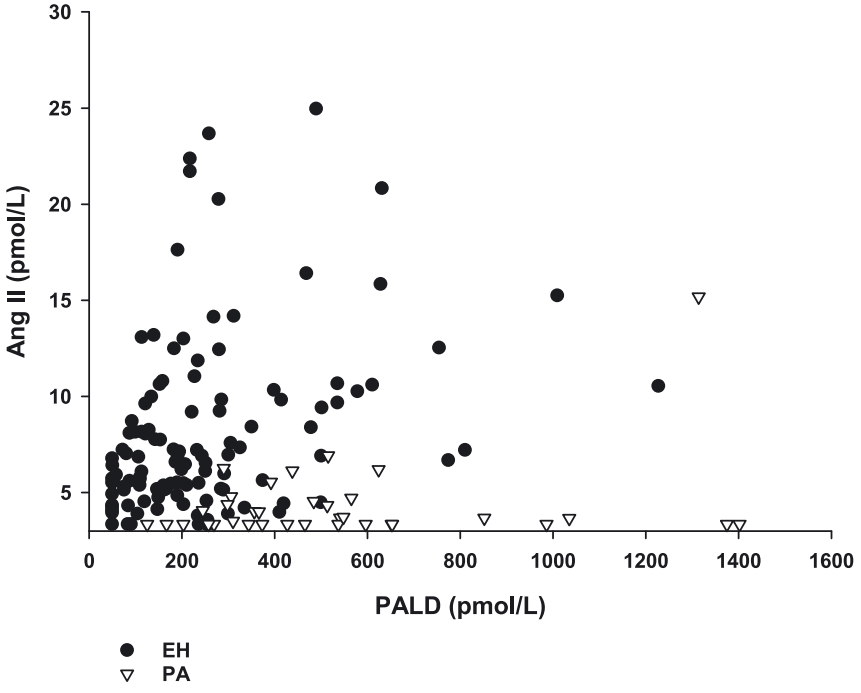

Figure 3: The correlation of Ang II and PALD in patients with EH or PA, Ang II–angiotensin II, EH–essential hypertension, PA–primary aldosteronism, PALD–plasma aldosterone concentration.

Comparing ARR and AAIIR

Characteristics of AAIIR in EH patients

The AAIIR was significantly higher in patients with PA compared to patients with EH (p-value <0.0001). Figure 4 shows the comparison of AAIIR in these two groups of patients.

Correlation of ARR and AAIIR

Figure 5 is a scatterplot of ARR and AAIIR, showing that the two ratios are significantly correlated (r=0.79, p-value <0.0001).

Comparison of diagnostic performance of AAIIR to ARR

The ROC analysis showed no significant difference in the AUC of ARR (0.935 (95 % CI:0.898 to 0.972)) and AAIIR [0.938

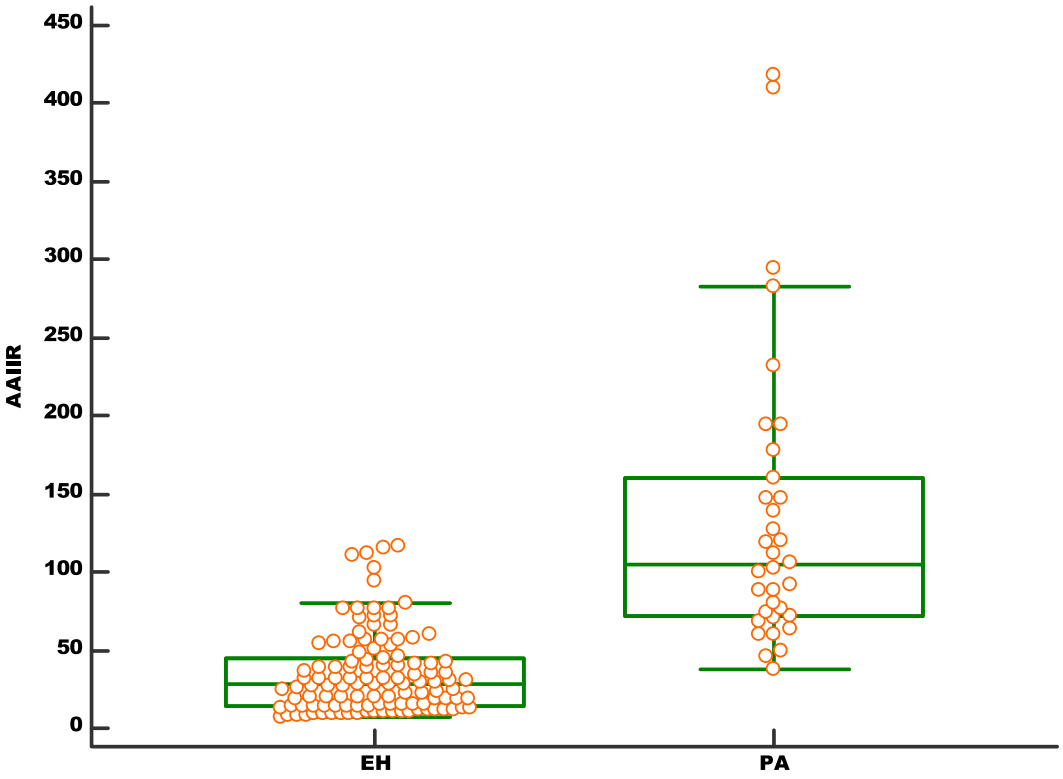

Figure 4: Comparison of AAIIR in PA and EH patients. AAIIR – aldosterone-to-angiotensin II ratio, EH–essential hypertension, PA–primary aldosteronism.

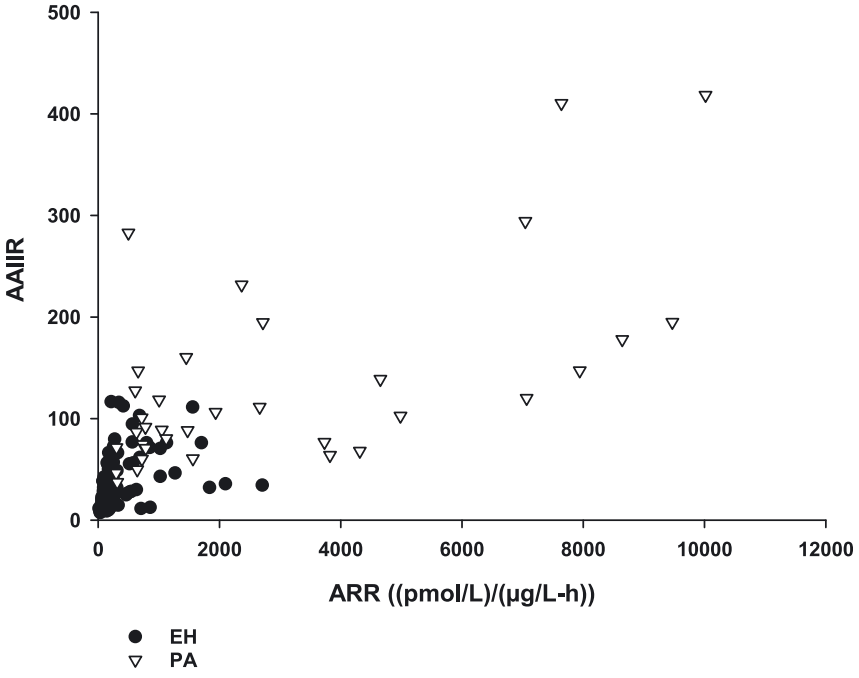

Figure 5: Correlation of AAIIR and ARR in EH and PA patients. AAIIR – aldosterone-to-angiotensin II ratio, ARR – aldosterone-to-renin ratio; EH–essential hypertension, PA–primary aldosteronism.

(95 % CI:0.901 to 0.975)], as shown in Figure 6. The optimal cutoff values were 577 (pmol/L)/(μg/L^{-h}) and 60 for ARR and AAIIR, respectively. The two ratios shared a similar specificity (85 % for AAIIR vs. 86 % for ARR), while AAIIR had a higher sensitivity (91 vs. 88 %). Youden indices were 0.77 and 0.75 for AAIIR and ARR, respectively. The positive and negative likelihood ratios for AAIIR were 6.3 and 0.10, respectively, while those for ARR were 6.5 and 0.14.

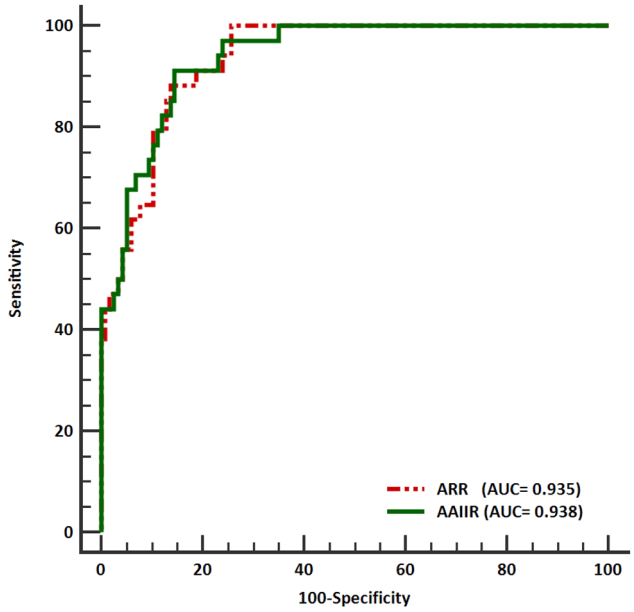

Figure 6: Comparison of ROC curves of AAIIR and ARR, AAIIR – aldosterone-to-angiotensin II ratio, ARR – aldosterone-to-renin ratio; ROC–receiver operating characteristic.

Discussion

Although the measurements of Ang II by immunoassays and LCMS have previously been reported in different studies, there is limited literature on evaluating AAIIR as a screening tool for PA. In 2020, Guo et al. evaluated eqAAIIR (i.e., PALD/eqAng II) to screen PA [13]. However, the efficacy of eqAng II in accurately representing RAAS status remains uncertain. Consequently, as far as we know, this study represents the first evaluation of AAIIR based on endogenous Ang II for the screening of PA.

Our study revealed significant correlations between Ang II and PRA in patients with EH or PA. In patients with EH, Ang II levels correlated significantly with PALD concentrations. However, this correlation was not observed in patients with PA. The AAIIR and the ARR demonstrated comparable efficacy in the screening for PA.

Ang II is the metabolite produced from Ang I through the enzymatic action of angiotensin-converting enzyme. As renin catalyzes the conversion of angiotensinogen to Ang I [17], an increase in PRA leads to a rise in Ang I and, subsequently, Ang II. Therefore, the concentrations of the two analytes are expected to exhibit a strong correlation in all patients as long as they are not receiving any medications which affect the RAAS. Conversely, Ang II is the active effector of the RAAS, responsible for stimulating the production of PALD in the adrenal glands [18]. In patients with EH, an increase in Ang II would result in a rise in PALD. However, in patients with PA, the secretion of PALD becomes independent of PRA [19] and Ang II due to autonomous secretion. The excess in PALD would lead to sodium retention and blood volume increase, thereby suppressing PRA and Ang II generation [20]. Consequently, as expected, there was no significant correlation between PALD and Ang II in patients with PA in our study.

In the ROC curves analysis of ARR and AAIIR, there was no significant difference in AUC, with both ratios demonstrating comparable capability in the screening for PA. As both ARR and AAIIR are screening parameters for PA, false positive and negative results were expected. False positive results of ARR and AAIIR would primarily be attributed to low PRA and Ang II levels. Low PRA was observed in 25 % of EH patients [21, 22], and the prevalence can increase with age. We expected a similar observation in Ang II. In our study, the percentages of samples with PRA and Ang II results that were less than the lower limits of quantitation were 8 and 17 %, respectively. The higher percentage of low Ang II could be explained by the low physiological Ang II concentration in even normal subjects [8], not to mention in PA patients in whom PRA and Ang II would be theoretically suppressed. There were 17 (15 % of EH patients) false positive ARR and 18 (15 % of EH patients) false positive AAIIR. Aldosterone levels in all false positive ARR cases and 14 false AAIIR cases were within the reference interval. The remaining 4 false positive AAIIR cases showed high aldosterone, borderline high/high renin (2.27–5.68(pmol/L)/(μg/L^{-h})) and normal/high Ang II (6.9–15.3 pmol/L). These cases are generally younger in age and obese, which might explain the high aldosterone results [23]. Therefore, the exact values of renin or Ang II might be worth considering for making clinical decisions. For false negative cases, ARR showed 4 cases (12 % of PA patients)., while AAIIR showed 3 cases (9 % of PA patients). Two cases were found to have negative ARR but positive AAIIR results. Therefore, AAIIR was a better marker for PA screening. All the false negative cases had high 24-h urine aldosterone, which prompted the endocrinologists to provide confirmatory studies. Therefore, including 24-h urine aldosterone in the screening of PA may decrease the false negative rate.

Due to the lack of uniformity in diagnostic protocols, different cutoff values of ARR, ranging from 550 to 830 (pmol/L)/(μg/L^{-h}) [24–26], were adopted in different clinical centres. According to the Endocrine Society Guideline, the optimal cutoff for ARR is 750 (pmol/L)/(μg/L^{-h}) [2]. In this study, the optimal cutoff value for ARR is 577 (pmol/L)/(μg/L^{-h}), which approximates the 550 (pmol/L)/(μg/L^{-h}) cutoff adopted by our endocrinologists.

According to the Endocrine Society guidelines [2], ARR calculated by PRA and PALD is recommended as a screening

method for PA. In our laboratory, online SPE has been utilized in the PRA LCMS method to eliminate matrix interference. The injection-to-injection time is 12 min. Each patient requires 2 injections to calculate a single PRA result. Completing one batch of 40 PRA samples typically requires 22 h, resulting in a suboptimal turnaround time for the ARR measurements. The measurement of Ang II and the utilization of AAIIR for PA screening would facilitate a reduction in the duration of the diagnostic procedure. Its low physiological concentration and short half-life in the plasma have previously impeded its application. However, LCMS measurements can achieve a detection limit of 3.3 pmol/L [7]. Only one single injection of Ang II is required for each sample from each patient. Each injection necessitates a running time of 5 min. Analyzing a batch of 40 Ang II samples requires less than 5 h. Therefore, if Ang II can substitute PRA for the screening of PA, the analysis time required will decrease significantly, expediting the PA screening process. PA patients may thus receive confirmatory tests and definitive treatment earlier, potentially reducing the risk of long-term complications. Compared to PRA, no incubation step was involved in measuring Ang II, which reduces the influences of various factors, including endogenous proteases which break down generated Ang I and incubation variability [3].

Nevertheless, introducing AAIIR measurements to routine clinical services may present significant challenges. Firstly, plasma Ang II concentration (measured in pmol/L) is substantially lower than that of Ang I (measured in nmol/L), necessitating highly sensitive analyzers to accurately quantify Ang II. Such sophisticated analytical instruments may only be readily available to some clinical laboratories. Furthermore, the unavailability of an EQAP for Ang II poses difficulties in monitoring the analytical performance of this assay.

In our study, 23 % of patients who underwent investigations for PA were eventually diagnosed with PA. The frequency of confirmed cases is marginally higher than that reported in the local and nearby districts [25, 27, 28]. The prevalence of PA in various studies ranged from 5 to 20 % [29–34], depending on the selection criteria for the study population [31]. In our study, the deliberate selection of patients being investigated by the endocrinologists (i.e., patients with UALD measured) may account for the higher proportion of PA cases in the cohort. Due to this potential selection bias, likelihood ratios, rather than sensitivity and specificity, were calculated as they would not be influenced by the prevalence rate of the disease [35, 36]. The negative likelihood ratio for AAIIR was 0.10, while that for ARR was 0.14. Both could generate moderate change for pretest probability to posttest probability in the screening of PA, indicating that both the ARR and AAIIR would be deemed acceptable for screening out PA [37].

Based on our study results, we propose AAIIR as a potential novel biomarker for the screening of PA, which requires further investigation in different clinical settings. Additional studies are necessary before incorporating this test as part of the procedures for screening for PA in routine service.

Conclusions

This study demonstrates that the AAIIR shows comparable diagnostic performance to the traditional ARR for PA screening in hypertensive patients. AAIIR exhibited a strong correlation with ARR and similar specificity and sensitivity. While AAIIR offers potential advantages such as shorter analysis time and reduced susceptibility to confounding factors, its implementation in routine clinical practice may face challenges. Further large-scale studies are needed to validate the efficacy of AAIIR and to establish its role in the screening for PA. If successfully implemented, AAIIR could improve the early diagnosis and treatment of PA, leading to better patient outcomes.

Research ethics: The Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee has approved this study (CRE-2011.601). Year of approval: 2011.
Informed consent: Not applicable.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interest: The authors state no conflict of interest.
Research funding: None declared.
Data availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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