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Screening for primary aldosteronism using the newly developed IDS-iSYS® automated assay system

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ABSTRACT

Background: The recommended approach to screening for primary aldosteronism (PA) in at-risk populations is to determine the ratio of aldosterone concentration (serum (SAC)/plasma (PAC)) to renin measured in plasma as activity (PRA) or concentration (DRC). However, lack of assay standardisation mandates the need for method-specific decision thresholds and clinical validation in the local population.

Aim: The study objective was to establish method-specific aldosterone: renin ratio (ARR) cut-offs for PA in men and women using the IDS-iSYS® assay system (IDS plc).

Methods: A prospective cohort study design was used. PAC and DRC were measured immunochemically in ethylenediamine-tetraacetic acid (EDTA) plasma on the IDS-iSYS® instrument.

Results: A total of 437 subjects (218 men, 219 women) were recruited including: healthy normotensive volunteers (n=266) and women taking the oral contraceptive pill (OCP; n=15); patients with essential hypertension (EH; n=128); confirmed PA (n=16); adrenal cortical carcinoma (ACC; n=3); Addison's disease (AD; n=4) and phaeochromocytoma/paraganglioma (PPGL; n=5). In this population, an ARR cut-off at >37.4 pmol/mIU provided 100% diagnostic sensitivity, 96% specificity and positive likelihood ratio for PA of 23:1. When the ARR decision threshold was stratified according to gender, a cut-off of >26.1 pmol/mIU in men and >113.6 pmol/mIU in women resulted in diagnostic sensitivity and specificity of 100%.

Conclusion: This study demonstrates that decision thresholds for PA should not only be method-specific but also gender-specific. However, given the small number of PA patients (n=16), particularly women (n=4), further validation through a prospective study with a larger PA cohort is required before the thresholds presented here could be recommended for routine clinical use.

1. Introduction

Primary aldosteronism (PA), first described by Jerome Conn [1], is a group of adrenal disorders in which aldosterone production

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is inappropriately high for sodium status, relatively independent of angiotensin II stimulation and nonsuppressible by sodium loading [2]. Prevalence rates in hypertensive populations vary from 3% to 32% [3–6]. Patients with PA have higher cardiovascular morbidity and mortality than age-, gender- and blood pressure- (BP) matched subjects with essential hypertension [7,8]. Timely identification is likely to lead to better outcome as specific management has been shown in observational studies to improve the impact of this condition on key patient outcomes [7,9,10]. The recommended approach to screening for PA in at-risk populations is to determine the aldosterone-to-renin ratio (ARR)[2,11]. The ARR is calculated from the concentration of aldosterone in serum (serum aldosterone concentration, SAC) or plasma (plasma aldosterone concentration, PAC) divided by plasma renin measured either as renin activity (PRA) or direct renin concentration (DRC)[11]. However, measurement of aldosterone and renin is analytically challenging, with several method combinations for both analytes in routine clinical use. This situation is further compounded by the lack of assay standardisation and the use of different reporting units for both aldosterone (ng/dL, ng/L, pg/mL and pmol/L) and renin (PRA: ng/mL/h, nmol/L/h and pmol/L/min; DRC: μIU/mL, mIU/L and ng/L). This underlies the need for method-specific decision thresholds and clinical validation in the local population.

The recent Endocrine Society Clinical Practice Guideline (ESCPG) for the management of PA provides assay-dependent ARR decision threshold values reflecting the use of renin activity and concentration, and expressed using different reporting units [2]. Notably, this table is unchanged from that previously published in 2008 [11]. From a clinical perspective this is challenging, as significant methodological improvements have occurred in the intervening eight years. There is no reference to clinical validation studies using these newer assays [12,13]. Moreover, it is often not appreciated that indiscriminate adoption of the ESCPG cut-offs has the potential to incorrectly classify patients [14].

Using the newly developed *Immunodiagnostics Systems Speciality Immunoassay Automated System* (IDS-iSYS® system; IDS plc, Boldon, UK) for aldosterone and renin measurement, we demonstrated that reference intervals for aldosterone, renin and the ARR are gender-specific [15]. The finding of significant differences between genders is an important consideration in relation to how these reference intervals are applied in the stratification of patients with refractory hypertension and optimisation of therapeutic management of patients with hypertension. The objective of this study was to establish method-specific ARR cut-offs for PA in men and women using the IDS-iSYS® assay system.

2. Methods

Research ethics approval for this collaborative study was obtained in accordance with the Declaration of Helsinki and was granted by each Institution's Clinical Research Ethics Committees prior to commencing patient recruitment.

2.1. Study design

2.1.1. Patient cohorts

A prospective cohort study design was conducted at the Centre for Endocrinology, Diabetes and Metabolism at Galway University Hospital (GUH) between December 2014 and September 2015. Redundant ethylene diamine-tetracetic acid (EDTA) plasma from patients presenting to GUH with hypertension (HTN; n=128) or an adrenal mass/pathology (n=28) and with an ARR requested was utilised. Study subjects were investigated according to routine standard medical/diagnostic care [2,16–18]. Clinical details were recorded on a standardised data collection form following chart review and interrogation of the electronic radiology and laboratory information systems.

The inclusion criteria were: age ≥18years, non-pregnant and either exclusion or confirmation of PA by standard criteria that were necessarily independent of the biochemical tests being evaluated (specifically, the aldosterone response to the Saline Infusion Test (SIT) or an established alternative diagnosis).

Those with specific diagnoses were included based on the following criteria:

PA: diagnosis confirmed by pathological SIT i.e., PAC >140 pmol/L post the infusion of 2 L of normal saline (0.9% NaCl) over 4 h [12]: Phaeochromocytoma/Paraganglioma (PPGL)/Adrenal Cortical Carcinoma (ACC): diagnosis confirmed histologically; Addison's disease (AD): confirmed by response to short synacthen test i.e., 30 min post synacthen cortisol <430 nmol/L (Method: Cobas® Cortisol assay [Roche Diagnostics, Basel, Switzerland); Treated essential hypertension (EH): Type 2 Diabetes Mellitus (T2DM) with haemoglobin A_{1C} ≤75 mmol/mol on a minimum of 2 anti-HTN agents excluding β-blockers; Treatment-naïve EH: non-diabetic with normal electrolytes and kidney function (Modification of Diet in Renal Disease Study [MDRD] equation eGFR >60 mL/min/1.73 m^2). Not all patients in the treated EH or treatment-naïve EH groups had a SIT to definitively exclude PA. The decision not to perform the SIT was based on the initial clinical presentation, the degree of hypertension and the number of antihypertensive medications required to control the hypertension.

The exclusion criteria were: insufficient sample volume (<500 μL) or gross haemolysis/ lipaemia.

2.1.2. Healthy volunteers

Data for ARR from 266 participants recruited from the local population with the objective of establishing reference intervals for PAC, DRC and the ARR and previously published were utilised in this study [15]. In brief, the inclusion criteria for healthy volunteers were; age ≥18years, BMI ≤30 kg/m^2, non-pregnant, BP <140/90 mm Hg, normal electrolytes and kidney function (MDRD equation eGFR≥60 mL/min/1.73 m^2), non-smoker, Irish Caucasian, and not taking prescribed/Over The Counter (OTC) medications for a minimum of 3 months. In female participants of reproductive age, no record of the stage of the menstrual cycle was taken at the time of sample collection.

2.1.3. Healthy female volunteers taking the oral contraceptive pill (OCP)

A total of 15 healthy normotensive female volunteers were recruited from the local population. The inclusion criteria were identical to that of the healthy volunteers except that these women had been taking the OCP for a minimum of 3 months.

2.2. Analytical methods

DRC and PAC were measured in EDTA plasma using the IDS/iSYS® Immunochemiluminometric assay (ICMA) platform. The DRC assay is a sandwich ICMA employing two monoclonal antibodies, a magnetic particle solid-phase capture antibody and an acridinium-labelled tag antibody. Concentration of renin is directly proportional to light (expressed in relative light units) emitted by the acridinium label and measured by the system luminometer. The DRC assay is calibrated to the WHO International Standard 68/356. The reportable range is 1.8 mIU/L to 550 mIU/L. The inter-assay imprecision expressed as coefficients of variation (CV_A%), at mean DRCs of 14 mIU/L, 100.3 mIU/L and 390.2 mIU/L were 7.7%, 8.4% and 4.9%, respectively [15].

The PAC assay is a competitive one-site ICMA that uses a biotinylated monoclonal antibody bound to streptavidin-coated magnetic particles. Acridinium-labelled aldosterone competes with sample aldosterone for the limited amount of biotinylated antibody. Concentration of aldosterone is inversely proportional to light emitted by the acridinium label and measured by the system luminometer. The IDS-iSYS® PAC assay is referenced to liquid chromatography-tandem mass spectrometry (LC-MS/MS). The reportable range is 102 pmol/L to 3656 pmol/L. The between-run CV_As at mean aldosterone concentrations of 238 pmol/L, 442 pmol/L and 1648 pmol/L were 9.71%, 9.37% and 3.83%, respectively [15]. Results from each assay were used to calculate the ARR as follows: Aldosterone in pmol/L divided by the DRC in mIU/L to give the ARR in pmol/mIU.

2.3. Laboratory sampling protocol for ARR

Routinely, biochemical testing for the ARR was carried out in ambulatory subjects with an unrestricted salt intake. Subjects were required to attend clinic for phlebotomy between 07:00–12:00. All subjects were seated for 10–15 min prior to having whole blood (10 mL) drawn and collected into appropriate specimen tubes (Becton Dickinson/Sarstedt plastic evacuated tubes containing EDTA anticoagulant; Becton-Dickinson, Franklin Lakes, NJ, USA; Sarstedt, Numbrecht, Germany) for the measurement of PAC/DRC. Specimen tubes were kept at room temperature (RT) and transported within 30 min of blood draw to the laboratory for immediate processing (centrifugation, separation and freezing of plasma). EDTA plasma was stored in a temperature-controlled freezer at −20° Celsius prior to batch analysis on the IDS-iSYS® instrument.

It is mandatory for clinical and medication details to be supplied on the laboratory request form. Prior to testing, the correction of pre-existing hypokalaemia and where medically safe, the withdrawal of any agents that markedly interfere with the renin-angiotensinogen-aldosterone system (RAAS) for a minimum of 6 weeks, i.e. renin inhibitors, mineralocorticoid receptor antagonists and amiloride is recommended [19]. Further, as β-blocker therapy is associated with a significant risk of false-positive ARR results their withdrawal for 2 weeks prior to measurement of ARR using DRC [20] is recommended. Angiotensin converting enzyme inhibitors and angiotensin II type 1 receptor blockers have the potential to cause false-negative ARR screens as these drugs lower aldosterone and raise renin [21]. In such patients an undetectable renin makes PA highly likely. By contrast, should the renin be detectable or the ARR low the diagnosis cannot be excluded [19]. In such instances ARR testing should be repeated having substituted blood pressure lowering medications with agents that negligibly interfere with the RAAS. Drugs that can be used as an alternative to control hypertension, and that minimally interfere with ARR include verapamil, doxazosin, prazosin hydrochloride and hydralazine.

2.4. Statistical analysis

Statistical analysis was performed using R (V3.2.0 R Foundation for Statistical Computing; accessible at www.r-project.org). Summary statistics for normally distributed continuous variables were given by mean (standard deviation) and frequencies (percentages) for categorical variables. Data that was not normally distributed was presented as median (range). Results below the limit of detection were set to each assay's respective analytical sensitivity (DRC=1.8 mIU/L and PAC=102 pmol/L). Median values for DRC, PAC and ARR between the study cohorts were compared using Mann-Whitney nonparametric one-way analysis of variance with Bonferroni correction for multiple comparisons post-test. A p-value <0.05 was deemed to be statistically significant.

The clinical utility of the IDS-iSYS® PAC, DRC and ARR in the study cohort was assessed using Receiver Operator Characteristics (ROC) curve analysis. The PAC, DRC and ARR values from those in whom PA was confirmed were used in the ROC curve analysis comparison with participants without PA to define the optimum IDS-iSYS® decision thresholds for each test. For each analyte, a ROC curve was constructed from the relationship between true-positive and false-positive results (i.e., sensitivity versus 1-specificity) at different criterion values. The Area Under the Curve (AUC) of each test was calculated as it is the summary measure of the diagnostic utility and is independent of reference limits. The AUC can be interpreted as the probability that a randomly selected individual from the positive group has a test result indicating greater suspicion than that for a randomly chosen individual from the negative group. The optimum AUC equals 1.0; in this instance the curve will reach the upper left corner of the plot. When the variable under study cannot distinguish between those with and without disease the AUC will be equal to 0.5 (the ROC curve will coincide with the diagonal).
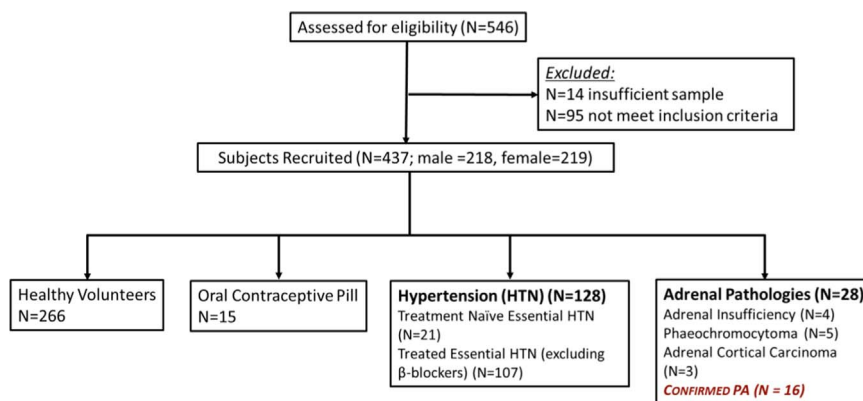
Fig. 1. Recruitment Schematic.

3. Results

A total of 546 participants from the local population were assessed for inclusion in this study (Fig. 1). 109 subjects were excluded (95 did not meet the inclusion criteria and a further 14 due to insufficient sample volume (<500 μL) to perform the analysis of both PAC and DRC). In total, 437 subjects (218 men, 219 women) were recruited including: healthy normotensive volunteers (n=266) and women taking the oral contraceptive pill (OCP: n=15); treatment-naïve patients with essential hypertension (EH; n=21); patients with EH and type 2 diabetes chronically treated with antihypertensive medications excluding β-blockers (EH+T2DM; n=107); confirmed PA (n=16); adrenal cortical carcinoma (ACC; n=3); Addison's disease (AD; n=4) and phaeochromocytoma/ paraganglioma (PPGL; n=5). Baseline clinical and biochemical parameters are outlined in Table 1.

Integrated comparison of sensitivity and specificity using ROC curves showed the diagnostic power of the ARR to be superior to either PAC or DRC (Fig. 2). ROC analysis for DRC provided an AUC of 0.97. The best DRC decision threshold for predicting PA in our study cohort was ≤14.2 mIU/L giving a diagnostic sensitivity and specificity of 100% and 84%, respectively and positive likelihood ratio of 6.2:1. When stratified according to gender, a cut-off of ≤4.5 mIU/L in women and ≤14.2 mIU/L in men resulted in a diagnostic sensitivity of 100% in both sexes and specificity of 98% and 89%, respectively.

In all subjects, a cut-off value for PAC >239 pmol/L resulted in a diagnostic sensitivity and specificity of 100% and 44%, respectively and positive likelihood ratio for PA of 1.8:1. In men, this cut-off resulted in a moderate improvement in the probability of PA to 2.0:1 (diagnostic sensitivity 100% and specificity 51%). In women, a cut-off for PAC of >602 pmol/L resulted in a likelihood ratio for PA of 7.7:1 (diagnostic sensitivity and specificity of 100% and 87%, respectively).

ROC curve analysis performed on the total cohort determined that the ARR provided the highest diagnostic efficiency for PA with an AUC of 0.99. An ARR cut-off in males and females of >37.4 pmol/mIU showed a diagnostic sensitivity and specificity of 100% and 96% respectively and positive likelihood ratio for PA of 23:1 (Fig. 2). The significance of the decision thresholds for PAC, DRC and ARR between patients with PA, EH, non-PA adrenal pathologies and healthy volunteers is detailed in Table 2.

When the ARR was stratified according to gender this provided the optimum AUC (1.0) for both men and women. A cut-off of >26.1 pmol/mIU in men and >113.6 pmol/mIU in women resulted in diagnostic sensitivity and specificity of 100%. Combining the PAC and ARR decision thresholds pertaining to the total cohort or according to gender did not improve diagnostic accuracy for PA. An ARR of <26 pmol/mIU made the diagnosis of PA highly unlikely (Fig. 3).

The ARR in healthy normotensive women (n=15) taking the OCP is shown in Fig. 4. The ARR in 2 subjects was found to be above the reference interval for women set at <64 pmol/mIU [15] and below the newly established decision threshold for women for the case detection of PA set at >113.6 pmol/mIU.

Table 1
Baseline clinical and biochemical parameters of the study cohorts.

	PA	EH+T2DM	EH	ACC; AD; PPGL	OCP	Healthy volunteers
Total number (n)	16	107	21	12	15	266
Men (%)	12 (75)	69 (64)	11 (52)	4 (33.3)	0 (0)	122 (46)
Age (years)[*]	55(26–76)	67(53–70)	54(33–72)	52(18–70)	32(23–44)	35(18–65)
Sodium (mmol/L)[*]	142 (140–147)	139 (132–145)	140 (135–143)	140 (138–143)	140 (136–144)	140 (135–146)
Potassium (mmol/L)[*]	3.7 (2.6–4.6)	4.5 (3.7–5.6)	4.3 (3.5–4.7)	4.2 (3.9–4.8)	4.0 (3.7–4.4)	4.2 (3.5–5.1)

Median (range)*.
PA=primary aldosteronism; EH=essential hypertension; T2DM=type 2 diabetes mellitus; ACC=adrenal cortical carcimona; AD=Addison's disease; PPGL=phaeo-chromocytoma/paraganglioma; OCP=oral contraceptive pill.
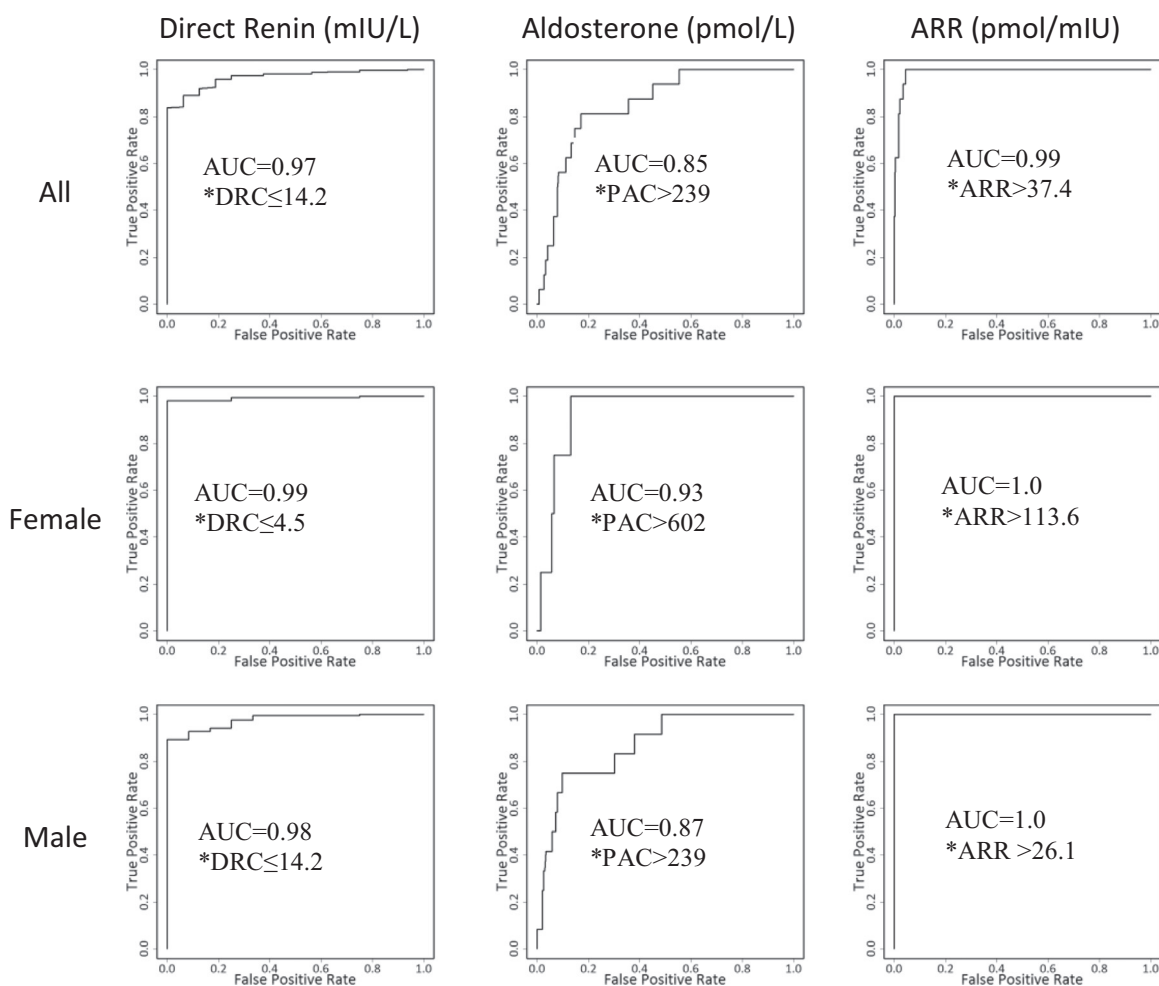Non-PA adrenal pathologies: ACC; AD; PPGL.

Fig. 2. Receiver Operator Characteristic (ROC) curves for direct renin concentration (DRC) (mIU/L), plasma aldosterone concentration (PAC) (pmol/L) & aldosterone:renin ratio ARR (pmol/mIU) in the diagnosis of primary aldosteronism for male and female participants both individually and together. AUC: area under the curve (optimum=1.0 the curve will reach the upper left corner of the plot); when the variable under study cannot distinguish between those with and without disease the AUC will be equal to 0.5 (the ROC curve will coincide with the diagonal). *=cut-off point.

4. Discussion

PA is the most frequent cause of secondary hypertension worldwide [21,22]. Early detection has the potential to prevent or attenuate cardiovascular complications if treated appropriately [7,9,23]. Case detection necessitates the use of validated and cost-effective screening protocols [2,24]. In 1976, Dunn and Espiner [25] first suggested the ARR as a potentially useful screening test for PA. Five years later Hiramatsu et al. [26] proposed it as the screening test of choice for PA. Currently, the ARR is the recommended approach for case detection of PA [2,11] in at-risk populations [3,23]. This is despite the ARR being a highly variable test, with diagnostic sensitivity ranging from 64% to 100% and specificity from 87% to 100%. Variability of the ARR can be attributed to the high degree of within-subject variation, differences in laboratory assays, reporting units, [27,28] pretesting patient preparation protocols [3], the influence of medication [19,20,29,30] and the population characteristics used to establish the decision thresholds [31]. Probably, the most important consideration regarding the interpretation of the ARR is the establishment of clinically useful thresholds [32]. We previously established gender-specific reference intervals for PAC, DRC and ARR using the IDS-iSYS® system for plasma aldosterone and renin [15]. In the present study, we evaluated the diagnostic performance of these tests for PA in males and females. Evaluating diagnostic tests in a group of patients already known to have the disease and in a group of healthy volunteers can lead to overestimation of diagnostic accuracy [33,34]. Hence, we assessed the clinical utility of the IDS-iSYS® PAC, DRC and ARR for PA in a population that included patients in whom it was reasonable to suspect PA (i.e., patients with hypertension and various adrenal pathologies).

In accordance with the literature, ROC curve analysis determined the ARR to be more sensitive and specific than screening by either determination (renin or aldosterone) considered singly [26,35,36]. In our population, the ARR cut-off in males and females of >37.4 pmol/mIU provided a diagnostic sensitivity and specificity of 100% and 96%, respectively with a positive likelihood ratio for PA of 23:1. This ARR threshold value for PA is consistent with that of Manolopoulou et al. [12], using these same assays in healthy

Table 2

Matrix table showing DRC, PAC & ARR in patients with PA, EH, non-PA adrenal pathologies (AD; PPGL; ACC) & healthy volunteers. Abbreviations as Figure 2 and Table 1.

Study cohorts	Test	PA (n=16)	EH+T2DM (n=107)	EH (n=21)	OCP (n=15)	ACC; AD; PPGL. (n=12)	Healthy volunteers (n=266)
PA	DRC	6.3 (1.8–14.2)					
	PAC	637 (241–2000)					
	ARR	86 (37.7–1111)					
EH+T2DM	DRC	<0.001	122.8 (6.5–550)				
	PAC	<0.001	210 (102–877)				
	ARR	<0.001	2.1 (0.2–22.3)				
EH	DRC	<0.001	<0.001	12.5 (4.3–61.2)			
	PAC	<0.001	0.448	177(102–341)			
	ARR	<0.001	<0.001	11.3 (3.8–57.8)			
OCP	DRC	<0.001	<0.001	0.092	26.5 (6.2–96.7)		
	PAC	0.423	<0.001	0.005	490 (113.6–3091)		
	ARR	<0.001	<0.001	0.025	18.3 (3.0–80.9)		
ACC; AD; PPGL	DRC	<0.001	0.009	0.002	0.167	32.7 (7.8–550)	
	PAC	<0.001	0.011	0.041	0.001	106 (102–701)	
	ARR	<0.001	0.009	0.056	0.003	6.1 (0–16.8)	
Healthy volunteers	DRC	<0.001	<0.001	<0.001	0.437	0.18	27 (3.5–113.2)
	PAC	<0.001	<0.001	<0.001	0.054	<0.001	293 (102–2063)
	ARR	<0.001	<0.001	0.64	0.004	0.036	12 (0.9–113.6)

Median (range) along diagonal; p-values below diagonal; DRC mIU/L; PAC pmol/L; ARR pmol/mIU.

controls (n=147), essential hypertensives (n=152) and larger numbers of PA patients (n=93). These authors determined that an ARR at 31 pmol/mIU discriminated between EH and PA subjects with a diagnostic sensitivity and specificity of 98.9% and 78.9%, respectively [12]. Moreover, our study confirms the previous clinical validation of considerably lower ARR cut-off values with these newly developed immunoassays [12] to that currently recommended by the ESCPG [2]. The import being the risk of false negative classification if laboratories using these assays apply the proposed ESPCG cut-off of 103 pmol/mIU [14].

Perhaps most useful to clinicians in clinical practice is the finding that in our population an ARR of <26 pmol/mIU irrespective of gender, makes the diagnosis of PA highly unlikely (Fig. 3). In men and women, the DRC ROC curve analyses (AUC=0.97) demonstrated the high predictive power of this biochemical marker to select out those patients without PA. A cut-off for DRC ≤14.2 mIU/L resulted in a diagnostic sensitivity and specificity of 100% and 84%, respectively and likelihood ratio of 6.2:1. In females, a cut-off for PAC of >602 pmol/L resulted in a likelihood ratio for PA of 7.7:1 (diagnostic sensitivity and specificity of 100% and 87%, respectively). Notably, stratification of the ARR according to gender resulted in perfect discrimination (optimum AUC of 1.0) and improved diagnostic accuracy at decision thresholds for men: >26.1 pmol/mIU and women: >113.6 pmol/mIU resulting in diagnostic sensitivity and specificity of 100%. Despite the correct characterization of all our patients with PA (n=16) we do acknowledge that the numbers are relatively small and that only 4 of these 16 patients were women. Hence, before the thresholds presented here could be recommended for implementation into routine clinical use further validation through prospective study with a larger PA cohort is required.

Of interest, in our study two healthy normotensive women taking the OCP had an ARR above the reference interval for women set at <64 pmol/mIU [15], and below the newly established PA decision threshold for women set at >113.6 pmol/mIU (Fig. 4). Both were taking an OCP preparation containing drospirenone/ethinylestradiol. Drospirenone (a progestogenic agent), like endogenous progesterone, acts as a potent mineralocorticoid receptor antagonist [37]. This is consistent with the findings of Pizzolo et al. [38], who previously reported an elevated ARR in a 34 year old normotensive woman also taking a combined preparation of drospirenone and ethinylestradiol and tested on day 28 of her cycle with renin measured as concentration. The ARR in the latter woman normalised when the OCP was withdrawn. False-positive ARRs have been found in normal women tested during the luteal phase of their menstrual cycle when measurement of renin concentration is used. [38,39] This serves to illustrate the importance of understanding the clinical and biochemical context of the tests being interpreted and in particular the influence of medications. We suggest that, when using DRC to calculate the ARR, ovulating females should be screened during the follicular phase of the menstrual cycle. Moreover, the ARR must be interpreted with caution in individuals taking the OCP or hormone replacement therapy. This necessitates evaluation of the pharmacological content of the medication, mode of administration, and the method of measurement of renin prior to screening [3,30,40,41]. Cessation of oral contraceptives (minimum period of 6 weeks) in advance of further screening should only be considered when other effective measures can be taken to avoid an unwanted pregnancy [41].

There is overlap in the ARR between individuals with and without PA. The use of a single cut-point value for the ARR to determine whether a test is considered positive or negative should not exclude evaluation of the pre-test probability of PA, and the
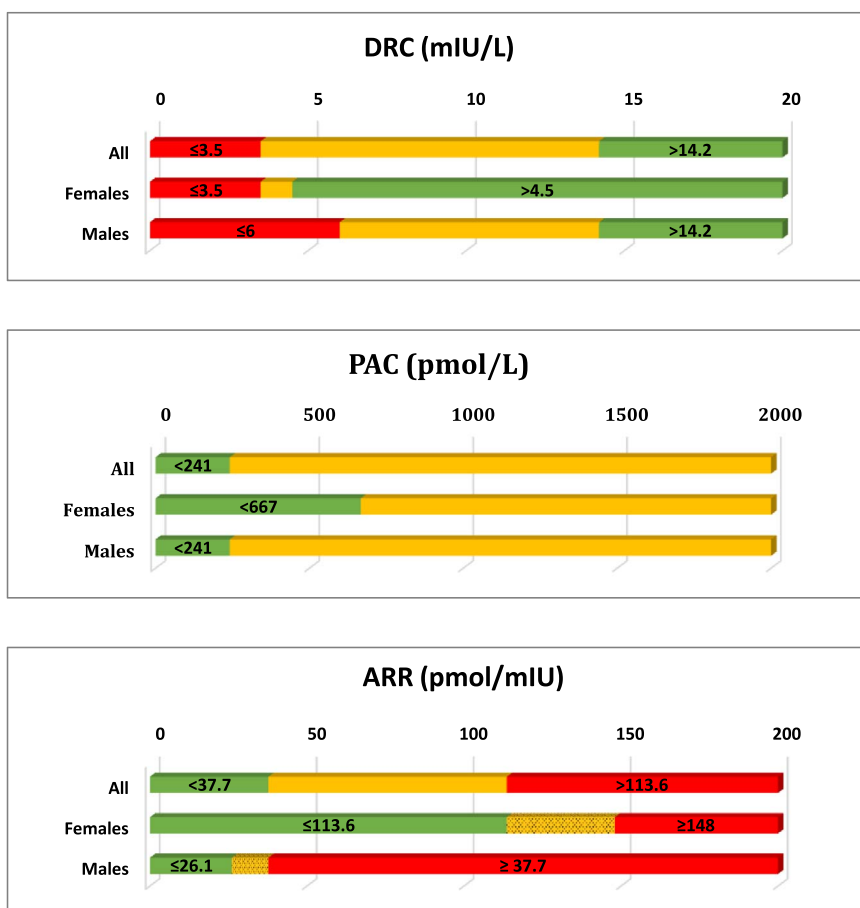
Fig. 3. Traffic Light plot: green illustrates the absolute values for DRC, PAC and ARR at which study participants in our cohort did not have PA (i.e. PA is highly unlikely), orange represents the absolute values for DRC, PAC and ARR at which some participants had and others did not have PA (i.e. PA indeterminate), orange with shading represents "No Data" and red represents the absolute values for DRC, PAC and ARR at which all participants had PA (i.e. PA highly likely). For example, for DRC (total cohort), all values >14.2 mIU/L are not PA, all values ≤3.5 mIU/L are PA, with values >3.5 mIU/L and ≤14.2 mIU/L indeterminate for PA. For ARR (total cohort), all values <37.7 pmol/mIU are not PA, all values ≥37.7 pmol/mIU and ≤113.6 pmol/mIU are indeterminate for PA, and all values >113.6 pmol/mIU are PA. The junction of the red and orange bar represents the value for the test at which it is 100% specific. The junction of the green and orange bar represents the value for the test at which it is 100% sensitive. Once validated in other cohorts, traffic light plots maybe a useful tool to aid in the interpretation of DRC, PAC and ARR. Abbreviations as Fig. 2.

independent assessment of PAC and DRC. It is essential when interpreting the ARR that physicians and laboratory specialists do so in the context of the clinical presentation (family history, the degree of hypertension, resistance to and response to antihypertensive medications, the presence of any electrolyte abnormalities and/or an adrenal adenoma) [27] with special attention paid to the analytical methods used, the influence of medications, posture, dietary salt intake and gender.

It is imperative to appreciate that the uncritical adoption of the ESCPG cut-offs may lead to patient misclassification [14]. Laboratories should only use cut-offs from the literature when using analytical methods that are identical to those of the published studies [27]. Further, literature-sourced cut-offs require validation in the local population. The lack of internationally accepted pre-testing patient preparation criteria, accepted standardised analytical methodologies, standard reference materials and assay reporting units obligates the continued use of method-specific reference intervals for PAC, DRC and the ARR [2,3,27].

It has previously been suggested that the ARR decision thresholds between the sexes are different [42–44]. The current study provides evidence of significant differences in the ARR between men and women. Should the findings presented here be prospectively validated using a larger cohort of PA patients this will have important implications for the case detection of PA. In young women, it will protect against the probability of inappropriately undergoing more complex, unnecessary and potentially harmful follow-up investigations, while in men, the use of method- and gender-specific decision values will attenuate the risk of missing a diagnosis of PA.

5. Conclusions

This study demonstrates that ARR decision thresholds for PA should not only be method-specific but gender-specific. However, given the small number of PA (n=16) patients, in particular females (n=4), further validation through prospective study with a larger
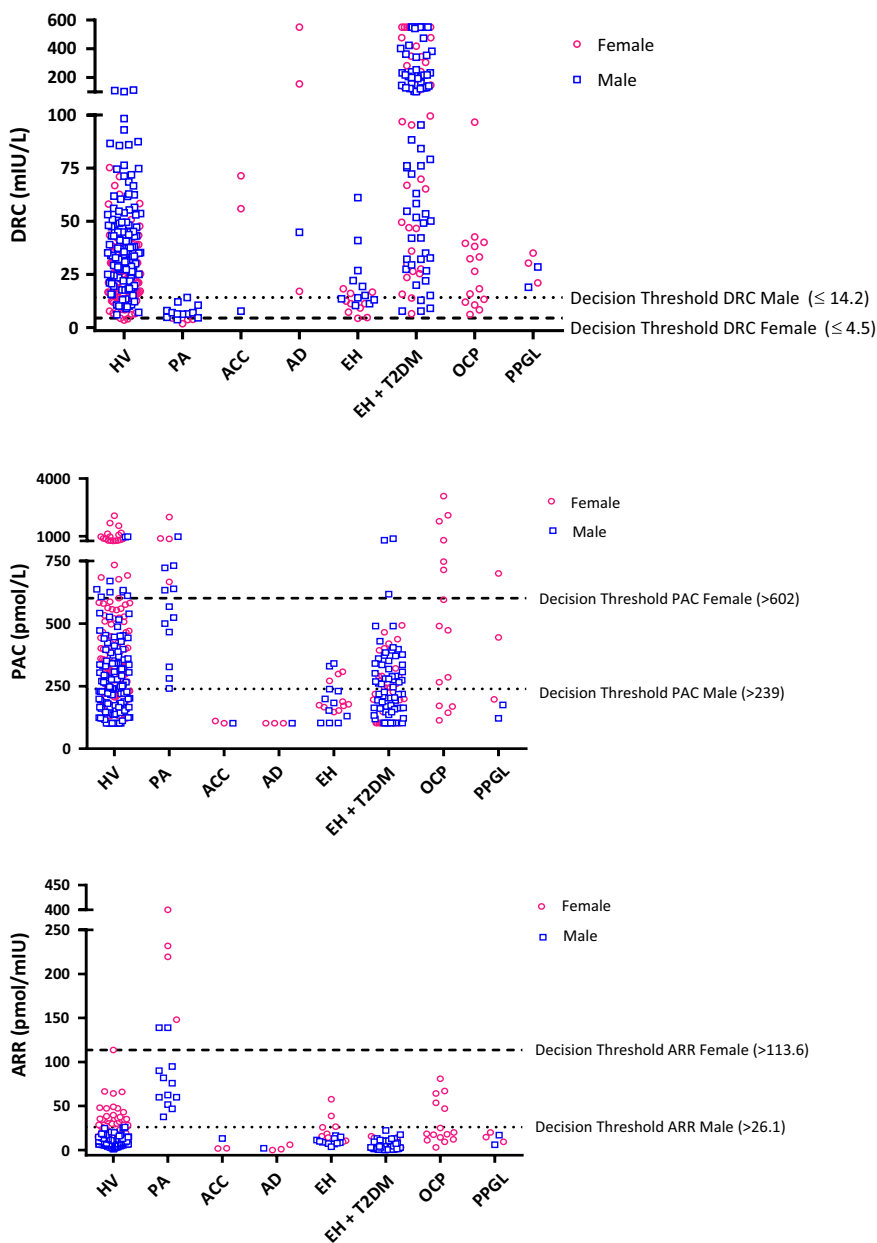
Fig. 4. IDS-iSYS® DRC, PAC and ARR values observed in primary aldosteronism (PA, n=16), healthy volunteers (HV, n=266), healthy females taking the oral contraceptive pill (OCP, n=15), patients with essential Hypertension (EH, n=21), EH+Type 2 diabetes mellitus (T2DM, n=107) and non-PA adrenal (adrenal cortical carcinoma, ACC; Addison's disease, AD; phaeochromocytoma & paraganglioma, PPGL) pathologies (n=12). Abbreviations DRC, PAC and ARR as Fig. 2.

PA cohort is required before the thresholds presented here could be recommended for routine clinical use. Notwithstanding, it suggests the validity of the recently established gender-specific reference intervals for the IDS-iSYS® ARR in our population [15]. Hence, we advocate that the ESCPG recommend the adaptation of method- and gender-specific reference intervals for PAC, DRC and the ARR. Furthermore, we advocate the inclusion of the ARR cut-off values established using these newly developed automated assays into future Endocrine Society guidelines for the diagnosis of PA.

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