UNIVERSITÀ DEGLI STUDI DI PADOVA

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SERIES XXXIV

NEurological Involvement in Primary Aldosteronism

NEPAL study

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For my father and brother

When I was writing first time my thesis, I never imagined that I will add here also my mother

For my best friend, real support, and for the true love of my life

Ээждээ хайртай шүү (translation from Mongolian I love you, mum)

CONTENTS

ACKNOWLEDGEMENTS

ABBREVIATIONS:

ABPM: ambulatory blood pressure measurement

AF: atrial fibrillation

ACTH: adrenocorticotropic hormone

AHI: apnea hypopnea index

AME: apparent mineralocorticoid excess

AP: antero-posterior

APA: aldosterone producing adenoma

ARAS: atherosclerotic renal artery stenosis

ARR: aldosterone-to-renin ratio

AVS: adrenal venous sampling

BAH: bilateral adrenal hyperplasia

BMI: body mass index

BP: blood pressure

CAC: coronary artery calcium

CCB: calcium channel blocker

CCT: captopril challenge test

CE: closed eye

CI: confidence interval

CKD: chronic kidney disease

CMAP: compound motor action potential

CNS: central nervous system

CoPv: center of pressure velocity

CT: computed tomography

CV: cardiovascular

DOR: diagnostic odds ratio

DRC: direct renin concentration

DT-EO: dual-task eye open

ENG: electroneurography

ESH: European Society of Hypertension

FMD: fibromuscular dysplasia

FST: fludrocortisone suppression test

FUT: furosemide upright test

HT: hypertension

HE-OE: Head extended eyes open

HMOD: hypertension mediated organ damage

iAME: iatrogenic apparent mineralocorticoid excess

iSHT: isolated systolic hypertension

LMICs: low middle income countries

MI: myocardial infarction

NLR: negative likelihood ratio

OE: open eye

OLT: oral sodium loading test

OSA: obstructive sleep apnea

PA: primary aldosteronism

PAC: plasma aldosterone concentration

PET: positron emission tomography

PH: primary hypertension

PNS: peripheral nervous system

PPGL: pheochromocytoma and paraganglioma

RAAS: renin angiotensin aldosterone system

RI: resistivity index

RIA: radioimmunoassay

ROC curve: receiving operation characteristic curve

SA: sway area

sAUC: summary area under the ROC curve

SBI: silent brain infarction

SIT: saline infusion test

SNAP: sensory nerve action potential

STARD: Standards for Reporting Diagnostic Accuracy

TSH: thyroid stimulating hormone

SBP: systolic blood pressure

uPA: unilateral PA

WML: white matter lesion

YI: Youden index

1. ABSTRACT

Hypertension (HT) is a leading cause of death worldwide, and it is classified as primary (or essential), when no specific cause is identified, or secondary, when it is due to an identified cause and, therefore, can be resolved by removing the underlying cause. Clinical studies suggest that the prevalence of essential hypertension accounts for 90-95% of adult cases, and secondary hypertension (SH) accounts for 2-10% of cases, accumulating evidence have demonstrated that if SH is systematically sought for, its prevalence is much higher, involving a proportion of the hypertensive patients that ranges from about 35% in general to higher rates in those with drug-resistant hypertension. Of note, primary aldosteronism (PA) was considered a rare cause of SH, but recently its prevalence is estimated to be 5% to 10% among all persons with hypertension. PA is the most common curable form of HT and is associated with an excess rate of hypertension-mediated organ damage (HMOD) and cardiovascular (CV) complications as compared to primary hypertension with a similar degree of blood pressure (BP). To date, only a small fraction of patients is screened for PA, probably because screening for PA requires more time, and sources, than are available to many primary care physicians and other non hypertension specialists. Patients with PA present with HT with classical hypokalemia, and may present hypokalemia-associated symptoms like muscle weakness, and paresthesia, identically to patients with hypokalemic periodic palsy who develop shortness of motor action potential amplitude of the peripheral nerve.

Thus, in chapter 2 we aimed to determine the prevalence of secondary hypertension in patients referred to our specialized center for hypertension. In chapter 3 we assessed postural balance, and sensory nerve conduction of lower limbs in PA patients to clarify if there are postural balance abnormalities due to peripheral sensory nerve conduction changes before and after MR antagonist and/or surgical cure.

In chapter 4, we assessed the accuracy of exclusion tests in the work-up of PA using the diagnosis of unilateral PA as a reference.

In chapter 5, the screening of PA among the Mongolian population was never done before. We aimed to investigate the prevalence of PA in the next year in Ulaanbaatar, Mongolia using a predefined diagnostic workup.

A total of 462 patients were retrospectively reviewed for the main cause of HT. Applying a predefined diagnostic workup to the cohort of consecutive hypertensive patients, we found primary hypertensive, secondary hypertensive, and patients with a pending final diagnosis with a prevalence of 24.3% 59.6%, and 16.4% respectively. The most common causes of secondary HT were PA, and obstructive sleep apnea.

A total of 4,242 patients were meta-analyzed for the accuracy of exclusion tests and pooled accuracy estimates (sAUC) showed no differences between the ARR (0.95, 95% CI: 0.92-0.98), the captopril challenge test (CCT) (0.92, 95% CI: 0.88-0.97), and the saline infusion test (SIT) (0.96, 95% CI: 0.94-0.99).

Further, PA patients in comparison to the same patients who were cured of PA were having poor postural sway, especially without vision control (p=0.002), moreover, these results associated with sural nerve amplitude and serum potassium level. Nonetheless, patients with PA had a reduction of sural nerve amplitude comparable to patients with diabetic polyneuropathy, meaning that PA patients might have sub-clinical neuropathy.

CONCLUSION:

Results of the current study show that secondary forms of hypertension are common in comparison to primary hypertension, highlighting the necessity for hypertensive patients to be screened in a more detailed way.

Exclusion of PA patients from further invasive subtyping, both the captopril challenge test and the saline infusion test showed high diagnostic accuracy, however, as neither test furnished a diagnostic gain over the aldosterone to renin ratio, no evidence supporting their systematic use in clinical practice could be found.

PA patients have higher postural sway in comparison to the same patients who were cured of PA under the gold diagnostic standard. Moreover, primary PA regardless of the treatment might have sub-clinical neuropathy.

2. CHAPTER 1. NEPAL STUDY GENERAL INTRODUCTION

2.1 Essential hypertension is underdiagnosed secondary hypertension

Hypertension (HT) is a major public health problem worldwide because it is the most important modifiable risk factor for cardiovascular (CV), cerebrovascular, and renal disorders. Accounting for 10.4 million deaths per year HT remains a leading cause of death globally(1,2). The awareness of HT varies, in American and European countries, people are aware of their condition at 70% and in contrast in Asian and African countries 40%(3). The cause of HT is multifactorial, however, it is classified as essential and secondary HT. The essential, primary, or idiopathic HT is defined as high blood pressure (BP) in which, secondary causes such as renovascular disease, chronic kidney disease (CKD), pheochromocytoma and paraganglioma (PPGL), primary aldosteronism (PA), or other causes of secondary hypertension (SH) are not present and its prevalence accounts for 95% of all cases(4). SH is a form of HT that is due to an identified cause and, therefore, can be resolved by removing the underlying cause(5). However, the term Essential Hypertension (Essenktielle hypertonie) reflects an old and obsolete pathophysiological concept related to the need to maintain high-pressure regimen in the organs to allow an appropriate flow. And the term implies that primary hypertension (PH) is not a diagnosis, but rather the exclusion of a diagnosis (G.P.R. personal communication). The European Society Guideline recommends(6) screening of HT patients for secondary causes in the presence of suggestive symptoms and/or signs and, when those criteria are applied they might lead to underdiagnosing the SH forms. Moreover, when SH systematically looked for, its prevalence is much higher(7), and differs across studies ranging from 5 to 10%(8). In resistant hypertension (RH), SH prevalence rises up to 31.1%, and primary aldosteronism (PA) accounts for 65% cases(9). Moreover, prevailing causes for SH differ in different age groups. For an example in patients over 65 years atherosclerotic renal artery stenosis (ARAS), CKD, and hypothyroidism are the most common causes of SH, and for patients under 65 years PA, obstructive sleep apnea (OSA), Cushing syndrome, and pheochromocytoma and

paraganglioma (PPGL) the most frequent causes of high BP, even though, the prevalence rates may also differ by ethnicity(10). Identification of the underlining pathophysiological mechanism of HT is crucial for the target pharmacological treatment choice aimed at further cure or reduction of BP. PA is the most common form of SH. PA is associated with excess risk of cardiovascular (CV) complications and hypertension mediated organ damage (HMOD) in comparison to PH patients(11,12), timely diagnosed and treated PA allows to prevent CV related complications and HMOD.

2.2 Meta-Analysis of diagnostic gain exclusion tests over baseline aldosterone-renin ratio in diagnosing unilateral primary aldosteronism

In about half of the hypertensive patients on medications, the control of high BP values remains disappointing despite improvements in awareness and treatment in the last decades(13). A major reason for this poor high BP control is overlooking secondary hypertension(13,14), of which PA is the most common(15,16).

PA remains markedly underdiagnosed and undertreated owing to poor clinical awareness, with ensuing "under suspicion", unduly complex diagnostic work-up(17,18), limited availability of invasive investigations for localizing unilateral PA (uPA), constrained surgical capacity, and uncertainties about clinical outcomes(17).

The strategy for case detection of PA currently relies on the aldosterone-to-renin ratio (ARR) with the use of low cut-off values to maximize sensitivity(18,19). To exclude from further subtyping procedures guidelines recommend(17,20) exclusion tests including the captopril challenge test (CCT), the fludrocortisone suppression test (FST), the saline infusion test (SIT), and the oral sodium loading test (OLT), and the furosemide upright test (FUT). However, applying current

recommendations for patients with a high probability of PA we are at risk to assess patients with false-positive exclusion tests for invasive subtyping. Moreover, the negative predictive value of those tests exceeds their positive (confirmatory) predictive value(21) since they serve as "exclusion" tests. The validation of these tests, which increase complexity and costs of the diagnostic work-up, and involve the risk of overlooking the angiotensin II-responsive PA patients(22–24), was affected in many studies by a vicious circle type of bias, in that it relied on using another arbitrarily chosen exclusion test, as reference(25). In fact, only a few studies used an unambiguous diagnosis of unilateral primary aldosteronism (uPA) as a reference, and thus followed the Standards for Reporting Diagnostic Accuracy(26) (STARD) statement; furthermore, many preselected patients based on a positive ARR result, which, by increasing the rate of PA, led to overestimating specificity, and accuracy. Thus, we aimed this meta-analysis to provide diagnostic gain of baseline ARR over exclusion tests in diagnosing PA in the Chapter 3.

2.3 Postural balance and lower limbs nerve conduction in patients with unilateral primary aldosteronism

HMOD is defined as the structural or functional alteration of the arterial vasculature and/or the organs including the heart, kidneys, central and peripheral arteries, the retina, and the brain it supplies that are caused by high BP(1).

Adequate detection of HMOD can provide important therapeutic guidance for the management of hypertensive patients in terms of assessment of overall cardiovascular (CV) risk and preferential selection of treatment based on the impact of specific classes of drugs on HMOD.

Primary aldosteronism (PA) is the most common and curable form of hypertension. Aldosterone excess induces inflammation, oxidative stress, and endothelial dysfunction leading to arterial

stiffening which plays a fundamental role in the CV complication of PA(27). The prevalence of left ventricular hypertrophy, atrial fibrillation (AF), and myocardial infarction (MI) is higher in patients with PA than in BP- and age-matched patients with primary hypertension (PH)(28,29). Furthermore, after a median of 8.8 years from the diagnosis of hypertension, PA patients had an increased risk of heart failure, coronary artery disease (CAD), AF, and stroke(12). One important predictor of atherosclerotic CV disease events is coronary artery calcium (CAC). The Multi-Ethnic Study of Atherosclerosis found a positive association between high plasma aldosterone concentration (PAC) level and higher CAC score in the general population; moreover, this association was more prominent in subjects with suppressed renin phenotype, thus further corroborating the evidence for increased CV risk in PA patients, who by definition, show low plasma renin(30).

Thus, we aimed to conduct lower limb sensory nerves conduction with help of electroneurography (ENG) and postural balance using stabilometry force platform in patients with PA before and after treatment in the Chapter 4.

2.4 The prevalence of primary aldosteronism among the Mongolian population (PAMP study)

Projections estimate that three-quarters of the world's hypertensive population will reside in low- and middle-income countries (LMICs) within the next decades(31). The rate of HT in Mongolian population is 27%(32), moreover, the core cause of hemorrhagic stroke among this population is arterial hypertension. Even though, the mortality range from hemorrhagic stroke among the Mongolian population is 186 per 100.0 population, which is the highest in the world(33).

PA is the most common cause of SH. From the clinical standpoint, PA is classified into surgically curable and surgically not curable forms(27). Surgically curable forms represent APA, primary multinodular unilateral adrenocortical hyperplasia, ovary aldosterone secreting tumor, APA or bilateral adrenal hyperplasia (BAH) with concomitant pheochromocytoma and aldosterone-producing carcinoma. Surgically not curable forms include BAH, unilateral APA with BAH, familial hyperaldosteronism(27). Diagnosis of PA with further identification of aldosterone secreting side have great importance since adrenalectomy guided by AVS resolves or improves arterial hypertension(34).

Thus, we aimed because many patients with undetected PA develop stage III and/or drug resistant hypertension, leading to HMOD, cardiovascular sudden insufficiencies, and deaths, along with screening programs for PA were never conducted in Mongolia we aimed to investigate the prevalence of PA in adult population of Ulaanbaatar Mongolia.

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CHAPTER 2. ESSENTIAL HYPERTENSION IS UNDERDIAGNOSED SECONDARY

HYPERTENSION

3.1 INTRODUCTION

Secondary hypertension (SH) is a form of hypertension (HT) that is due to an identified cause, therefore, can be resolved by removing the underlying cause(1). Possible causes for SH comprises primary aldosteronism, renovascular disease, chronic kidney disease (CKD), pheochromocytoma and paraganglioma (PPGL), Cushing syndrome and hypothyroidism.

Recent guidelines recommend to screening certain categories of patients for possible secondary causes, such patients with increased blood pressure (BP) at young age, hypertension-mediated organ damage (HMOD), sudden worsening of BP control which previously was stable, and resistant HT(2). However, accumulating evidences indicates when causes for SH systematically searched show higher rates and the prevalence rate differs across centers especially for PA(3,4).

PA is the most common and curable form of SH. Patients with PA are at higher risk of cardiovascular complications and HMOD in comparison to PH patients with the same degree of BP level(5). The prevalence of PA ranges from 6% up to 20% depending on selected patients and referred centers(3,6). The final diagnosis of PA is established retrospectively when biochemical values are normalized after adrenalectomy, and beforehand patients are subtyped for the aldosterone excess lateralization using adrenal vein sampling (AVS). The most common subtype of PA is aldosterone-producing adenoma (APA) and bilateral adrenal hyperplasia is the third leading cause of PA(7). However, there are also genetically inherited forms of PA, and these forms of PA are named Familial hyperaldosteronism (FH), most of them due to germline mutations (8), important is FH-1 which is also named as a glucocorticoid remediable aldosteronism is treated by a low dose of dexamethasone.

Obstructive sleep apnea (OSA) is an independent risk factor for HT an cardiovascular (CV) diseases(9). Recent guidelines recommend screening for OSA patients with snoring, daytime sleepiness, and resistant HT. OSA affects more obese people with increased neck circumstance, and its prevalence ranges from 20% to 50%(10). Moreover, the documentation for continuous positive airway pressure on lowering BP in patients with hypertension has produced mixed results(10).

The prevalence of renovascular hypertension (RVH) differs among different age groups and sexes, however mainly it raises BP due to activation of renin-angiotensin-aldosterone system (RAAS) as a result of decreased renal perfusion induced by renal artery stenosis(1). In young aged women, RVH due to fibromuscular dysplasia and atherosclerotic renal artery stenosis (ARAS) is common in older adults. Its prevalence ranges between 1 to 8 %, and prevalence might be higher in the pre-selected population(11).

Cushing syndrome is a disorder that is characterized by excess levels of cortisol and leads to physical, mental, muscular, and cardiovascular complications. The incidence of Cushing syndrome is 0.2% to 5.0% per year, and nearly 80% of patients with Cushing syndrome are affected by high BP(1,12).

Apparent mineralocorticoid excess (AME) is a rare genetic disorder that is caused by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD) deficiency. Nevertheless, that excess, and chronic usage of licorice may induce iatrogenic apparent mineralocorticoid excess (iAME), which mimics AME. Clinical features include HT, polyuria, low renin, low aldosterone, and hypokalemia due to sodium and water retention. Mutations or inhibition of 11βHSD2 by licorice have been clearly shown to produce a congenital or acquired syndrome of mineralocorticoid excess, moreover studies in patients with essential hypertension showed a prolonged half-life of

cortisol and an increased ratio of urinary cortisol to cortisone metabolites, suggesting a deficient 11βHSD2 activity(13). Limited data concerning the prevalence of iAME, but timely diagnosed iAME with quick discontinuation of licorice leads to the full recovery of symptoms within 4 weeks(14).

Detection of SH forms is crucial since developing further right treatment strategy reduces BP levels, preventing for CV complications and HMOD, thus we aimed to investigate the prevalence of SH in consecutive newly-diagnosed hypertensive patients referred to our center following a predefined search strategy.

3.2 METHODS

Clinical and biochemical data of all consecutive hypertensive patients who referred to Hypertension Center of the University Hospital of Padua were retrospectively reviewed from January 1st 2017 till December 31st 2021.

Symptoms or signs suggestive of secondary forms of HT (Table 1), if present, were systematically recorded, and collected in the database. However, the diagnosis work-up was applied regardless of the presence of signs and/symptoms (Figure 1).

We retrospectively recruited the systematic biochemical work-up including measurements of plasma aldosterone concentration (PAC), direct renin concentration (DRC), renal function, catecholamines, plasma levels and urinary excretion of electrolytes. The patients were maintained on routine sodium intake for measurement of 24h urinary Na$^+$ and K$^+$ excretion. Before measuring DRC and PAC any drug interfering with renin angiotensin aldosterone system (RAAS) was withdrawn for at least 4 weeks, and replaced with calcium channel blocker (CCB) and/or to α-blocker. Hypokalemia was corrected with potassium supplements i.v. or orally. Patient was

instructed to maintain the supine position for at least 1.5 hours before blood collection, following the Practice Recommendations for Diagnosis and Treatment of the Most Common Forms of Secondary Hypertension with recommendation to switch antihypertensive drugs with proper wash-out period(1), moreover, as in our recent meta-analysis upright position did not showed any diagnostic gain over supine position(16.17). The diagnostic criteria, suggestive symptoms and diagnostic work-up for each causes of SH reported in Table 1.

Primary aldosteronism (PA) diagnosis established on recent guidelines(2,15), moreover regarding to the recent two meta-analysis(16,17) confirmatory tests were skipped. Obstructive sleep apnea (OSA) diagnosis made on polysomnography results with an apnea-hypopnea index (AHI) of >5 following recent guidelines(2,15). The of chronic kidney disease (CKD) established on KDIGO guideline referring to the eGFR <60 ml/min/1.73m² with albuminuria > for last 3 months. The following American heart association recommendations and European society guideline patients with more severe phenotype, heart failure, young onset of HT especially in women were screened renal vascular HT(2,10,15). Diagnostic criteria for renal vascular HT described in detail in Table 1. *Isolated systolic HT (iSHT)* defined as brachial systolic blood pressure ≥140 mmHg and diastolic BP is ≤90 mmHg. Cushing's syndrome, iatrogenic apparent mineralocorticoid excess (iAME), drug induced HT, and *white coat HT*.

The patients referred to the Centre, but with no persistent raise of BP values after the first visit were excluded from analysis.
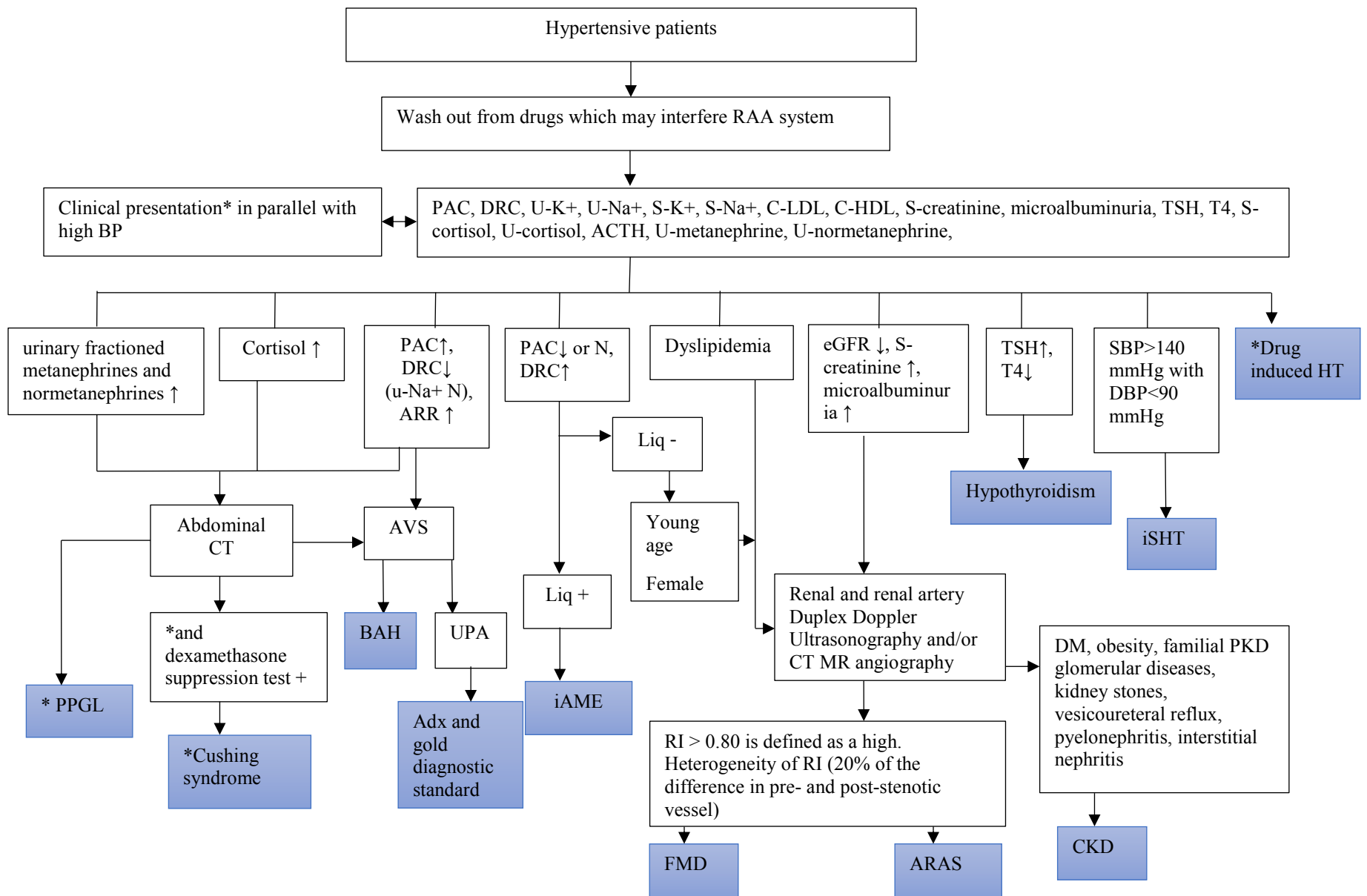
Figure 1. Diagnostic work-up of secondary hypertension. Abbreviations: ACTH, adrenocorticotropic hormone; Adx, adrenalectomy; ARAS; atherosclerotic renal artery stenosis, AVS, adrenal vein sampling; BAH, bilateral adrenal hyperplasia; BP, blood pressure; DRC, direct renin concentration; CKD, chronic kidney disease; CT, computed tomography; FMD, fibromuscular dysplasia; iAME, iatrogenic apparent mineralocorticoid excess; iSHT, isolated systolic hypertension; Liq, liquorice; RAA, renin angiotensin aldosterone; RI, resistivity index; TSH, thyroid stimulating hormone; PPGL; pheochromocytoma paraganglioma; UPA, unilateral primary

Essential, primary, or idiopathic HT was defined as high BP in absence of any causes of HT. BP measured on the brachial artery, in the middle of the tendon of the biceps in standing, and in supine position at first visit, and at follow-up. HT definition referred to the European Society Guideline(18).

The diagnosis was defined pending when patient did not complete the diagnostic work-up suggested at the first visit.

Only BP, and heart rate at the baseline were reported in the database; echocardiography and laboratory tests, if not available at first visit, were collected later. Left ventricular hypertrophy was defined as: Left ventricular mass index (LVMI) $\geq$115 g/m2 in male patients and $\geq$95 g/m2 in female patients. For the LVMI calculation we used following formula: LVMI = LVM (left ventricular mass)/body surface area. left ventricular mass $= 0.8\{1.04[([LVEDD + IVSd + PWd]^3 - LVEDD^3)]\} + 0.6$. Atrial fibrillation (AF) established after electrocardiography (ECG) confirmation, and classified as paroxysmal new-onset, persistent or long-standing, and permanent AF. History of HT arbitrarily separated in four classes 1 to 5 years, 6 to 10 years, 11 to 15 years and more than 15 years.

Divergences on the final diagnosis were resolved by consensus of senior investigators. Previous diagnosis of SH were accepted only if supported by documentation.

Table 1. Signs and symptoms suggestive of secondary hypertension

Condition	Suggestive signs/symptoms Risk factors	Work-up	Diagnostic criteria
Primary Aldosteronism (PA)	Hypokalemia Adrenal mass Adrenal incidentaloma	1. Proper pharmacological wash-out 2. Measurement of ARR 3. Abdominal CT/MRI 4. AVS for subtyping	ARR > 20 ng/dL/mIU* Gold† (PAC value of 2-9 ng/dL, serum potassium >3.8 mEq/L and DRC >6 mU/L with ARR <20 ng/dL considered as a gold reference of PA diagnosis) or Golden® diagnostic standard
Obstructive Sleep apnea (OSA)	Daily sleepiness Snoring with/without episodes of apnea	1. Epworth scale > 5 2. ABPM (non-dipping or reverse dipping) 3. PSNG results with attention on saturation	AHI > 5 Improvement of BP with same medications or with less antihypertensive medications with CPAP, or with sleeping position change
Isolated systolic hypertension	Age	1. ABPM 2. Repeated measurement of office blood pressure with proper interval	SBP>140 mmHg with DBP<90 mmHg by 24h ABPM and/or repeated office blood pressure measurement at least 3 times with 5 minutes of interval between measurements
Hypothyroidism	Bradycardia Fatigue Sleepiness	1. Thyroid hormones measurement 2.	Increase of TSH level (n.v. 0.20-4.0 mU/L), decrease of T4 (n.v. 9.00-22.00 pmol/L)
Iatrogenic apparent mineralocorticoid excess (iAME)	History of excess and chronic use of licorice	1. Serum K^+, PAC, 2. DRC measurement 3. Urinary cortisol level	Hypokalemia <3.5 mmol/L, low renin activity (n.v. 2.8-39.9 mIU/L), and low and/or normal plasma aldosterone concentration (n.v. 48.7-643 pmol/L)
Chronic kidney disease	Diabetes mellitus, obesity, familial polycystic kidney disease (PKD) glomerular diseases, kidney stones (with known history of obstruction), vesicoureteral reflux, pyelonephritis, interstitial nephritis	1. Abdominal ultrasound	eGFR < 60 ml/min/1.73m² and/or albuminuria which lasted at least 3 months
Drug-induced hypertension	Use of drugs that can raise BP		Use of: Anti-infective, anti-inflammatory, chemotherapeutic, illicit, immunosuppressive agents, psychiatric agents, sex hormones, steroids.

Atherosclerotic renal artery stenosis	Age, hypercholesterolemia		RI with normal value 0.50- 0.70 and RI > 0.80 defined as a high. Heterogeneity of RI (20% of difference in pre- and post-stenotic vessel), increase in peak systolic velocity in the renal artery (the post-stenotic threshold for significant RAS is 100 cm/sec to 200 cm/sec), a renal-to-aortic peak systolic velocity ratio of greater than 3.5 and turbulent post-stenotic site were additional signs of ARAS and FMD.
Fibromuscular dysplasia	Young age Female sex	Plasma renin activity Renal artery Duplex Doppler Ultrasonography and/or CT MR angiography	
Pheochromocytoma and paraganglioma	High rate of arrhythmias, diaphoresis	Abdominal CT/MRI Measurement of plasma free metanephrines or urinary fractioned metanephrines and normetanephrines within the proper medical washout	Increase of urinary fractioned metanephrines for 24h normetanephrines for 24h on top of CT positive results
Cushing's syndrome	Round face Purple striae Central obesity Skin ulcers Sexual dysfunctions	Serum cortisol, ACTH level measurement with dexamethasone suppression test Abdominal CT/MRI	Cortisol level> 5ug/dL on top of CT positive results

* If computer tomography results was negative or pending but DRC was decreased under normal or restricted sodium intake with proper pharmacological wash-out was considered as a PA; ®"golden" standard comprised excess aldosterone production, adrenal imaging and/or adrenal venous sampling; †"gold" standard comprised biochemical cure of PA; ABPM, ambulatory blood pressure measurement; ACTH, adrenocorticotropic hormone; AHI, apnea hypopnea index; ARAS, atherosclerotic renal artery stenosis; ARR, aldosterone to renin ratio; AVS, adrenal venous sampling; BP, blood pressure; CT, computed tomography; DBP, diastolic blood pressure; DRC, direct renin concentration; FMD, fibromuscular dysplasia; MRI, magnetic resonance imaging; RI, resistance index;

Statistical analysis

Normal distribution was assessed with Kolmogorov–Smirnov test, and quantitative variables that showed a skewed distribution underwent appropriate transformation to achieve a normal distribution, as required. One-way ANOVA followed by Bonferroni's posthoc test, or t test, were used to compare quantitative variables among/between groups. The distribution of categorical variables was compared by chi-square analysis.

3.3 RESULTS

3.3.1 Prevalence rates of secondary and primary hypertension

Total of 462 patients were retrospectively evaluated for the main cause of HT. A form of SH was found in 56% of patients; a pending diagnosis was assigned to 17%, thereby, appointing diagnosis of primary hypertension to 26.4%. Patients with final diagnosis were reviewed for further analysis (Table 2). No significant differences in age, sex or BMI between PH, and SH groups were found (Table 2). SBP levels in both supine and standing positions was higher in SH than in PH patients. Higher prevalence of AF was found in the SH group (p=0.03).

Two or more causes of high BP were found in 8.8% of patients with final diagnosis, with PA and OSA being the main overlapping forms of SH. Moreover, 9.2% of whole cohort of the patients had resistant hypertension (RH) by recent ESH guidelines(18), however, with no significant difference between groups (p=0.14).

Table 2. Clinical and biochemical characteristics of the patients with primary and secondary hypertension

Demographics	Primary HT patients (n=122)	Secondary HT patients (n=261)	p value
Age, years	60±16	62±15	ns
Sex female, n (%)	54 (44)	134 (51)	ns
BMI, kg/m²	26.2±3.6	27±4.6	ns
SBP, supine (mmHg)	148±19	159±23	<0.0001
DBP, supine (mmHg)	87±10	88±15	ns
SBP, standing (mmHg)	141±21	152±23	<0.0001
DBP, standing (mmHg)	91±13	80±14	ns
Resistant HT, n (%)	7 (5.7)	30 (11.5)	ns
S-K (mmol/L)	4.1±0.4	4.1±0.5	ns
S-Na (mmol/L)	140.2±3.1	140.2±3.2	ns
C-LDL (mmol/L)	110.9±34	107.4±30.9	ns
C-HDL (mmol/L)	55.6±20.3	52.8±14	ns
24h-U-Na$^+$ (mmol/day)	152±81	144±63	ns
LV hypertrophy, n (%)	27 (22.6)	80 (30.8)	ns
AF, n (%)	4 (3.17)	26 (10.2)	0.03

Mean ± SD, or median (range), as appropriate. AF, atrial fibrillation; BMI, Body Mass Index; DBP, diastolic blood pressure; HT, hypertension; LV, left ventricle;

3.3.2 Causes of secondary hypertension

After systematically applying the diagnostic algorithm we found that most frequent form of SH was PA with prevalence of 16%, followed by OSA and iSHT with prevalence of 11% and 9% respectively. In total 77 patients with PA, 21 (27.6%) received the diagnosis of unilateral primary aldosteronism (uPA) and, were cured after unilateral adrenalectomy.
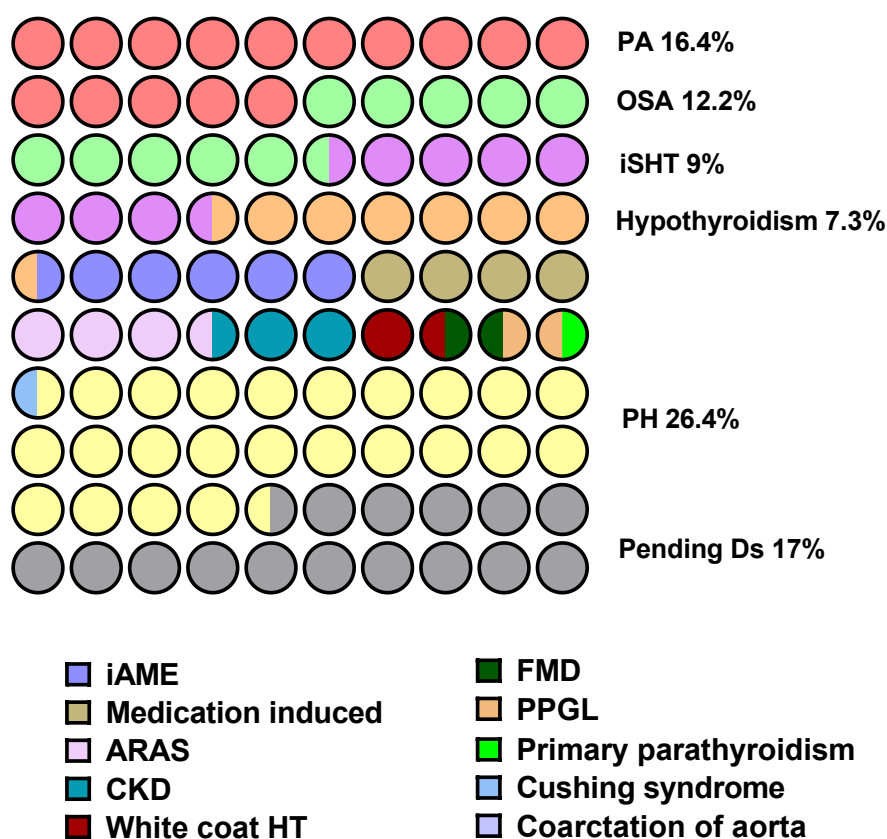
Figure 1. Prevalence of etiologic causes of hypertension. iatrogenic apparent mineralocorticoid excess 6%; medication induced hypertension 4%; atherosclerotic renal artery stenosis 4%; Chronic kidney disease 2.6%; White coat HT 1.2%; Fibromuscular dysplasia 1%; Pheochromocytoma and paraganglioma 1%; Primary parathyroidism 1%; Cushing syndrome 0.4%; Coarctation of aorta 0.2%; ARAS, atherosclerotic renal artery stenosis; CKD, Chronic kidney disease; FMD, Fibromuscular dysplasia; iAME, iatrogenic apparent mineralocorticoid excess; HT, hypertension; PPGL, Pheochromocytoma and paraganglioma;

Considering the most prevalent forms of HT, which included secondary forms PA, OSA, iSHT and PH, we found that the patients with iSHT were the oldest, and had highest SBP (Table 3). PA patients had lower level of serum potassium and sodium levels and, DBP than the others. OSA group showed higher BMI and lower C-HDL levels than other groups.

Table 3. Characteristics of the patients with most prevalent causes of secondary hypertension, and primary hypertension

Demographics	PA (n=76)	OSA (n=50)	iSHT (n=42)	PH (n=113)	p value
Age, years	57.2±13.5	60.4±13.9	76±11.6	59.8±15.8	PH vs iSHT 0.0001
Sex, female, n (%)	34 (44.7)	15 (30)	14 (33)	54 (47.8)	ns
BMI, kg/m²	27.1±4.5	29.7±4.8	25.2±3.4	26.2±3.6	PH vs OSA 0.0002 PA vs OSA 0.03 PA vs iSHT 0.04 OSA vs iSHT 0.0002
SBP, supine (mmHg)	161±24	158±24	166±23	148±19	PH vs PA 0.001 PH vs iSHT <0.0001
DBP, supine (mmHg)	93±13	88±15	77±12	87±10	PH vs PA 0.01 PH vs iSHT 0.0005 PA vs iSHT <0.0001 OSA vs iSHT 0.001
SBP, standing (mmHg)	152±23	151±23	156±24	141±21	PH vs PA 0.009 PH vs iSHT 0.001
DBP, standing (mmHg)	96±13	91±14	79±13	91±13	PH vs PA 0.008 PH vs iSHT 0.002
Resistant HT, n (%)	11 (14.5)	5 (10)	4 (9.5)	9 (7.9)	ns
S-K (mmol/L)	3.9±0.6	4.2±0.4	4.2±0.5	4.1±0.4	PA vs iSHT 0.01
S-Na (mmol/L)	140.9±2.6	140.8±2.5	138.8±3.9	140.2±3.1	PA vs iSHT 0.03
C-LDL (mmol/L)	107±29.8	95.6±31.4	104.8±34.8	110.9±34	ns
C-HDL (mmol/L)	47.9±12.5	45.2±9.2	64±14.6	55.6±20.3	PH vs PA 0.01 PH vs OSA 0.003 PH vs iSHT 0.02 PA vs iSHT <0.0001 OSA vs iSHT <0.0001
24h-U-Na$^+$ (mmol/day)	151±68	156±66	129±48	152±81	ns
LV hypertrophy, n (%)	8 (10.5)	2 (4)	2 (4.7)	7 (6.2)	ns
AF, n (%)	10 (13)	3 (6)	8 (19)	9 (8)	ns

Mean ± SD, or median (range), as appropriate. AF, atrial fibrillation; BMI, body mass index; iSHT, isolated systolic hypertension; HT, hypertension; LV, left ventricle; OSA, obstructive sleep apnea; PA, primary aldosteronism; PH, primary hypertension; SBP, systolic blood pressure;

3.4 Discussion

In this study, by applying a strict predefined algorithm for the diagnosis of secondary forms of hypertension in patients consecutively referred to a specialized center for hypertension, we found a prevalence rate of 56%. That is markedly high considering that most studies previously reported prevalence of SH approximately 5%(19). Such high prevalence of SH might be explained due current study conducted in those group of patients who referred to HT center, moreover the prevalence of SH especially for PA differs across primary care hospitals, and referral centers(3,6). Hence, when the patients are accurately investigated for SH, it can be found in most hypertensive patients.

Of note, 40.7% of PA were unilateral forms, i.e. surgically curable forms(7). This means that, if not recognized, such patients would be classified as primary hypertensive and, therefore, any chance of cure would be missed.

The guidelines(20,21) restrict the work-up for PA to selected categories of hypertensive patients at high probability of PA and, in those selected, recommend a complex algorithm that includes, after measurement of ARR, the confirmatory tests. This complex algorithm can be managed only by trained physicians, making difficult the screening for PA in most patients. Moreover, the confirmatory, better renamed exclusion tests, have been challenged because the diagnostic gain over carefully performed ARR was never demonstrated(22,23). Hence, a simplified algorithm for the work-up of PA, not including the exclusion tests, has been recently suggested(24).

Taking advantage of this algorithm, a work-up of PA was applied to all patients referred for HT to the center of Padua, leading to the identification of PA in 16.4% patients of the entire cohort. Of them, 29% had unilateral PA, whose diagnosis was established based on the adrenal vein sampling (AVS), and then conclusively with the biochemical and/or clinical cure at the follow-up. AVS, the key test recommended by available guidelines to identify unilateral PA(20,21), being technically challenging, difficult to interpret, and thus available in only a few centers, represents the major hurdle "bottleneck," in the subtyping of PA patients. However, it is a mandatory step for the PA subtyping. In our survey, imaging failed to detect the culprit adrenal in 28% of the surgically cured unilateral PA patients.

11% of patients had OSA, with stabilization of hemodynamics improvement of AHI after use of CPAP or oropharyngeal surgical reconstruction, under the with same or less number of antihypertensive drugs. Nearly 64% of hypertensive patients are suffering from OSA. The high prevalence of OSA in previous studies, that 90% of male patients and 77% of female patients with resistant hypertension had OSA(25) and, also that 30% of OSA patients had masked HT(26). In our study nearly 11% of patients had OSA, and, the mean BMI of whole cohort was 26.6±4.4 so this value might be the explanation of lower rate of OSA in compare to other studies(26).

In our study fourth leading cause of SH was hypothyroidism. The mechanism underlying the rise of BP in hypothyroidism have been clarified only in part. One conception is that the plasma renin activity leads to an increased salt sensitivity and as a consequence renal sodium reabsorption and volume expansion(27). In the study that

conducted relationship between high BP and hypothyroid state in 30% of patients with hypothyroidism had raised DBP(28), in contrast another group found no relation between BP level and hypothyroid and euthyroid state (29)(30). However, in current study 7.3% of HT patients had hypothyroidism without having any other cause for high BP.

HT is both a cause and an effect of CKD, moreover, contributes to its progression. CKD affects 10–15% of the population worldwide, and its prevalence is increasing. For the CKD we applied an estimated glomerular filtration rate [eGFR] < 59 mL/min/1.73, microalbuminuria ≥ 3 months duration(31), and presence of secondary cause for kidney damage. In our study we found quite low rate of CKD in comparison to other study(32), however to make conclusive diagnosis HT induced CKD or CKD induced HT is difficult.

AME induced by licorice abuse is the much more common than its genetic forms(33). To the best of our knowledge, there are no reports which conducted prevalence of licorice-induced AME, in the current cohort iAME prevailed in 5.6 % of the whole cohort. Furthermore, carefully collected dietary status in parallel with biochemical examination including PAC, DRC, serum potassium, and urinary cortisol could lead to differential diagnosis of low-renin HT to prevent high BP related CV complications and HMOD timely avoiding excess consumption of excess licorice.

The prevalence rate of RAS in the hypertensive patients ranges from 1 to 5%(34). The rate has been reported increases to 10-15% in the hypertensive patients over the age of

50 years, and up to 50-60% in the elderly patients in particular in those with atherosclerotic coronary and peripheral artery diseases, and renal dysfunction(35).

The major strength of our study is the strictly pre-defined algorithm for the diagnostic work-up: the major limitation is that the survey was performed at only one center.

In conclusion, this study shows that prevalence rate of secondary hypertension is twofold higher than the rate of essential hypertension suggesting that most an accurate diagnosis is usually omitted in a great proportion of the hypertensive patients.

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4. CHAPTER 3. META-ANALYSIS OF DIAGNOSTIC GAIN
EXCLUSION TESTS OVER BASELINE ALDOSTERONE-RENIN
RATIO IN DIAGNOSING UNILATERAL PRIMARY
ALDOSTERONISM

4.1 INTRODUCTION

Arterial hypertension is the most prevalent risk factor for cardiovascular disease worldwide. Despite substantial improvement in awareness and treatment of hypertension during the past decades, the BP values are still above the normal range in about half of the hypertensive patients on medication. One of the main reasons for this poor BP control according to Lancet Hypertension Committee, is the missing or delayed diagnosis of secondary hypertension[1].

Primary aldosteronism (PA) is the most common secondary surgically curable cause of arterial hypertension[2],[3]. However, it remains markedly underdiagnosed and undertreated for multiple reasons, including lack of clinical awareness with ensuing "under suspicion" complexity of the recommended diagnostic work-up, limited availability of invasive investigations for subtyping aimed of localizing the source of aldosterone excess, constrained surgical capacity and uncertainties about clinical outcomes[4]. In clinical practice, the screening for PA relies on the aldosterone-to-renin ratio (ARR). As to maximize the sensitivity, low cut-off values, for example 26 ng/dl/ng/ml/h (corresponding to 20·6 ng/mIU), are chosen[5]. This approach generates a high rate of false positive results, which must be identified and excluded from the costly and invasive subtyping procedures which should preserved to only patients with PA, who have an increased prior (pre-test) probability of harboring a unilateral surgically curable form of PA and be plausible candidates to adrenal surgery. Multiple tests, including the captopril challenge test (CCT), the fludrocortisone suppression test (FST), the saline infusion test (SIT), the oral sodium loading test (OLT), and the

furosemide upright test (FUT), have been proposed(4,6). Although generally referred to as "confirmatory", in reality, these tests serve to rule out false positive cases and thus function as "exclusion" tests. In fact, unambiguous evidence exists that at the prevalence rate of PA, ranging from 10% to 30% encountered in hypertension referral centers, their negative predictive value exceeds by far their positive (confirmatory) predictive value has been provides(7). Therefore, the term "exclusion" will be used to indicate these tests throughout this paper.

The preposition for using such tests stand on the premise that in PA excess aldosterone production is autonomous from the renin–angiotensin system, a contention that is not evidence-based(8). Moreover, evidence has been provided of expression of the angiotensin type I receptor in human aldosterone-producing adenoma (APA), the receptor mediating the aldosterone secretion in response to angiotensin II and of angiotensin II - induced aldosterone biosynthesis in APA strips and cells as vivo.(9)·(10) Moreover, use of the exclusion tests was generally supported by studies, which disregarded the Standards for Reporting Diagnostic Accuracy (STARD) statement(11) and were affected by selection and tautology biases.

In fact, some studies preselected patients based on positive result of a screening test, which obviously affected assessment of sensitivity, specificity, and accuracy. Besides, they attempted to validate the test vs another arbitrarily chosen test used as the reference. As, by nature, the latter was not 100% accurate, this created a vicious circle type of bias(12).

As exclusion tests increase complexity and costs of the diagnostic work-up, their

systematic use might not be justified in the clinical decision-making if their diagnostic gain over the simpler ARR is not proven beyond any reasonable doubts. Moreover, use of these tests involves the risk of overlooking the PA patients who are angiotensin II-responsive, whose existence is well documented(8,13,14). Therefore, the latest Endocrine Society Clinical Practice Guidelines suggested the feasibility of skipping these tests in PA patients, who showed a florid phenotype, defined as spontaneous hypokalemia, PRA below detection levels, and a PAC > 20 ng/dl.(4) To date, published studies that compared the screening test and the exclusion test(s) are few(7), and, therefore, the diagnostic gain of the exclusion test(s) over baseline ARR remains uncertain.

Based on these premises, it is altogether evident that it would be important to perform a meta-analysis of that aimed at determining the accuracy of exclusion tests and the diagnostic gain over baseline ARR in the work-up of PA.

4.2 AIMS

Considering the lack of adequately powered studies conclusively proving the accuracy and diagnostic gain of exclusion tests over the ARR, we conceived this meta-analysis to determine if the systematic use of exclusion tests is justified in the work-up of PA.

4.3 METHODS

This meta-analysis entailed two phases based on a different level of diagnostic uncertainty: in the exploratory phase, we used a "golden" standard (comprising excess aldosterone production, adrenal imaging and/or adrenal venous sampling [AVS]). In the validation phase we exploited use of uPA as the "gold" reference standard for

validation of the exclusion test(s) by the "four corners" criteria (Table 1).

Table 1. "Four corners" criteria for the diagnosis of unilateral primary aldosteronism.

1. Excessive aldosterone production
2. Lateralization of aldosterone secretion at AVS or adrenal scintigraphy
3. Adenoma/hyperplasia demonstration at pathology
4. Correction of biochemical values and fall of blood pressure after adrenalectomy

AVS: adrenal vein sampling.

4.3.1 Data Sources

The study followed the preferred reporting items for systematic review and meta-analysis (PRISMA) statement(15).

4.3.1 Study Selection

After removal of duplicates, articles eligibility was assessed by two reviewers (RZ, and TS), and three senior investigators (TMS, GR, and GPR). The study inclusion criteria were: (1) prospective or retrospective design; (2) uPA diagnosis established by the gold or a golden standard; (3) reported diagnostic accuracy of tests; (4) sufficient data to construct a 2x2 diagnostic table. Exclusion criteria comprised: (1) reviews, case reports, case-control studies; (2) duplicated data.

4.3.3 Data Extraction

Data extraction was performed by two investigators (RZ, and TS) using a predefined standardized form. When accuracy of ARR and/or exclusion test(s) was reported for different cut-off values, the value that provided the best combination of sensitivity and specificity was selected.

4.3.4 Quality Assessment

Given the limitations of the QUADAS-2 method, to assess the quality of the studies we

developed a novel quantitative scoring method, which comprised 9 items in 4 domains: study design and patient selection, index test, reference standard, and flow and timing (Table 2). Quality was independently assessed by 3 senior investigators; divergences were resolved by consensus. Each item received a score from 0 to 10 on a digital scale; thus, the overall score ranged from a minimum of 0 to a maximum of 90. We determined beforehand that studies should fulfil the following quality criteria: enrolment of consecutive newly-diagnosed hypertensive patients, pre-specified cut-off values for the index tests, gold or golden reference standard for uPA diagnosis, and appropriate follow-up. Moreover, all patients should have received the same reference and no exclusion from the analysis was accepted.

Table 2. Adapted QUADAS-2 Criteria

Domain	Patient Selection	Index Test	Reference Standard	Flow and Timing
Signaling questions (yes, no, or unclear)	Q1: Was a consecutive or random sample of patients enrolled? Q2: Did the study avoid inappropriate exclusions?	Q3: Were the index test results interpreted without knowledge of the results of the reference standard?	Q4: Is the reference standard likely to correctly classify the target condition? Q5: Were the reference standard results interpreted without knowledge of the results of the index tests?	Q6: Did all patients receive a reference standard? Q7: Did all patients receive the same reference standard? Q8: Were all patients included in the analysis?
Risk of bias (high, low, or unclear)	Could the selection of patients have introduced bias?	Could the conduct or interpretation of the index test have introduced bias?	Could the reference standard, its conduct, or its interpretation have introduced bias?	Could the patient flow have introduced bias?
Concerns about applicability (high, low, or unclear)	Are there concerns that the included patients do not match the review question?	Are there concerns that the index test, its conduct, or its interpretation differ from the review question?	Are there concerns that the target condition as defined by the reference standard does not match the review question?	

4.3.5 Data Synthesis and Analysis

The threshold effect was assessed by the Spearman correlation coefficient between Logit(sensitivity) and Logit (1-specificity) with p value > 0.05 indicating a non-threshold effect. I^2 was used to evaluate heterogeneity among studies. The random effects model was used when I^2 was > 30%.(16)

Summary receiving operation characteristic (sROC) curves and area under the curve (sAUC), pooled specificity, negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) with 95% confidence intervals (CI) were computed.(17)

Meta-regression was performed to identify covariates that affected heterogeneity.

A sensitivity analysis was performed to evaluate the quality and consistency of results by sequentially excluding each single study at a time.(18) Potential publication bias was evaluated by the p value of Deeks' funnel plot.(19)

All analyses were performed with Meta-Disc version 1.4 and STATA version 12.0 (Stata Corp, College Station, TX); statistical tests were two-sided, with a p value < 0.05 denoting statistical significance.

4.4 RESULTS

4.4.1 Study selection

We found 3,010 articles in the databases, but only 1,623 remained after removal of duplicates, irrelevance based on abstract (n=1,563) and on full-text reading (n=40). Therefore, a total of 4,242 patients in 20 articles, entailing 31 separate datasets were eligible for the meta-analysis: 11 in the exploratory phase, and 20 in the validation phase (Figure 1).

Figure 1. PRISMA Flow Chart
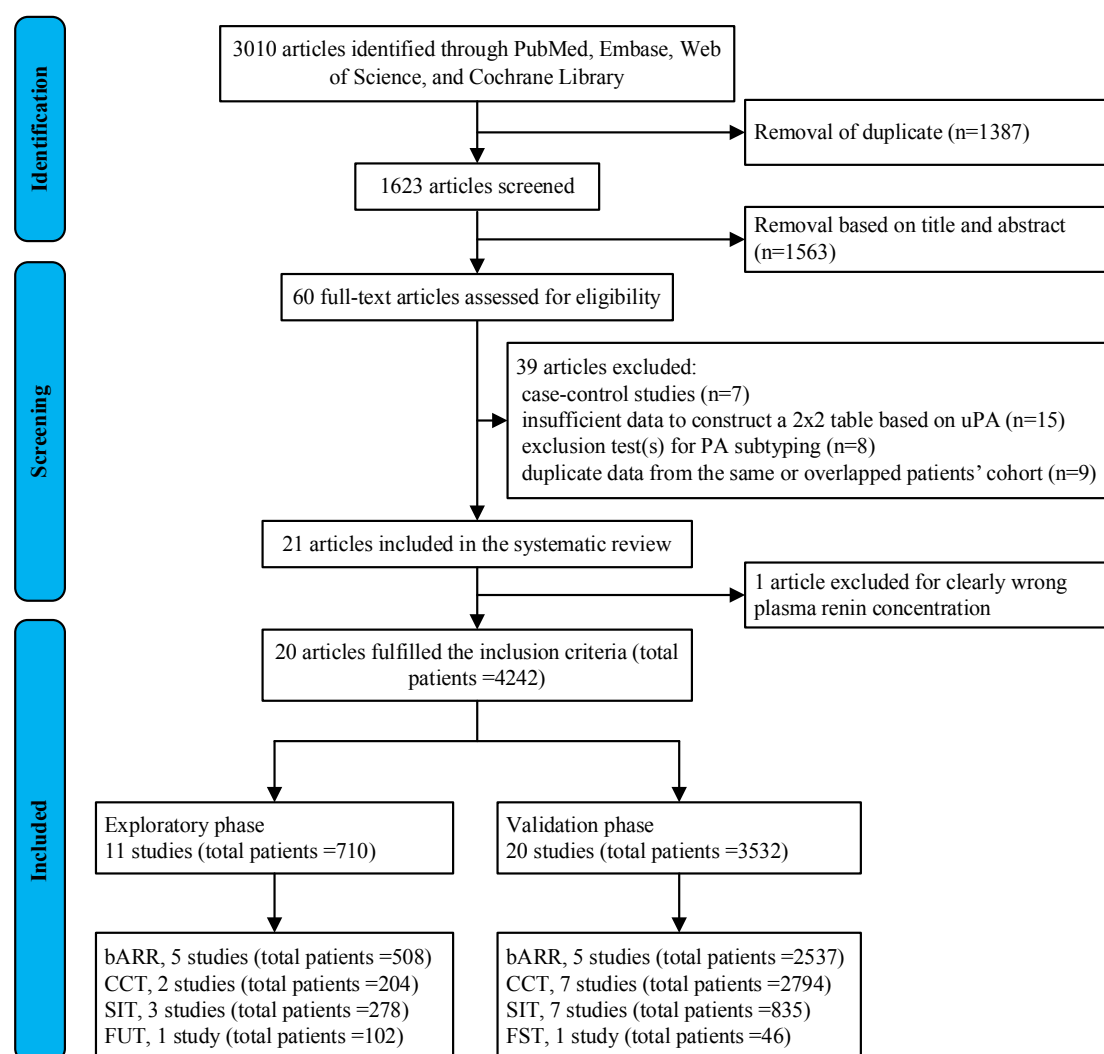
4.4.2 Study Characteristics and Quality Assessment

Notwithstanding our strict inclusion criteria, the overall scores of study quality showed

a wide range (Table 3 and Figure 2). The main characteristics of these studies are

detailed in Table 4: all were conducted in referral hypertension centers located in 3

continents (Europe, Asia, and Oceania). Of these, 6 assessed the diagnostic accuracy

of ARR,(20–25) 3 of CCT,(26–28) 4 of SIT,(29–32) and 7 of multiple tests using a

head-to-head comparison:(7,33–38) one study compared ARR and CCT;(7) one ARR

and SIT;(33) one ARR, CCT, SIT, and FUT;(35) one SIT and FST,(38) and 3 CCT and

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SIT(34,36,37).

Table 3. Quantitative Scoring for the Quality of Each Single Study

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9
ARR									
Bernini 2008	7	8	4	5	6	5	3	3	6
Burrello 2016	6	4	10	5	4	4	4	4	5
Ducher 2012	8	4	8	8	5	3	5	7	6
Fries 2020	9	6	2	6	4	2	3	3	4
Giacchetti 2006	3	4	4	5	3	3	3	3	5
Maiolino 2017a	10	9	9	10	9	7	5	7	6
Maiolino 2017b	10	9	9	10	9	7	5	7	6
Okamoto 2018	9	2	10	5	6	5	4	6	3
Vorselaars 2018	9	7	6	6	1	2	5	2	6
Weickert 2009	2	3	1	5	6	2	2	2	5
CCT									
Kim 2016	5	2	9	5	1	5	4	4	8
Maiolino 2017[a]	10	10	9	10	10	9	5	7	6
Maiolino 2017[b]	10	10	9	10	10	9	5	7	6
Meng 2018	3	6	9	7	7	8	3	5	9
Okamoto 2019	9	2	10	5	6	5	4	6	3
Rossi 2007	10	10	9	8	8	9	6	7	7
Song 2018	6	3	8	6	4	7	5	5	7
Wu 2009	7	4	9	7	6	5	3	4	8
Wu 2010	8	4	10	7	8	5	4	3	8
SIT									
Fries 2020	9	6	2	6	4	2	3	3	4
Fuss 2021	7	8	4	5	6	5	3	3	6
Meng 2017	3	6	9	6	7	8	3	5	9

Mulatero 2006	5	2	9	5	2	6	2	2	8
Okamoto 2019	9	2	10	5	6	5	4	6	3
Rossi 2007	10	9	8	9	9	9	7	8	7
Song 2017	6	3	8	6	4	7	5	5	7
Stowasser 2018	4	3	9	7	8	7	5	5	5
Vivien 2019	6	6	8	7	7	5	5	4	5
Zhang 2020	3	5	6	7	2	1	1	1	1
FST									
Okamoto 2019	9	2	10	5	6	5	4	6	3
FUT									
Stowasser 2018	4	3	9	7	8	7	5	5	5

ARR, aldosterone-to-renin ratio; CCT, captopril challenge test; SIT, saline infusion test; FST, fludrocortisone suppression test; FUT, furosemide upright test. a and b represent the exploratory and the validation cohort in Maiolino's study, respectively.

None assessed the diagnostic accuracy of OLT and FST vs the ARR. Thus, on the whole 10 datasets on ARR(7,20–25,33,35), 9 on CCT(7,26–28,34–37), and 10 SIT(29–38) were analyzed.

Figure 2. Mean Average of Quantitative Scoring of the ARR, CCT, and SIT
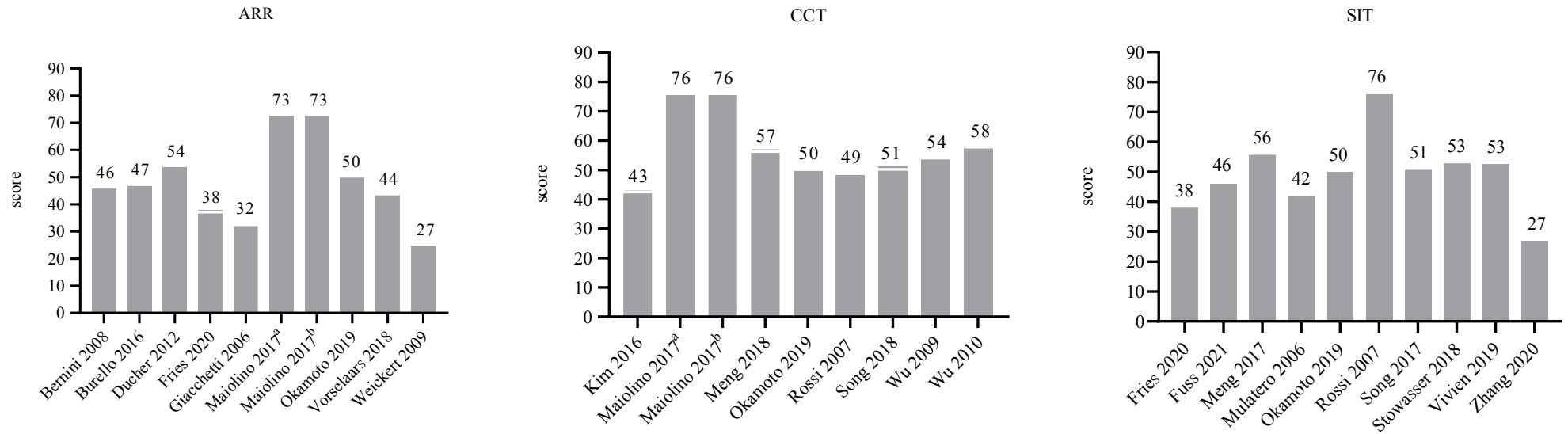
ARR, aldosterone-to-renin ratio; CCT, captopril challenge test; SIT, saline infusion test. a and b represent the exploratory and the validation cohort in Maiolino's study, respectively

4.4.3 Target population

The study population comprised a total of 4,242 patients, of whom 16% (n=677) had

uPA (Table 4). They comprised newly diagnosed hypertensives in two studies(7,20);

patients with a high prior probability of PA in 8(21–25,27,28,34); patients with a

positive ARR in 7(26,29–31,33,35); patients with positive ARR plus one-third/fourth

of patients with negative ARR in 2(36,37); and patients with positive recumbent SIT in

one(32).

Table 4. Main Characteristics of the Studies that were Meta-analyzed

Author, year	Country	Population	Dates	Index test(s)	Assay	uPA	Controls	PA diagnosis	uPA diagnosis
Bernini 2008(20)	Italy	New diagnosed PT	1998-2003	ARR	PAC by RIA (DiaSorin); PRA by RIA (DiaSorin)	30	100	Baseline PAC > 35 ng/dL and PRA < 0.5 ng/mL/h	Biochemical cure after surgery
Burrello 2016(21)	Germany	Suspected PA	2014	ARR	PAC by RIA amd CLIA (DiaSorin); PRA by RIA (DiaSorin); DRC by CLIA (DiaSorin)	5	75	Pos. SIT [PAC > 5 ng/dL (> 38.7 pmol/L)], or Pos. CCT [ARR > 30 ng/dL/ng/mL/h (832.2pmol/L/ng/mL/h) and ADRR > 3.7 ng/dL/mU/L (102.6 pmol/L/mU/L)]	Biochemical cure after surgery
Ducher 2012(22)	France	Suspected PA	2006-2007	ARR	N.A.	12	167	An outcome committee	Pathology after surgery
Fries 2020(33)	Germany	Pos. ARR (cutoff N.A.)	2016-2019	ARR, SIT	PAC by CL-MS/MS (Chromsystems); DRC by CLIA (DiaSorin)	9	67	Pos. ARR [PAC > 550 pmol/L (20 ng/dL), s-k$^+$ ↓, and PRA↓) or Pos. SIT [PAC > 140 pmol/L (5 ng/dL)]	Biochemical cure after surgery
Fuss 2021(29)	Germany	Pos. ARR (cutoff > 20 ng/dL/ng/mL/h)	2009-2018	SIT	PAC by RIA (Siemens)) or CLIA (IDS-iSYS) or LC-MS/MS (SCIEX); DRC by RIA (Cisbio)) or CLIA (IDS-iSYS	56	84	Pos. SIT	Biochemical cure after surgery
Giacchetti 2006(23)	Italy	Suspected PA	1996-2000	ARR	PAC by RIA (Biodata); UA by RIA (DiaSorin); PRA by RIA (Radim)	26	96	At least two of the following: (a) PAC↑, UA↑; (b) upright PRA↓ (≤ 1.0 ng/mL/h); (c) Pos. SIT (PAC ≥ 10 ng/dL); (d) an adrenal mass by imaging	Pathology after surgery

Kim 2016(26)	Korea	Pos. ARR (cutoff > 20 ng/dL/ng/mL/h)	2011-2014	CCT	PAC by RIA (TFB Inc.); PRA by RIA (TFB Inc.)	36	13	Pos. SIT (PAC ≥ 10 ng/dL)	Biochemical cure after surgery
Maiolino 2017(7)[a]	Italy	New diagnosed PT	2000-2005	ARR, CCT	PAC by RIA (Mirya); PRA by RIA (DiaSorin, or Radim)	51	991	Pos. ARR 40 ≥ ng/dL/ng/mL/h or Pos. CCT (ARR ≥ 30 ng/dL/ng/mL/h), or a logistic discriminant function ≥ 0.5	Biochemical cure after surgery
Maiolino 2017(7)[b]	Italy	New diagnosed PT	2012-2015	ARR, CCT	PAC by RIA (Mirya); PRA by RIA (DiaSorin, or Radim)	30	1028	Pos. ARR 40 ≥ ng/dL/ng/mL/h or Pos. CCT (ARR ≥ 30 ng/dL/ng/mL/h) or a logistic discriminant function ≥ 0.5	Biochemical cure after surgery
Meng 2018(34)	China	Suspected PA	2011-2016	CCT, SIT	PAC by RIA (Jiuding Bio); PRA by RIA (Northern Bio)	70	49	Pos. ARR > 30 ng/dL/ng/mL/h	Biochemical cure after surgery
Mulatero 2006(30)	Italy	Pos. bARR (cutoff chosen by each center)	2004	SIT	PAC by RIA (DiaSorin, or DCS California); PRA by RIA (DiaSorin)	18	31	Pos. FST (PAC > 5 ng/dL)	Biochemical cure after surgery
Okamoto 2019(35)	Japan	Pos. bARR (cutoff > 20 ng/dL/ng/mL/h)	2012-2018	ARR, CCT, SIT, FUT	N.A.	16	86	At least two of the following: Pos. CCT (ARR > 20 ng/dL/ng/mL/h), or Pos. SIT (PAC> 6.0 ng/dL), or Pos. FUT (PRA < 2.0 ng/mL/h)	AVS, LI ≥ 4
Rossi 2007(36)	Italy	Pos. bARR (cutoff ≥ 40 ng/dL/ng/mL/h) or pos. CCT (ARR ≥ 30 ng/dL/ng/mL/h) or a logistic discriminant function ≥ 0.50 + 1/4	2000-2005	CCT, SIT	PAC by RIA (Mirya); PRA by RIA (DiaSorin)	46	197	Pos. ARR 40 ≥ ng/dL/ng/mL/h or Pos. CCT (ARR ≥ 30 ng/dL/ng/mL/h) or a logistic discriminant function ≥ 0.5	Biochemical cure after surgery

		not fulling the above criteria							
Song 2018(37)	China	Pos. bARR (cutoff >37 ng/mIU) + 1/3 neg. bARR	2013-2016	CCT, SIT	PAC by CLIA (DiaSorin); DRC by CLIA (DiaSorin)	71	101	Pos. FST (PAC > 8 ng/dL)	Biochemical cure after surgery
Stowasser 2018(38)	Austr alia	Pos. bARR (cutoff >70 pmol/mIU by RIA or >55 pmol/mIU by HPLC-MS/MS)	2012-2017	SIT, FST	PAC by RIA kit or HPLC-MS/MS; DRC by CLIA (DiaSorin)	25	17	Pos. FST (PAC ≥ 133 pmol/L)	Biochemical cure after surgery
Vivien 2019(31)	Franc e	Pos. bARR (> 64 pmol/mIU)	2010-2015	SIT	PAC by RIA (Immunotech); DRC by IRMA (Cisbio)	24	76	Pos. SIT (PAC > 5 ng/dL) or Pos. CCT (PAC suppressed < 30%)	AVS /CT/MRI
Vorselaars 2018(24)	Nethe rland	Suspected PA	2015-2017	ARR	PAC by RIA (Siemens); PRA by RIA (In-house)	10	217	Pos. SIT (PAC >280 pmol/L and PRA > 100 fmol/L /s)	AVS/patholog y
Weickert 2009(25)	Germ any	Suspected PA	2005-2006	ARR	PAC by RIA (Immunotech); PRA by RIA (DiaSorin)	7	22	Pos. ARR (PAC↑, UA↑, PRA↓, s-k$^+$↓, and PRA↓)	Pathology after surgery
Wu 2009(27)	Taipe i	Suspected PA	2003-2006	CCT	PAC by RIA (Adaltis); PRA by RIA (Cisbio)	47	64	Pos. SIT (PAC > 10 ng/dL) or UA ≥ 12 μg/24h	Biochemical cure after surgery
Wu 2010(28)	Taipe i	Suspected PA	2008	CCT	PAC by RIA (Adaltis); PRA by RIA (Stillwater)	39	63	Pos. SIT (PAC > 10 ng/dL)	AVS, LI ≥ 4 or scintigraphy
Zhang 2020(32)	China	Pos. SIT (PAC >11.2 ng/dL)	2018-2019	SIT	PAC by RIA (Jiuding Bio); PRA by RIA (Northern Bio)	46	20	Pos. SIT (PAC > 11.2 ng/dL)	Biochemical cure after surgery

ARR, aldosterone-to-renin ratio; AVS, adrenal vein sampling; BP, blood pressure; bARR, baseline aldosterone-to-renin ratio; CCT, captopril challenge test; CLIA, chemiluminescence immunoassay; CT, computed tomography; FUT, furosemide upright test; LC-MS/MS , liquid chromatography tandem mass spectrometry; LI, lateralization index; MRI, magnetic resonance tomography; NA, not available; Neg., negative; P, prospective; PA, primary aldosteronism; PAC, plasma aldosterone concentration; PH, primary hypertension; Pos., positive; PRA, plasma renin activity; R, retrospective; RIA, radioimmunoassay; SIT, saline infusion test; UA, urinary aldosterone; uPA, unilateral primary aldosteronism. a and b represent the exploratory and the validation cohort in Maiolino's study, respectively.

Controls comprised mainly patients with primary (essential) hypertension (PH) except in 3 studies: one that used patients with PH and bilateral adrenal hyperplasia (BAH)(35); one that used patients with PH plus UAH plus BAH(22); and one that used patients with non-PA(38). Interfering medications were withheld for at least two weeks, and switched to calcium channel antagonists and/or α-blockers in all but one study(21).

4.4.4 Reference index

The diagnosis of PA was established by a positive result of the ARR in 3 studies,(20,25,34) on the ARR and/or exclusion test(s) in 4 studies(7,23,33,36), and on positive exclusion test(s) result in 13 studies(21,22,35,37,38,24,26–32). The exploratory phase, comprised 6 studies in 133 patients with uPA(22,23,25,28,31,35). The validation phase entailed in 14 studies in 544 patients with uPA(7,20,34,36–38,21,24,26,27,29,30,32,33).

The studies on the ARR were 10, equally split into the exploratory (22,23,25,33,35) and the validation(7,20,21,24) phase (Table 5). The mean cut-off ARR value of 48.1 ng/mIU (39.4 ng/dL/ng/mL/h) with a range from 19.1 to 96.4 ng/mIU (corresponding to 15.7 to 79.0 ng/dL/ng/mL/h)(7,20–25,33,35). ROC curve and Youden index (YI) analysis for the uPA diagnosis was used to determine the cut-off value only in 5 studies(7,22,35,39).

The studies on the CCT were 9(7,26–28,34,35,37,40). All except used a captopril dose of 50 mg except one study(34) that used 25 mg; patients were kept in a sitting position for 1-2 hours after drug challenge. The test readout differed: 5 studies used post-captopril ARR cut-off values (ranging from 15.6 to 81.6 ng/mIU [12.8 to 66.9 ng/dL/ng/mL/h]);(7,27,28,35) 4 used the post-captopril PAC values with cut-offs

ranging between 13.0 and 19.0 ng/d(26,34,37,40). Seven studies selected the cut-offs by ROC curve and YI analysis for uPA diagnosis(7,27,28,35–37). 2 were in the exploratory phase(28,35) and 7 studies in the validation phase(7,26,27,34,36,37) (Table 5).

Table 5. 2x2 Table Table Reporting TP, FP, TN, and FN Using the Gold or Golden Diagnosis of uPA as Reference

Author, year	Procedure, dosage, time interval	Cut-off value (original/converted)	Reference (golden/gold)	Cut-off by ROC curve	TP	FP	FN	TN
ARR								
Bernini 2008(20)	Morning, after upright for at least 2 h and seated for 5-15 min	ARR > 96.4 ng/mIU	Gold	Yes	29	13	1	87
Burrello 2016(21)	Morning, after upright for at least 2 h and seated for at least 15 min	ARR ≥ 37.0 ng/mU and PAC ≥ 10.0 ng/dL	Gold	No	5	1	0	74
Ducher 2012(22)	Morning, after supine for 1 h	ARR ≥ 32.0 ng/ng / 20.2 ng/mIU	Golden	Yes	11	13	1	154
Fries 2020(33)	Morning, after seated for 15 min	ARR ≥ 53.0 pmol/mU / 19.1 ng/mIU	Golden	No	9	27	0	40
Giacchetti 2006(23)	Morning, after upright for 2 h and seated for 5-15 min	ARR ≥ 40.0 ng/dL/ng/ml/h / 48.8 ng/mIU	Golden	No	26	15	0	81
Maiolino 2017a(7)	Morning, after seated for 1 h	ARR ≥ 33.3 ng/dL/ng/ml/h / 40.6 ng/mIU	Gold	Yes	40	117	11	874
Maiolino 2017b(7)	Morning, after supine for 1 h	ARR ≥ 30.9 ng/dL/ng/ml/h / 37.3 ng/mIU	Gold	Yes	28	64	1	964
Okamoto 2019(35)	Morning, after seated for 15 min	ARR ≥ 52.8 ng/dL/ng/ml/h / 64.4 ng/mIU	Golden	Yes	12	22	4	64
Vorselaars 2018(24)	Morning, after upright for at least 2 h and seated for 5-15 min	ARR > 7 pmol/fmol / 65.6 ng/mIU	Gold	No	10	23	0	194
Weickert 2009(25)	Morning, after upright for 30 min	ARR ≥ 425 pg/ml/ ng/ml/h / 51.8 ng/mIU	Golden	No	6	4	0	18
CCT								
Kim 2016(26)	50 mg 1.5 h seated	PAC ≥ 19.0 ng/dL	Gold	No	27	0	9	13
Maiolino 2017(7)[a]	50 mg 2 h seated	ARR ≥ 13.9 ng/dL/ng/mL/h / 17.0 ng/mIU	Gold	Yes	40	120	51	871
Maiolino 2017(7)[b]	50 mg 2 h seated	ARR ≥ 12.8 ng/dL/ng/mL/h / 15.6 ng/mIU	Gold	Yes	28	227	2	801
Meng 2018(34)	25 mg 2 h seated	PAC ≥ 15.0 ng/dL	Gold	No	68	9	2	40
Okamoto 2019(35)	50 mg 1.5 h seated	ARR ≥ 42.2 ng/dL/ng/mL/h / ≥ 51.5 ng/mIU	Golden	Yes	12	16	4	70
Rossi 2007(36)	25 mg 2 h seated	ARR ≥ 30.0 ng/dL/ng/mL/h / PAC ≥ 13.9 ng/dL	Gold	Yes	32	51	14	146

					TP	FP	FN	TN
Song 2018(37)	50 mg 2 h seated	PAC ≥ 13.0 ng/dL	Gold	Yes	68	5	3	96
Wu 2009(27)	50 mg 1.5 h seated	ARR ≥ 23.9 ng/dL/ng/mL/h / 29.2 ng/mIU	Gold	Yes	28	6	11	57
Wu 2010(28)	50 mg 1 h seated	ARR ≥ 39.6 pmol/ng / 9.0 ng/mIU	Golden	Yes	39	8	8	56
SIT								
Fries 2020(33)	2 L 4 h supine	PAC ≥ 83 pmol/L / 3.0 ng/dL	Golden	No	9	5	0	62
Fuss 2021(29)	2 L 4 h supine	PAC ≥ 5.0 ng/dL	Gold	Yes	47	9	9	75
Meng 2017(34)	2 L 4 h supine	PAC ≥ 10.0 ng/dL	Gold	No	69	10	1	39
Mulatero 2006(30)	2 L 4 h supine	PAC ≥ 5.0 ng/dL	Gold	No	18	5	0	26
Okamoto 2019(35)	2 L 4 h supine	PAC ≥ 15.2 ng/dL	Golden	Yes	14	9	2	77
Rossi 2007(36)	2 L 4 h supine	PAC ≥ 6.8 ng/dL	Gold	Yes	38	49	8	148
Song 2018(37)	2 L 4 h supine	PAC ≥ 10.0 ng/dL	Gold	Yes	68	4	3	97
Stowasser 2018(38)	2 L 4 h seated	PAC ≥ 162 pmol/L / 5.8 ng/dL	Gold	Yes	26	1	2	17
Vivien 2019(31)	2 L 4 h supine	PAC ≥ 5.7 ng/dL	Golden	No	22	4	2	74
Zhang 2020(32)	2 L 4 h seated	PAC ≥ 12.9 ng/dL	Gold	No	41	2	5	18
FST								
Stowasser 2018(38)	0.1 mg every 6 h	PAC > 162 pmol/L / 5.8 ng/dL	Gold	Yes	24	0	4	18
FUT								
Okamoto 2019(35)	40 mg 2 h upright	PRA ≤ 0.55 ng/mL/h	Golden	Yes	13	23	3	63

ARR, aldosterone-to-renin ratio; CCT, captopril challenge test; FN, false negatives; FP, false positives; FST, fludrocortisone suppression test; FUT, furosemide upright test; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ROC, receiver-operating characteristic curve; SIT, Saline infusion test; TN, true negatives; TP, true positives. All units were converted to ng/mIU for ARR, ng/dL for PAC, ng/mL/h for PRA. To homogenize studies, all units of ARR, PAC, and PRA were converted with help of ARR smartphone application (ARR-APP). a and b represent the exploratory and the validation cohort in Maiolino's study, respectively.

The studies on the SIT were 10(29–38); all were performed in the supine position except two(32,38) that used a seated position. The post-saline PAC cut off value was chosen according to ROC curve and YI analysis for uPA diagnosis only in 5 studies; it ranged from 3.0 to 15.2 ng/dL, with a mean value of 8.1 ng/dL(29,35–38); 3 belonged to the exploratory phase(31,33,35) and 7 studies to the validation phase(29,30,32,34,36–38) (Table 5).

We could find only one eligible study for the FST(38) and the FUT(35). For FST, uPA was diagnosed by the gold standard, and the cut-off value of post-fludrocortisone PAC was 162 pmol/L (5.8 ng/dL); for the FUT, uPA was diagnosed by the golden standard, and the cut-off value of post-furosemide PRA was 0.55 ng/mL/h.

4.4.5 Meta-Analysis

I^2 values of pooled specificity, NLR, DOR for ARR, CCT, and SIT were all > 30%, denoting a heterogeneous non-threshold effect for all tests. As no evidence for a diagnostic threshold was detected, the heterogeneity analysis for ARR, CCT and SIT was performed by non-threshold effects and the random effects model was used.

Given the scope of the exclusion tests, the results are herein reported as specificity, NLR, sAUC, as a prevalence-dependent DOR, and as a prevalence-independent measure of diagnostic accuracy.

For ARR, the pooled specificity, NLR, and DOR were 0.90 (95% CI: 0.85-0.94), 0.12 (95% CI: 0.06-0.24), and 67.61 (95% CI: 27.05-168.99), respectively (Figure 2). The sAUC was 0.95 (95% CI: 0.92-0.98) (Figure 5).

For CCT, the pooled specificity, NLR, and DOR were 0.83 (95% CI: 0.79-0.87), 0.19 (95% CI: 0.12-0.31), and 36.89 (95% CI: 16.16-84.17), respectively (Figure 3). The

sAUC was 0.92 (95% CI: 0.88-0.97) (Figure 5).

For SIT, the pooled specificity, NLR, and DOR were 0.87 (95% CI: 0.81-0.89), 0.11 (95% CI: 0.07-0.17), and 99.3 (95% CI: 40.9-241.79), respectively (Figure 4). The sAUC was 0.96 (95% CI: 0.94-0.99) (Figure 5).

Overall, these results would indicate a high accuracy (sAUC) for ARR, CCT, and SIT, with no significant differences among tests ($p = 0.328$ for ARR vs CCT; $p = 0.566$ for ARR vs SIT; $p = 0.125$ for CCT vs SIT). Due to the limited available studies no conclusion was feasible for FST and FUT.

Figure 2. Forest Plots of Specificity, NLR, and DOR for the Aldosterone-to-renin ratio in the Exploratory and the Validation Phase

	TP	FP	FN	TN	Specificity (95% CI)	NLR (95% CI)	DOR (95%CI)
Exploratory phase							
Ducher 2012	11	13	1	154	0·92 (0·87 - 0·96)	0·13 (0·03 - 0·57)	87.74 (14.65 - 525.57)
Fries 2020	9	27	0	40	0·60 (0·47 - 0·72)	0·08 (0·01 - 1·26)	27.98 (1.56 - 500.86)
Giacchetti 2006	26	15	0	81	0·84 (0·76 - 0·91)	0·02 (0·00 - 0·34)	278.68 (16.12 - 4,818.05)
Okamoto 2019	12	22	4	64	0·74 (0·64 - 0·83)	0·36 (0·16 - 0·80)	7.96 (2.45 - 25.90)
Weickert 2009	7	4	0	18	0·82 (0·60 - 0·95)	0·08 (0·01 - 1·15)	61.67 (2.94 - 1,292.31)
Subtotal	**65**	**81**	**5**	**357**	**0.82 (0.70 - 0.93)**	**0.13 (0.04 - 0.42)**	**40.11 (9.42 - 170.83)**
					$\chi^2 = 35.86$, p < 0.001 (d.f.= 4); $I^2 = 88.8$ %	Cochran-Q = 8.03, p = 0.090 (d.f.= 4); $I^2 = 50.2$ %	Cochran-Q = 8.95, p = 0.063 (d.f.= 4); $I^2 = 53.3$ %
Validation phase							
Bernini 2008	29	13	1	87	0.87 (0.79 - 0.93)	0.04 (0.01 - 0.26)	127.47 (22.42 - 724.58)
Burrello 2016	5	1	0	74	0.99 (0.93 - 1.00)	0.09 (0.01 - 1.21)	546.33 (19.84 - 15,047.16)
Maiolino 2017[a]	40	117	11	874	0.88 (0.86 - 0.90)	0.24 (0.14 - 0.41)	26.21 (13.24 - 51.87)
Maiolino 2017[b]	29	64	1	964	0.94 (0.92 - 0.95)	0.04 (0.01 - 0.24)	294.09 (55.93 - 1,546.29)
Vorselaars 2018	10	23	0	194	0.89 (0.85 - 0.93)	0.05 (0.00 - 0.76)	173.81 (9.86 - 3,062.87)
Subtotal	**113**	**218**	**13**	**2193**	**0.91 (0.90 - 0.92)**	**0.08 (0.02 - 0.28)**	**115.63 (29.58 - 452.02)**
					$\chi^2 = 30.09$, p < 0.001 (d.f.= 4); $I^2 = 87.1$ %	Cochran-Q = 9.87, p = 0.043 (d.f.= 4); $I^2 = 59.5$ %	Cochran-Q = 112.05, p = 0.017 (d.f.= 4); $I^2 = 59.5$ %
Total	**178**	**299**	**18**	**2550**	**0.90 (0.85 – 0.94)**	**0.12 (0.06 - 0.24)**	**67.61 (27.05 - 168.99)**
					$\chi^2 = 96.47$, p < 0.001 (d.f.= 9); $I^2 = 90.7$ %	Cochran-Q = 16.86, p = 0.051 (d.f.= 9); $I^2 = 46.6$ %	Cochran-Q = 22.05, p = 0.008 (d.f.= 4); $I^2 = 59.8$ %

DOR, diagnostic odds ratio; FN, false negatives; FP, false positives; NLR, negative likelihood ratio; TN, true negatives; TP, truth positives. Error bars on the plots represent the 95% confidence intervals. Square size is proportional to the weight of the study. a and b represent the exploratory and the validation cohort in Maiolino's study, respectively. *, p < 0.05; †, p < 0.01; ‡, < 0.001 the exploratory vs the validation phase.

Figure 3. Forest Plots of Specificity, NLR, and DOR for the Captopril Challenge Test in the Exploratory and the Validation Phase

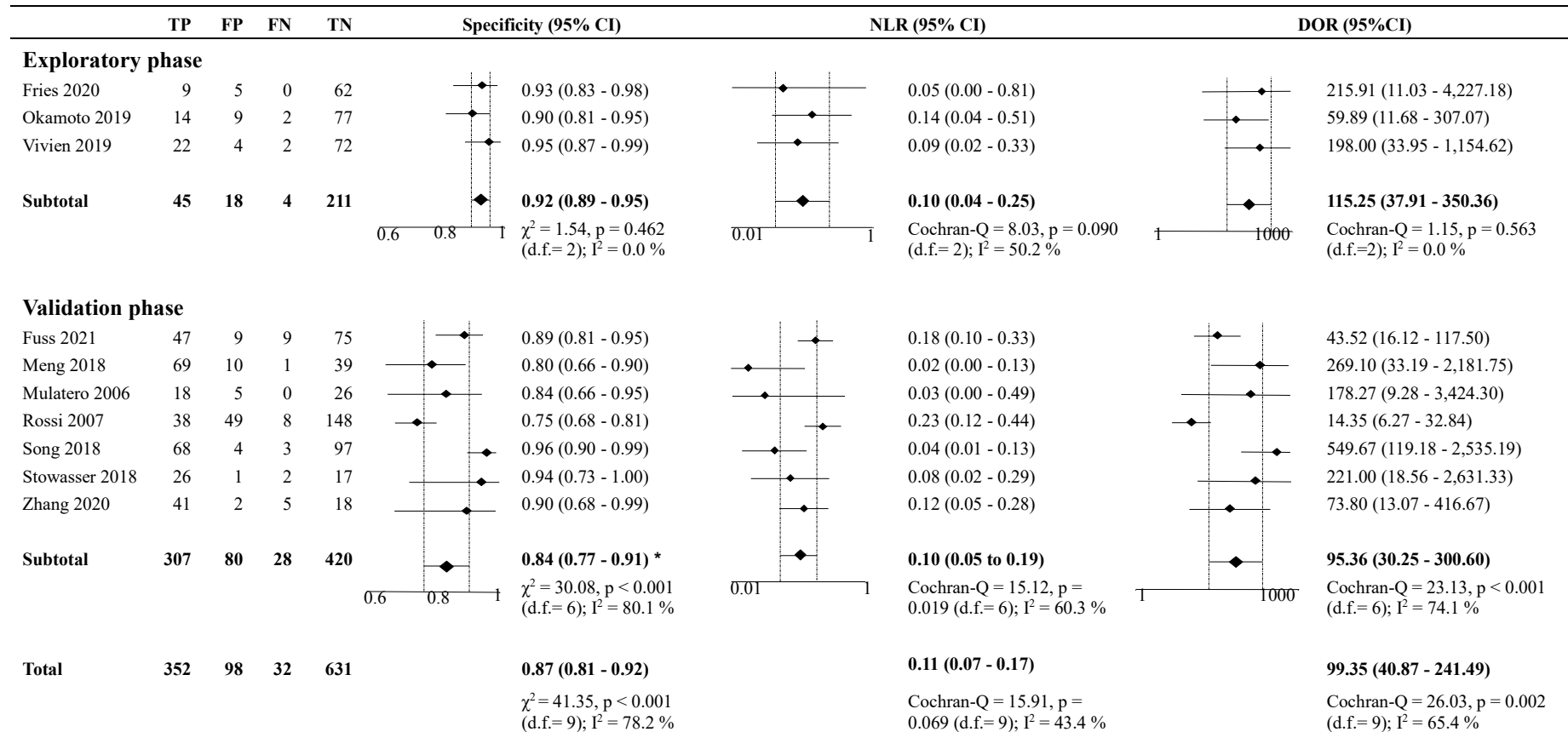|	TP	FP	FN	TN	Specificity (95% CI)	NLR (95% CI)	DOR (95%CI)
Exploratory phase							
Ducher 2012	11	13	1	154	0·92 (0·87 - 0·96)	0·13 (0·03 - 0·57)	87.74 (14.65 - 525.57)
Fries 2020	9	27	0	40	0·60 (0·47 - 0·72)	0·08 (0·01 - 1·26)	27.98 (1.56 - 500.86)
Giacchetti 2006	26	15	0	81	0·84 (0·76 - 0·91)	0·02 (0·00 - 0·34)	278.68 (16.12 - 4,818.05)
Okamoto 2019	12	22	4	64	0·74 (0·64 - 0·83)	0·36 (0·16 - 0·80)	7.96 (2.45 - 25.90)
Weickert 2009	7	4	0	18	0·82 (0·60 - 0·95)	0·08 (0·01 - 1.15)	61.67 (2.94 - 1,292.31)
Subtotal	**65**	**81**	**5**	**357**	**0.82 (0.70 - 0.93)**	**0.13 (0.04 - 0.42)**	**40.11 (9.42 - 170.83)**
					$\chi^2 = 35.86$, p < 0.001 (d.f.= 4); $I^2 = 88.8$ %	Cochran-Q = 8.03, p = 0.090 (d.f.= 4); $I^2 = 50.2$ %	Cochran-Q = 8.95, p = 0.063 (d.f.= 4); $I^2 = 53.3$ %
Validation phase							
Bernini 2008	29	13	1	87	0.87 (0.79 - 0.93)	0.04 (0.01 - 0.26)	127.47 (22.42 - 724.58)
Burrello 2016	5	1	0	74	0.99 (0.93 - 1.00)	0.09 (0.01 - 1.21)	546.33 (19.84 - 15,047.16)
Maiolino 2017[a]	40	117	11	874	0.88 (0.86 - 0.90)	0.24 (0.14 - 0.41)	26.21 (13.24 - 51.87)
Maiolino 2017[b]	29	64	1	964	0.94 (0.92 - 0.95)	0.04 (0.01 - 0.24)	294.09 (55.93 - 1,546.29)
Vorselaars 2018	10	23	0	194	0.89 (0.85 - 0.93)	0.05 (0.00 - 0.76)	173.81 (9.86 - 3,062.87)
Subtotal	**113**	**218**	**13**	**2193**	**0.91 (0.90 - 0.92)**	**0.08 (0.02 - 0.28)**	**115.63 (29.58 - 452.02)**
					$\chi^2 = 30.09$, p < 0.001 (d.f.= 4); $I^2 = 87.1$ %	Cochran-Q = 9.87, p = 0.043 (d.f.= 4); $I^2 = 59.5$ %	Cochran-Q = 112.05, p = 0.017 (d.f.= 4); $I^2 = 59.5$ %
Total	**178**	**299**	**18**	**2550**	**0.90 (0.85 – 0.94)**	**0.12 (0.06 - 0.24)**	**67.61 (27.05 - 168.99)**
					$\chi^2 = 96.47$, p < 0.001 (d.f.= 9); $I^2 = 90.7$ %	Cochran-Q = 16.86, p = 0.051 (d.f.= 9); $I^2 = 46.6$ %	Cochran-Q = 22.05, p = 0.008 (d.f.= 4); $I^2 = 59.8$ %

DOR, diagnostic odds ratio; FN, false negatives; FP, false positives; NLR, negative likelihood ratio; TN, true negatives; TP, truth positives. Error bars on the plots represent the 95% confidence intervals. Square size is proportional to the weight of the study. a and b represent the exploratory and the validation cohort in Maiolino's study, respectively. *, p < 0.05; †, p < 0.01; ‡, < 0.001 the exploratory vs the validation phase.

Figure 4. Forest Plots of Specificity, NLR, and DOR for the Saline Infusion Test in the Exploratory and the Validation phase

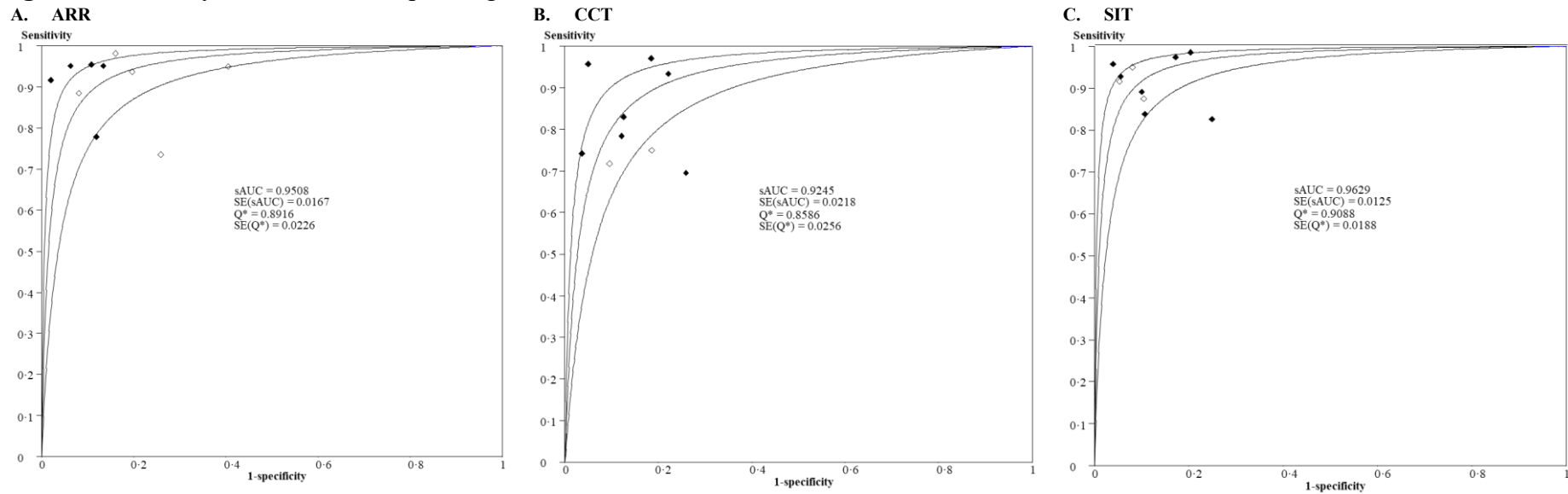|	TP	FP	FN	TN	Specificity (95% CI)	NLR (95% CI)	DOR (95%CI)
Exploratory phase							
Fries 2020	9	5	0	62	0.93 (0.83 - 0.98)	0.05 (0.00 - 0.81)	215.91 (11.03 - 4,227.18)
Okamoto 2019	14	9	2	77	0.90 (0.81 - 0.95)	0.14 (0.04 - 0.51)	59.89 (11.68 - 307.07)
Vivien 2019	22	4	2	72	0.95 (0.87 - 0.99)	0.09 (0.02 - 0.33)	198.00 (33.95 - 1,154.62)
Subtotal	**45**	**18**	**4**	**211**	**0.92 (0.89 - 0.95)**	**0.10 (0.04 - 0.25)**	**115.25 (37.91 - 350.36)**
					$\chi^2 = 1.54$, p = 0.462 (d.f.= 2); $I^2 = 0.0$ %	Cochran-Q = 8.03, p = 0.090 (d.f.= 2); $I^2 = 50.2$ %	Cochran-Q = 1.15, p = 0.563 (d.f.=2); $I^2 = 0.0$ %
Validation phase							
Fuss 2021	47	9	9	75	0.89 (0.81 - 0.95)	0.18 (0.10 - 0.33)	43.52 (16.12 - 117.50)
Meng 2018	69	10	1	39	0.80 (0.66 - 0.90)	0.02 (0.00 - 0.13)	269.10 (33.19 - 2,181.75)
Mulatero 2006	18	5	0	26	0.84 (0.66 - 0.95)	0.03 (0.00 - 0.49)	178.27 (9.28 - 3,424.30)
Rossi 2007	38	49	8	148	0.75 (0.68 - 0.81)	0.23 (0.12 - 0.44)	14.35 (6.27 - 32.84)
Song 2018	68	4	3	97	0.96 (0.90 - 0.99)	0.04 (0.01 - 0.13)	549.67 (119.18 - 2,535.19)
Stowasser 2018	26	1	2	17	0.94 (0.73 - 1.00)	0.08 (0.02 - 0.29)	221.00 (18.56 - 2,631.33)
Zhang 2020	41	2	5	18	0.90 (0.68 - 0.99)	0.12 (0.05 - 0.28)	73.80 (13.07 - 416.67)
Subtotal	**307**	**80**	**28**	**420**	**0.84 (0.77 - 0.91) ***	**0.10 (0.05 to 0.19)**	**95.36 (30.25 - 300.60)**
					$\chi^2 = 30.08$, p < 0.001 (d.f.= 6); $I^2 = 80.1$ %	Cochran-Q = 15.12, p = 0.019 (d.f.= 6); $I^2 = 60.3$ %	Cochran-Q = 23.13, p < 0.001 (d.f.= 6); $I^2 = 74.1$ %
Total	**352**	**98**	**32**	**631**	**0.87 (0.81 - 0.92)**	**0.11 (0.07 - 0.17)**	**99.35 (40.87 - 241.49)**
					$\chi^2 = 41.35$, p < 0.001 (d.f.= 9); $I^2 = 78.2$ %	Cochran-Q = 15.91, p = 0.069 (d.f.= 9); $I^2 = 43.4$ %	Cochran-Q = 26.03, p = 0.002 (d.f.= 9); $I^2 = 65.4$ %

DOR, diagnostic odds ratio; FN, false negatives; FP, false positives; NLR, negative likelihood ratio; TN, true negatives; TP, truth positives. Error bars on the plots represent the 95% confidence intervals. Square size is proportional to the weight of the study. *, p < 0.05; †, p < 0.01; ‡, < 0.001 the exploratory vs the validation phase.

Figure 5. Summary Area under the Operating Characteristic Curve of the ARR, CCT, and SIT

A. ARR

sAUC = 0.9508
SE(sAUC) = 0.0167
Q* = 0.8916
SE(Q*) = 0.0226

B. CCT

sAUC = 0.9245
SE(sAUC) = 0.0218
Q* = 0.8586
SE(Q*) = 0.0256

C. SIT

sAUC = 0.9629
SE(sAUC) = 0.0125
Q* = 0.9088
SE(Q*) = 0.0188

ARR, aldosterone-to-renin ratios; CCT, captopril challenge test; SIT, saline infusion test. Solid diamond: study by the gold standard; empty diamond: study by the golden standard.

4.4.6 Meta-Regression

A multivariable meta-regression analysis, including continent, populations (patients with suspected PA or with positive ARR), cut-off value (chosen by ROC curve or not), and reference standard for uPA diagnosis (gold or golden), was performed. The post-CCT readout variable (PAC or ARR) and posture (seated or supine) were also considered when exploring the heterogeneity of CCT and SIT results, respectively. For ARR, the selection of the populations (patients with suspected PA or with positive ARR) partly explained the heterogeneity (p= 0.024) (Table 6). For CCT, the readout variable had an impact on heterogeneity (p= 0.026) (Table 7). For SIT, none of these factors accounted for the heterogeneity (Table 8).

Table 6. Meta-regression of the Aldosterone-to-renin Ratio

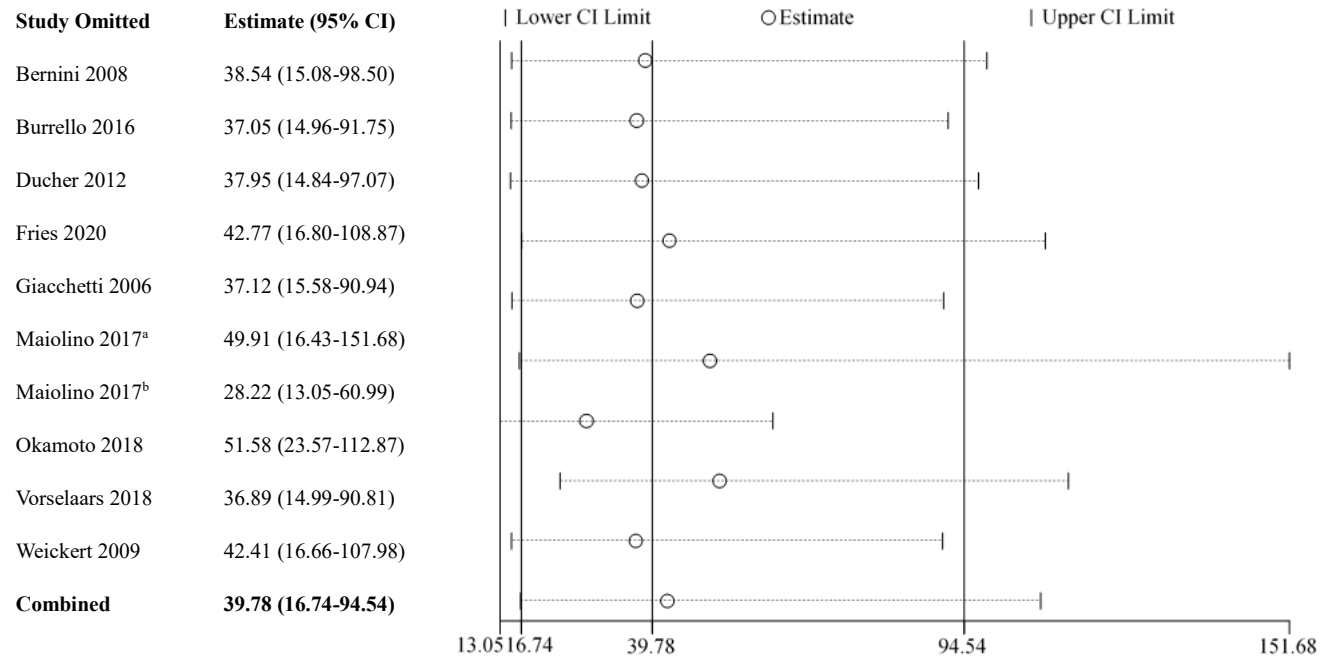| (1)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Continent (Europe or Asia)	9/1	-0.005	2.151	0.998	1.00 (0.00 - 390.56)
Population (suspected PA or positive ARR)	7/3	-2.357	1.829	0.267	0.09 (0.00 - 15.20)
Cutoff (chosen by ROC or not)	5/5	0.898	1.229	0.506	2.45 (0.08 - 74.50)
Reference (golden or gold)	5/5	0.010	1.299	0.994	1.01 (0.03 - 37.21)
(2)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Population (suspected PA or positive ARR)	7/3	-2.369	1.249	0.116	0.09 (0.00 - 2.32)
Cutoff (chosen by ROC or not)	5/5	0.882	1.014	0.425	2.41 (0.18 - 32.76)
Reference (golden or gold)	5/5	0.069	1.185	0.956	1.07 (0.05 - 22.55)
(3)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Population (suspected PA or positive ARR)	7/3	-2.275	0.827	**0.033**	0.10 (0.01 - 0.78)
Cutoff (chosen by ROC or not)	5/5	0.868	0.972	0.406	2.38 (0.22 - 25.66)
(4)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Population (suspected PA or positive ARR)	7/3	-2.209	0.766	**0.024**	0.11 (0.02 - 0.67)
(5)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Cutoff (chosen by ROC or not)	5/5	0.722	1.217	0.572	2.06 (0.12 - 36.62)

ARR, aldosterone-to-renin ratio; CI, confidence interval; No., number of studies; PA, primary aldosteronism; RDOR, relative diagnostic odds ratio; ROC, receiving operation characteristic curve; Std. Err., standard error.

Table 7. Meta-regression of the Captopril Challenge Test

(1)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Continent (Europe or Asia)	3/6	-0.151	1.979	0.946	0.86 (0.00 - 4293.95)
Population (suspected PA or positive ARR)	3/6	-0.863	1.591	0.642	0.42 (0.00 - 397.11)
Cutoff (chosen by ROC or not)	2/7	-0.537	2.193	0.829	0.58 (0.00 - 7310.15)
Reference (golden or gold)	3/6	-0.089	1.556	0.960	0.92 (0.00 - 739.01)
Readout variable (ARR or PAC)	6/3	2.405	1.601	0.272	11.07 (0.01 - 10841.02)
(2)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Continent (Europe or Asia)	3/6	-0.217	1.103	0.857	0.80 (0.02 - 26.95)
Population (suspected PA or positive ARR)	3/6	-0.912	1.181	0.496	0.40 (0.01 - 17.21)
Cutoff (chosen by ROC or not)	2/7	-0.506	1.815	0.799	0.60 (0.00 - 194.43)
Readout variable (ARR or PAC)	6/3	2.439	1.283	0.153	11.46 (0.19 - 678.66)
(3)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Population (suspected PA or positive ARR)	3/6	-0.756	0.751	0.371	0.47 (0.06 - 3.78)
Cutoff (chosen by ROC or not)	2/7	-0.676	1.482	0.672	0.51 (0.01 - 31.18)
Readout variable (ARR or PAC)	6/3	2.430	1.186	0.110	11.36 (0.42 - 305.70)
(4)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Population (suspected PA or positive ARR)	3/6	-0.911	0.631	0.208	0.4 (0.08 - 2.04)
Readout variable (ARR or PAC)	6/3	2.037	0.771	**0.046**	7.67 (1.06 - 55.66)
(5)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Readout variable (ARR or PAC)	6/3	2.283	0.779	**0.026**	9.81 (1.46 - 65.93)
(6)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Population (suspected PA or positive ARR)	3/6	-1.253	0.801	0.169	0.29 (0.04 - 2.03)

ARR, aldosterone-to-renin ratio; CI, confidence interval; No., number of studies; PA, primary aldosteronism; PAC, plasma aldosterone concentration; RDOR, relative diagnostic odds ratio; ROC, receiving operation characteristic curve; Std. Err., standard error.

Table 8. Meta-regression of the Saline Infusion Test

(1)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Continent (Europe or Asia)	6/4	0.647	0.910	0.528	1.91 (0.11 - 34.63)
Population (suspected PA or pos ARR)	3/7	1.349	1.606	0.463	3.85 (0.02 - 639.78)
Cutoff (chosen by ROC or not)	5/5	-0.205	1.429	0.895	0.81 (0.01 - 76.98)
Reference (golden or gold)	3/7	-0.207	1.286	0.883	0.81 (0.01 - 48.75)
Position (seated or recumbent)	5/5	1.459	0.966	0.228	4.30 (0.20 - 93.05)
(2)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Continent (Europe or Asia)	6/4	0.770	0.761	0.369	2.16 (0.26 - 17.84)
Population (suspected PA or positive ARR)	3/7	1.180	1.004	0.305	3.26 (0.20 - 52.88)
Reference (golden or gold)	3/7	-0.190	1.113	0.872	0.83 (0.04 - 18.16)
Position (seated or recumbent)	5/5	1.444	0.723	0.116	4.24 (0.57 - 31.56)
(3)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Continent (Europe or Asia)	6/4	0.904	0.645	0.220	2.47 (0.47 - 12.96)
Population (suspected PA or positive ARR)	3/7	1.048	0.632	0.158	2.85 (0.56 - 14.48)
Position (seated or recumbent)	5/5	1.456	0.573	0.052	4.29 (0.98 - 18.72)
(4)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Population (suspected PA or positive ARR)	3/7	1.276	0.743	0.137	3.58 (0.58 - 22.05)
Position (seated or recumbent)	5/5	1.440	0.725	0.094	4.22 (0.72 - 24.88)
(5)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Position (seated or recumbent)	5/5	1.082	0.845	0.241	2.95 (0.40 - 21.77)
(6)					
Variable	**No.**	**Coefficient**	**Std. Err.**	**p value**	**RDOR (95% CI)**
Population (suspected PA or positive ARR)	3/7	0.837	0.918	0.392	2.31 (0.26 - 20.22)

ARR, aldosterone-to-renin ratio; CI, confidence interval; No., number of studies; PA, primary aldosteronism; RDOR, relative diagnostic odds ratio; ROC, receiving operation characteristic curve; Std. Err., standard error.

4.4.7 Sensitivity Analysis and Publication Bias

A sensitivity analysis performed by omitting each single study, showed no significant difference in the pooled results for ARR, CCT, and SIT (Figure 6-8).

Neither Deek's funnel plot nor Deek's test showed evidence of publication bias (p= 0.73 for ARR, p= 0.81 for CCT, p= 0.76 for SIT) (Figure 9).

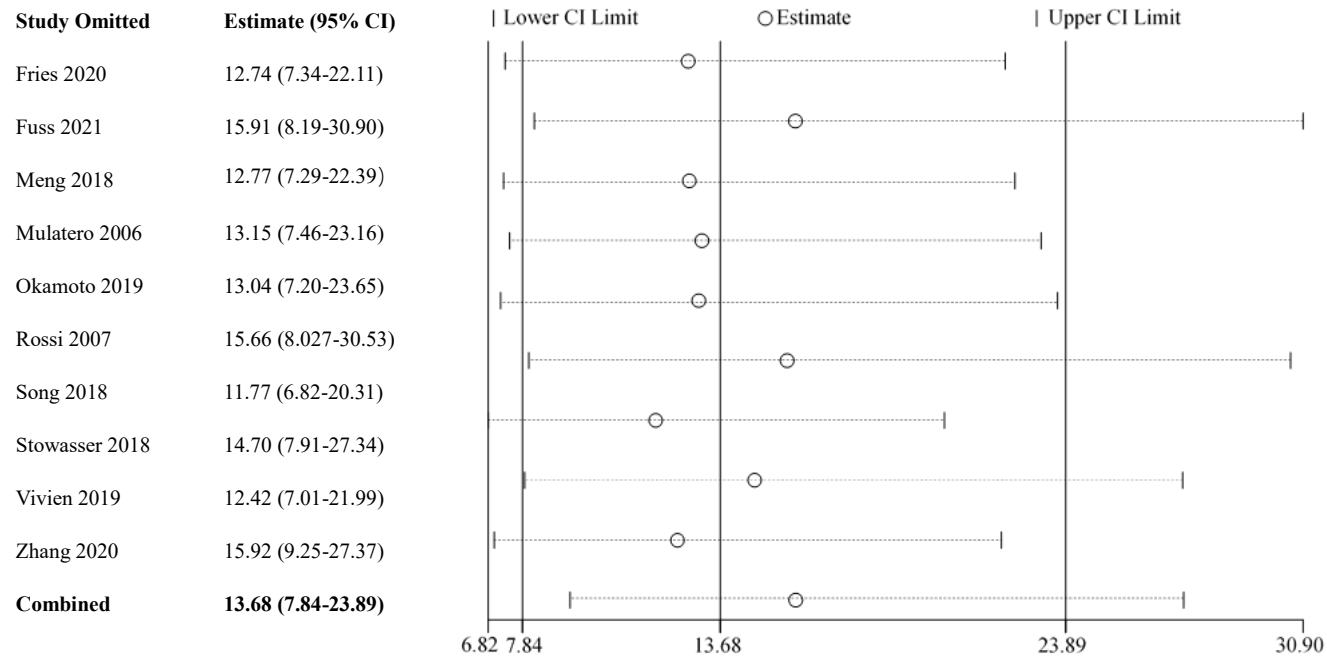**Figure 6.** Sensitivity Analysis of the Aldosterone-to-renin Ratio

Study Omitted	Estimate (95% CI)
Bernini 2008	38.54 (15.08-98.50)
Burrello 2016	37.05 (14.96-91.75)
Ducher 2012	37.95 (14.84-97.07)
Fries 2020	42.77 (16.80-108.87)
Giacchetti 2006	37.12 (15.58-90.94)
Maiolino 2017[a]	49.91 (16.43-151.68)
Maiolino 2017[b]	28.22 (13.05-60.99)
Okamoto 2018	51.58 (23.57-112.87)
Vorselaars 2018	36.89 (14.99-90.81)
Weickert 2009	42.41 (16.66-107.98)
Combined	**39.78 (16.74-94.54)**

Meta-analysis estimates, given named study is omitted. a and b represent the exploratory and the validation cohort in Maiolino's study, respectively.

Figure 7. Sensitivity Analysis of the Captopril Challenge Test
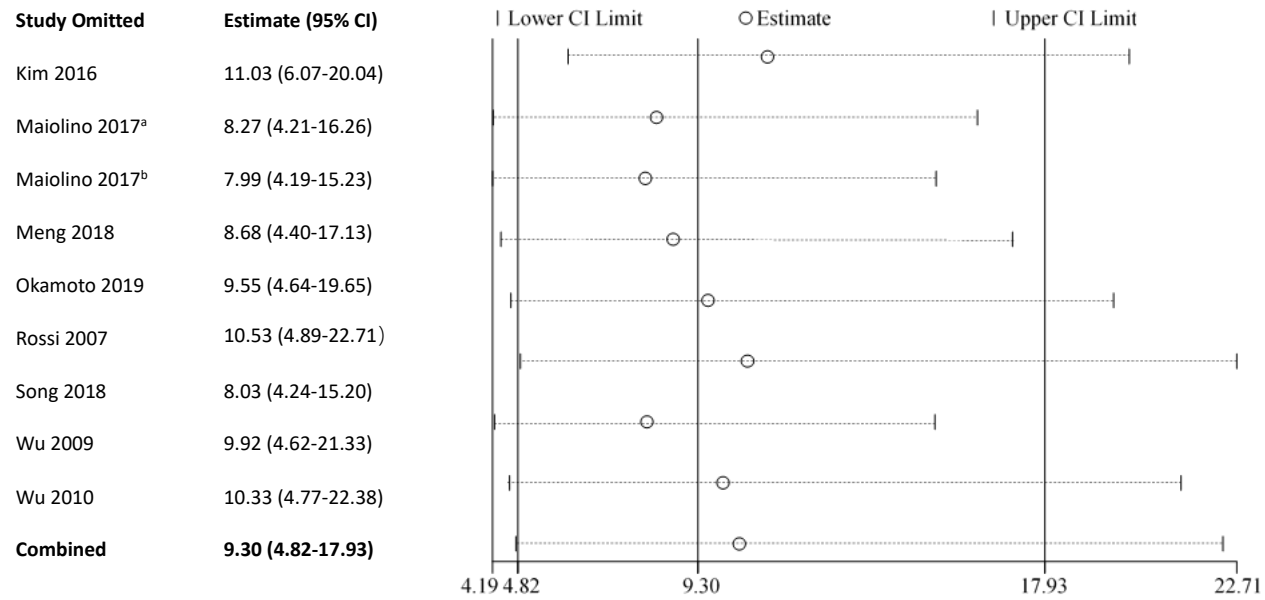
Study Omitted	Estimate (95% CI)
Kim 2016	11.03 (6.07-20.04)
Maiolino 2017[a]	8.27 (4.21-16.26)
Maiolino 2017[b]	7.99 (4.19-15.23)
Meng 2018	8.68 (4.40-17.13)
Okamoto 2019	9.55 (4.64-19.65)
Rossi 2007	10.53 (4.89-22.71)
Song 2018	8.03 (4.24-15.20)
Wu 2009	9.92 (4.62-21.33)
Wu 2010	10.33 (4.77-22.38)
Combined	**9.30 (4.82-17.93)**

Meta-analysis estimates, given named study is omitted. a and b represent the exploratory and the validation cohort in Maiolino's study, respectively.

Figure 8. Sensitivity Analysis of the Saline Infusion Test

Study Omitted	Estimate (95% CI)
Fries 2020	12.74 (7.34-22.11)
Fuss 2021	15.91 (8.19-30.90)
Meng 2018	12.77 (7.29-22.39)
Mulatero 2006	13.15 (7.46-23.16)
Okamoto 2019	13.04 (7.20-23.65)
Rossi 2007	15.66 (8.027-30.53)
Song 2018	11.77 (6.82-20.31)
Stowasser 2018	14.70 (7.91-27.34)
Vivien 2019	12.42 (7.01-21.99)
Zhang 2020	15.92 (9.25-27.37)
Combined	**13.68 (7.84-23.89)**

Meta-analysis estimates, given named study is omitted.

Figure 9. Funnel Plot Assessing Publication Bias of the ARR, CCT, and SIT

   A.   ARR
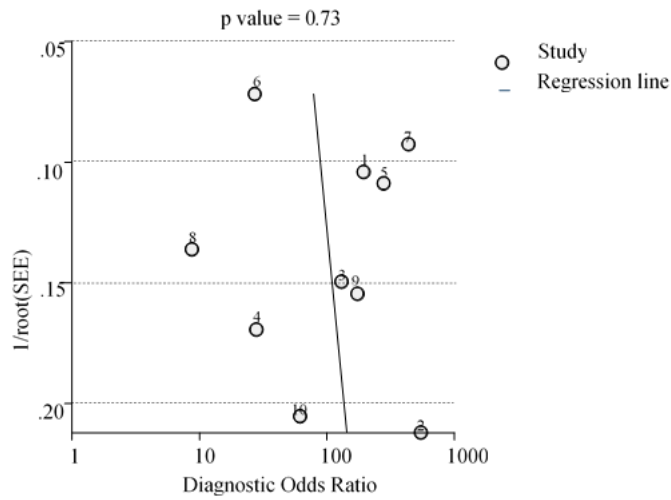
   B.   CCT
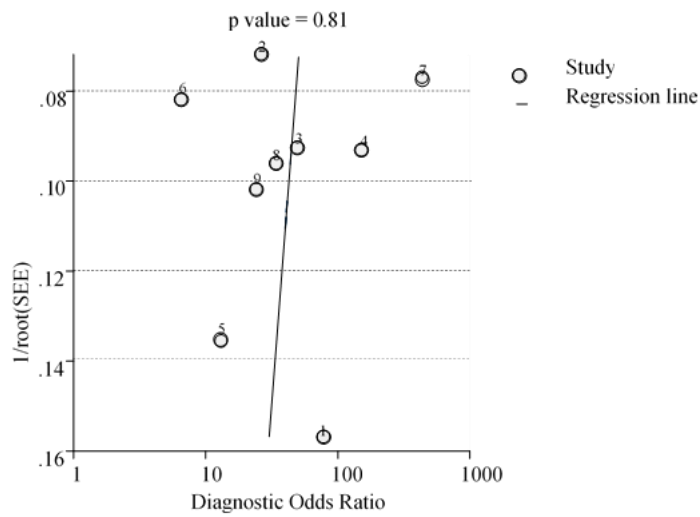
   C.   SIT
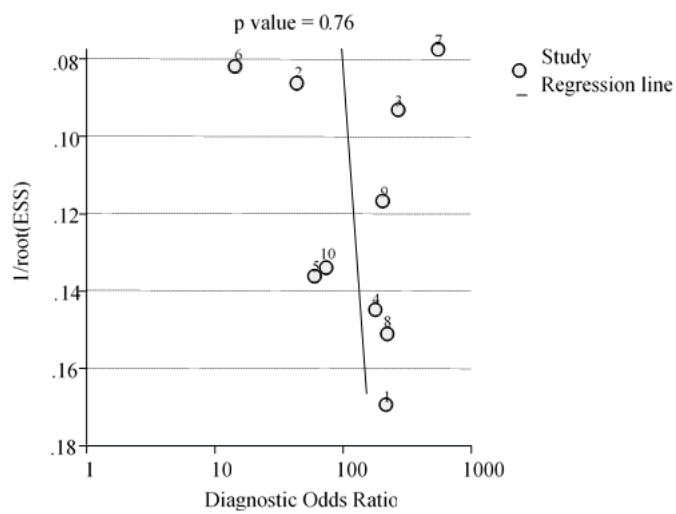
ARR, aldosterone-to-renin ratio; CCT, captopril challenge test; SIT, saline infusion test.

4.5 DISCUSSION

We meta-analyzed the diagnostic accuracy of the most popular tests for the screening and exclusion of PA by a novel quantitative approach to select eligible studies and following the STARD recommendation.(11) We determined beforehand to examine only studies that met predefined quality criteria in that they used a solid reference index and were not tautologically biased, by attempting to validate an exclusion test employing another non infallible test. Moreover, considering that the key issue is to identify unilateral surgically curable forms of PA because, like low-renin essential hypertension, bilateral PA requires medical treatment, we meta-analyzed only studies that used, as reference, the "gold" diagnosis of uPA confirmed by biochemical cure after surgery. In an exploratory survey, we also used a less certain (golden) diagnosis of uPA, entailing results of AVS, pathology, and BP fall after surgery.

Hence, based on these diagnostic criteria involving a different level of certainty, our meta-analysis involved two-phases: an exploratory and a validation phase that involved studies that used a golden and a gold reference, respectively.

The first most important findings were that i) the ARR, when carefully performed in a standardized way in referral centers, provided a high accuracy for identification of uPA; ii) neither CCT nor SIT furnished an additional diagnostic gain.

These results are in keeping with previous investigations: the largest study that prospectively examined with a standardized protocol more than two thousand newly-diagnosed referred hypertensive patients, 4% of whom received a diagnosis of uPA by the gold criterion, reported identical results(7).

Likewise, a prospective Japanese study of 102 patients with an elevated ARR (>20 ng/dL/ng/mL/h), where the accuracy of the ARR for discriminating uPA from PH and IHA patients was compared to CCT, SIT, and FUT(35), also reached the same conclusions. At

variance, Fries et al. using a golden reference standard, reported that a post-SIT PAC carried the highest accuracy, i.e. a sensitivity of 97% and a specificity of 92% in 104 consecutive patients with suspected PA(33). However, their PAC cut-off value, measured by liquid chromatography tandem mass spectrometry (LC-MS/MS), was 83 pmol/L (3 ng/dL), which corresponds to the lowest limit of detection of their assay for reliable PAC measurement. In the PAPY study that used Youden index analysis to determine post-SIT cut-off value with radioimmunoassay (RIA), the optimal cut-off was much higher (6.75 ng/dL),(36) close to that (5 ng/dL) independently found by Burello et al.(21). While owing to the lack of antibody cross-reactivity with other steroids(41), LC-MS/MS might give lower values than the immunoassays, in our experience 3 ng/dl value is a far too low PAC value for an exclusion test. In fact, in studies using the gold reference of uPA, by using such low cut-off, the overlap of post-SIT PAC values between uPA patients and PH, and/or bilateral PA patients is huge(36,40). This means that, although all uPA patients likely show PAC > 3 ng/dL, thus accounting for the very high sensitivity, many false positive ARR results will also exhibit values above this cut-off.

It must be underlined that studies of exclusion tests showed a prominent heterogeneity, whose source could not be entirely revealed by a meta-regression, owing to several plausible reasons. One, likely the most important, regards use of PA or uPA diagnosis, as reference. As discussed above, another one was the biochemical methods for measuring aldosterone: currently, the three methods for measuring aldosterone: LC-MS/MS, and the immunoassays (RIA, and chemiluminescence [CLIA]). Although expected to provide identical results, in reality, they exhibit significant inter-assay variabilities. A source of heterogeneity also comprises the patient's preparation. A two-week withdrawal of interfering drugs is too short, particularly for β-blockers and RAS inhibitors that, in our experience affect renin levels for more than 4 weeks.

A further source of variation relates to the calculation of the ARR, that can be performed with renin measured as PRA (by RIA) or as direct renin concentration by CLIA or by LC-MS/MS. These assays provides results in different units of measure and although values can be easily converted by an available APP(42), this was not systematically exploited in published studies: in fact, one had to be excluded from the meta-analysis because of obviously wrong renin data.

The thresholds also differed among studies: 45% of the investigators used arbitrarily chosen cut-off values and only 55% of them determined their cut-offs by ROC curve and Youden index analysis. Further heterogeneity originated from the choice of the readout of this test. For example, for the CCT it was either PAC or the ARR, or both.

A preselection bias was evident, at least in some studies, as the ARR was not done in consecutive hypertensive patients but mostly in patients with suspected PA with few exceptions(3,7,21,27,28,34). The exclusion tests were performed in patients selected for a higher prior probability of PA, mostly in patients with positive ARR. Accordingly, the uPA/controls ratio differed by 26.5-fold (from 2.8% to 73.5%, $32.94 \pm 24.20\%$), and by 5.9-fold (from 11.8% to 69.7%, $37.65 \pm 20.16\%$) for SIT.

Finally, factors as race, serum K^+ levels, salt intake at testing, might have also contributed to heterogeneity, although they did not emerge at meta-regression.

A further important methodological flaw needs to be mentioned: 48% of the studies performed an entire work-up only in the cases with a positive ARR and/or exclusion test result, and not in those with negative results. This verification bias by leaving the diagnosis uncertain in the negative cases, might lead to overestimating the test performance.

Surprisingly, use of the FST, which has been proposed as the most reliable exclusion test for PA, is supported by only one study;(38) the same applies to the FUT.(35) Thus, these tests did not lend themselves to a meta-analysis could.

Nonetheless, we would like to emphasize that this meta-analysis has major strengths. They comprise a painstaking selection of the eligible studies based on a novel quantitative analysis of their quality, the evaluation of the performance of each test using uPA as reference, and the fact that data were examined according to the level of diagnostic certainty.

In summary, this meta-analysis revealed that studies of exclusion tests for PA are markedly heterogenous. Importantly, albeit seemingly highly sensitive and specific, the exclusion tests did not provide any diagnostic gain over a well performed ARR. These tests are based on the premise of excess production of aldosterone autonomous from angiotensin II, whilst human aldosterone-producing adenoma (APA) were consistently found to express the angiotensin type I receptor, which mediates the secretagogue action of on aldosterone. Moreover, angiotensin II-induced aldosterone secretion from APA strips and cells ex vivo has been demonstrated(10). Thus, relying on exclusion tests may preclude the chance of long-term surgical cure to patients with angiotensin II-responsive uPA. This meta-analysis, even restricted to the studies that met the tightest quality criteria, showed no evidence to support the systematic use of exclusion tests in clinical practice. As these tests are time-consuming, not free of risks because of the need to keep patients on the "switch" antihypertensive treatment, and increase the costs and complexity of the diagnostic work-up of PA, thus contributing to the under-detection of PA, their usefulness should be proven in a large outcome-based prospective study comparing head-to-head strategies "with and without exclusion tests" before their systematic use can be recommended.

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**5. CHAPTER 4. POSTURAL BALANCE AND LOWER LIMBS NERVE
CONDUCTION IN PATIENTS WITH UNILATERAL PRIMARY
ALDOSTERONISM**

5.1 INTRODUCTION

Hypertension-mediated organ damage (HMOD) is defined as the structural or functional alteration of the arterial vasculature and/or the organs it supplies that is caused by high blood pressure (BP)(1). Primary aldosteronism (PA) is the most common cause of secondary hypertension (SH), cardiovascular complications and HMOD are more frequent then in those with essential hypertension(2,3).

In the study that studied relationship between number of white matter lesions (WML) in the subcortical white matter found negative correlation with PRA and positive with PAC, and ARR accordingly, using PET scan(4). Kunikata et.al reported that patients with PA are more likely to develop silent brain infarction (SBI) in comparison to age-matched PH patients(5), moreover, patients with aldosterone-producing adenoma (APA), and bilateral adrenal hyperplasia both are at higher risk for stroke and coronary artery disease(3). Silent brain damage, and diffuse WML in the periventricular region and the brain stem could lead to disruption of neuronal connections and secondary dysfunction of brain specific area. Postural balance is one of the potentially affected brain functions which is regulated with help of visual, musculoskeletal, and proprioceptive function.

5.1.1 Postural balance

Balance, or stability, is defined as the act of maintaining, achieving, or restoring a state of balance during standing or activity, and its controlled by the sensory and motor systems, and dependents on many circuits of the central nervous system (CNS)(6). Hausdorff et.al previously reported that hypertensive patients have reduced performance on balance and gait tests in comparison to age-matched normotensive control group(7). No such evidence is available for PA, or any other forms of HT due to an altered activity of the renin-angiotensin-aldosterone system (RAAS), the analysis of the cerebrospinal fluid of patients with

Normotensive Hydrocephalus, who typically show gait disturbances and imbalance, identified an excess aldosterone concentration(8).

Full understanding of postural balance also requires consideration of the peripheral nervous system including, sensory and motor nerves of lower limbs which mediate key inputs and outputs of the system.

Of note, in the elderly HT is an independent risk factor for peripheral neuropathy and chronic symmetric polyneuropathy(9,10), and in general for idiopathic peripheral neuropathy (11). The later typically presents with paresthesia, pain, weakness, and in severe cases loss of deep tendon reflexes. Notably, this very symptoms are typical of patients with hypokalemia, which is present in 20–25% of patients with PA(12)˙(13). Patients with chronic serum potassium depletion can present with hypokalemic periodic palsy, characterized by reduced compound motor action potential (CMAP) amplitude with hyperpolarization of axonal membrane(14).

In addition to the above peripheral systems somatosensory, motor, visual, and vestibular relays work synchronously to orient the body, and to maintain balance. During upright standing, somatosensory afferent impulses from the lower limbs drives bidirectional feedback, moreover, reduced somatosensory information from the lower limbs alter the ability of postural responses(15–17). In patients with severe peripheral neuropathy full compensation of visual, and vestibular inputs do not fully compensate for the impairment in proprioception with progressive deterioration of balance (17).

In general, studies on the association between BP, and standing balance by center of pressure velocity in healthy adults and elderly have furnished conflicting results and the performance of all above mentioned mechanisms in PA remains unknown.(7)˙(18,19) During my involvement in the diagnostic work-up of hypertensive patients as described in Chapter 2,

we made an observation that patients with PA, when investigated in detail, often reported mild sensory disturbances in the limbs which few cases, disappeared after surgical cure.

This led us to establish a clinical observation-driven diagnostic protocol in patients with PA, based on the hypothesis that those who, suffer from chronic depletion of serum potassium and at same time having systemic influence of high BP on the CNS and proprioceptors could show a reduction of action potential generation of sensory and motor nerve, and of nerve conduction velocity. As a result, we also expected PA patients to show an impairment in postural balance, which may represent subclinical and neglected risk factor for falling, probably in the context of subsequent "hits" like ageing. Finally, if there is worse postural balance performance and nerve conduction in PA patients we hypothesized that it would be resolved by cure of the aldosteronism.

5.2 STUDY AIMS:

1. To prospectively measure postural balance indexes (including center of pressure velocity, sway area, antero-posterior sway and medio-lateral sway) in patients with salt-dependent arterial hypertension due to PA before and after surgical cure and/or MR antagonist.

2. To evaluate lower limb motor and sensory nerve conduction velocity, amplitude and latency in the same patients.

3. To explore the impact of relevant covariables (i.e. sex, age, serum K+ level, plasma aldosterone concentration, direct renin concentration, and aldosterone to renin ratio) on these measures.

5.3 METHODS

5.3.1 Study design and participants

This was a prospective, single-center study conducted at the Arterial Hypertension Center and The Orthopedics and Rehabilitation Unit of the University of Padova, Italy, between May 2021 and July 2022.

Adult hypertensive patients (18-80 years old) referred to the Hypertension outpatient clinic and presenting with any feature listed in the SIIA 2020 guidelines for PA(20) were prospectively screened for PA. The whole screening and diagnostic workflow is described in Chapter 1 (Figure 1). A summary diagnostic work flow is presented in Figure 2.

Variables of interest were collected at this time, in washout from antihypertensive drugs that interfere with the RAAS, and therefore during pharmacological therapy with MR antagonists (≥1 month). This within-patient prospective design minimized the experimental variability and the post-adrenalectomy time point served as the most reliable control after PA resolution.

For patients with evidence of unilateral disease at adrenal vain sampling (AVS), who underwent adrenalectomy, post-operative study measures were collected after ≥3 months, upon conclusion of biochemical cure (normalization of PAC, DRC and serum K^+: i.e. PAC value of 2-9 ng/dL, DRC >6mU/L, and, serum K+ >3.8 mEq/L) and fulfillment of 5 corners criteria, as for Gold standard retrospective definition of unilateral PA (please see chapter 2). Patients with PA: (1) who has significant elevation of ARR; (2) who able to provide written informed consent; (3) who aged between 30 till 80 years old were included in this study. Patients with: (1) proven bilateral adrenal hyperplasia; (2) impaired cognitive status (<26 on MMSE score, obtained during the first visit study before postural balance assessment); (3) coexisting neurological disorders (stroke with disabling outcomes and/or with sensory deficits, Parkinson's disease, multiple sclerosis); (4) psychiatric disorders requiring drug treatment; (5) vestibule-cochlear diseases; (6) previous lower limb surgery; (7) patients with

severe thrombocytopenia (platelets below 50,000/mm3); (8) patients who denied to participate in the study and (9) diabetes requiring insulin or hypoglycemic drugs were excluded from this study.
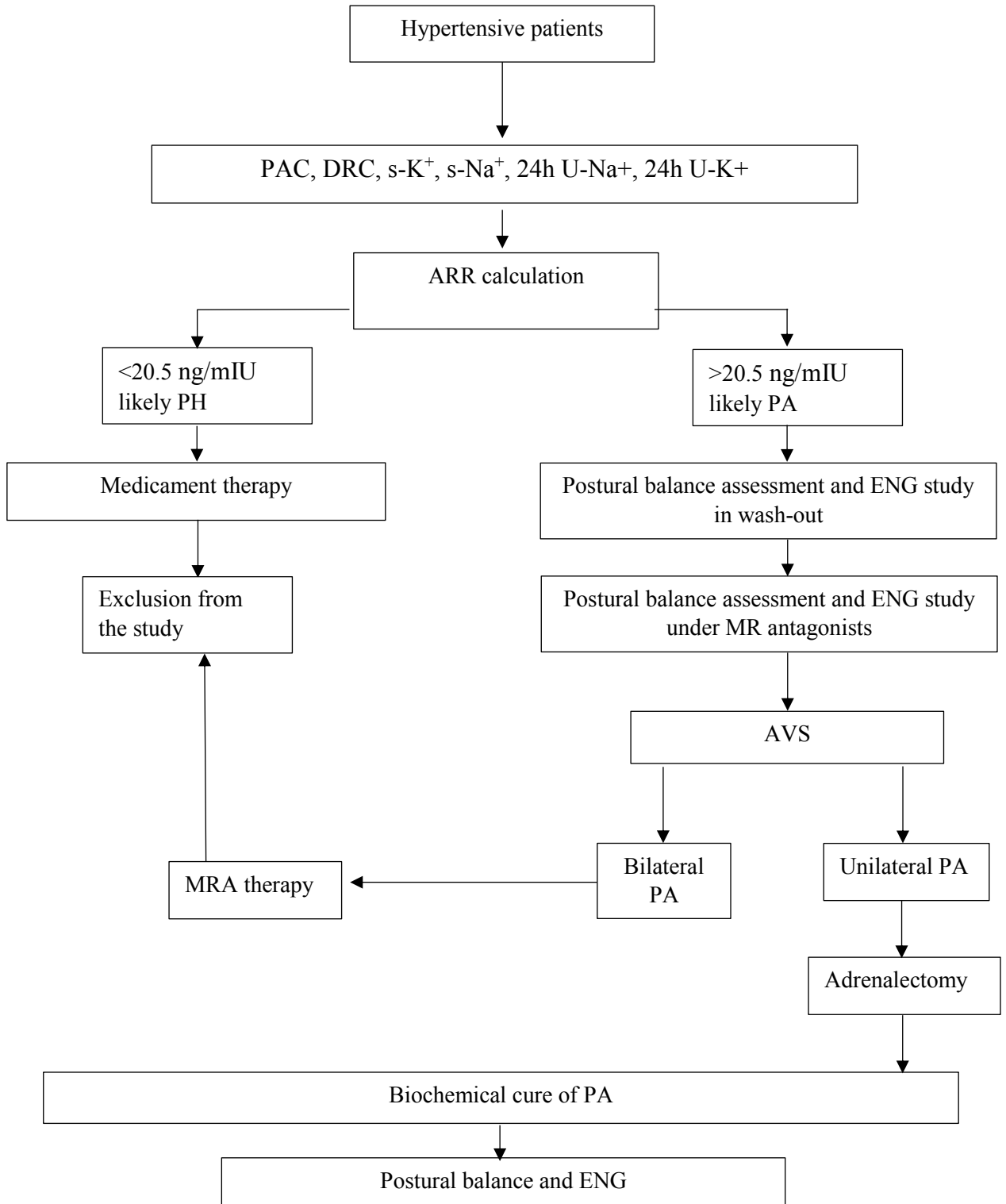
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┌─────────────────────────────────┐
│       Hypertensive patients     │
└─────────────────────────────────┘
                 │
                 ▼
┌──────────────────────────────────────────────────┐
│ PAC, DRC, s-K⁺, s-Na⁺, 24h U-Na+, 24h U-K+        │
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┌─────────────────────────────────┐
│         ARR calculation         │
└─────────────────────────────────┘
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$PAC, DRC, s\text{-}K^+, s\text{-}Na^+, 24h\ U\text{-}Na+, 24h\ U\text{-}K+$

ARR calculation

<20.5 ng/mIU likely PH

>20.5 ng/mIU likely PA

Medicament therapy

Postural balance assessment and ENG study in wash-out

Exclusion from the study

Postural balance assessment and ENG study under MR antagonists

AVS

MRA therapy

Bilateral PA

Unilateral PA

Adrenalectomy

Biochemical cure of PA

Postural balance and ENG

Figure 2. Study flowchart. ARR – aldosterone to renin ratio; APA – aldosterone producing adenoma; AVS, adrenal vein sampling; BAH – bilateral adrenal hyperplasia; DCR – direct renin concentration; ENG, electroneurography; MRA - mineralocorticoid antagonist; PA, primary aldosteronism; PAC – plasma aldosterone concentration; PH, primary hypertension;

5.3.2 Study measures

At each time point, consenting patients underwent postural balance assessment and ENG evaluation of the lower limbs. On the same day, blood and 24h samples were collected in standardized conditions (please see chapter 1) for RAAS and electrolyte biochemical measurements. At each time point patients asked for falling down history.

5.3.2.1 Postural balance assessment

The assessment of postural balance was performed by stabilometry, an objective study of body sway during quiet standing, in the absence of voluntary movements or external perturbations. For the study protocol patients were requested to stand on the stabilometric force platform (Argo plus MK1, Genova, IT) in following conditions: (1) single task eyes open (EO); (2) single task eyes closed (EC) (Figure 3); (3) head-extended open eyes (HE-OE) and (4) dual-task eyes open (DT-EO) conditions. Postural balance performance included assessment of center of pressure velocity (CoPv) (mm/s), sway area (cm^2) (SA), antero-posterior (cm) (AP) and medio-lateral (cm) (ML) sway. CoPv was measured at a frequency of 100 Hz for 40 seconds.

Single task

The single task was performed during the quiet standing on stabilometric force platform. The investigator asked patients to stand upright barefoot with feet together, eyes open for 40 sec. fixing eyes at reference point then after with closed eyes for 40 sec. duration in same position without any kind of voluntary movement.

Head-extended open eyes test

Patients were standing in upright barefoot with feet together on the force platform with maximal neck extension with open eyes for 40 sec.

Dual-task

Standing in upright barefoot with feet together on the force platform, while postural balance assessment patients were asked to count backwards as fast and accurately as possible counting backwards from 100 with provided starting number for the duration of the trial. Balance control was evaluated barefoot maintaining stable posture fixing eyes at reference point located at eye level during 40 seconds. Patients performed 3 trials with eyes open (single and dual task conditions) and 1 trial with eyes closed.
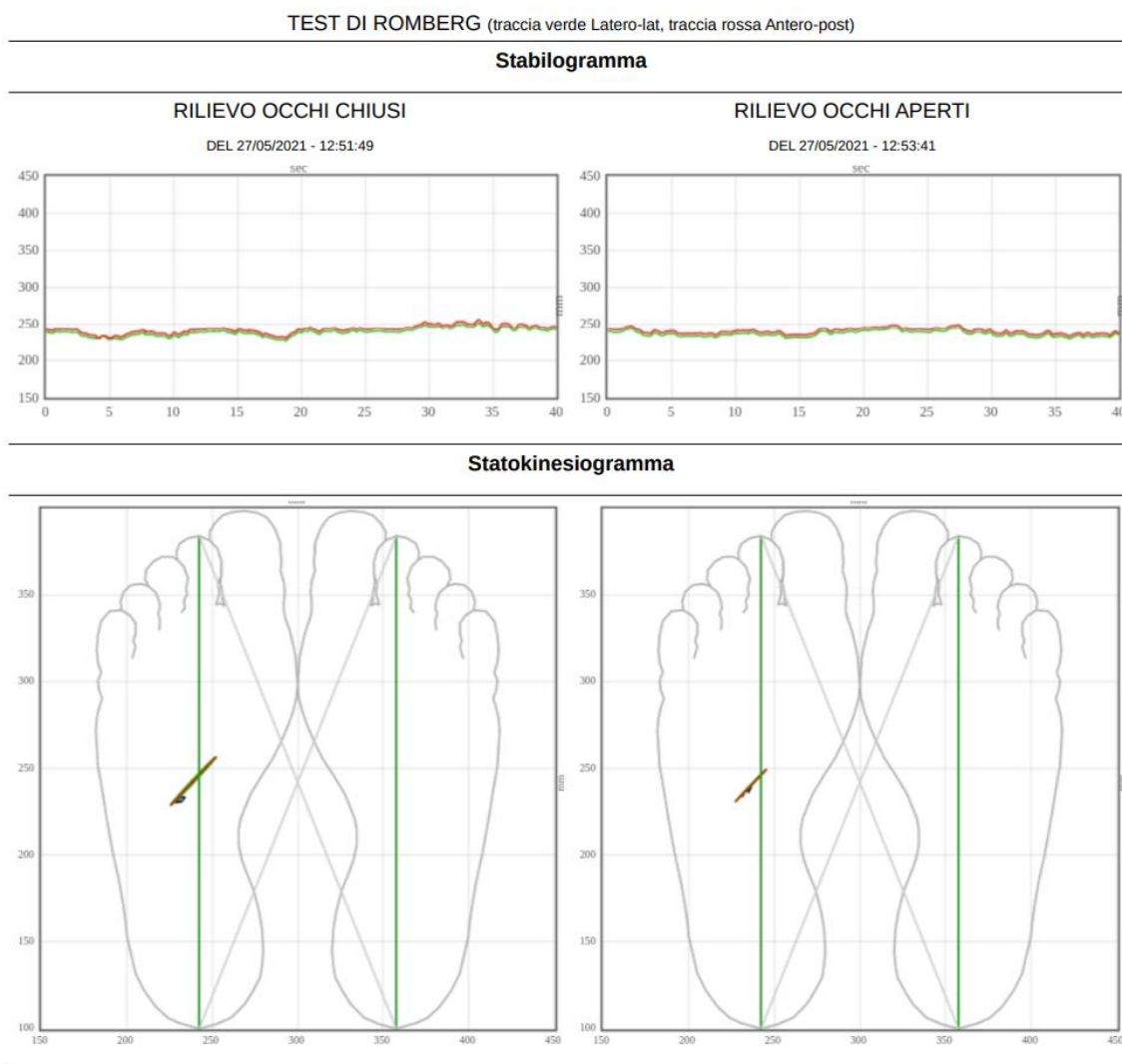
Figure 3. Postural balance assessment in EO and EC conditions.

To avoid possible effect of orthostatic hypotension (OH) on static balance performance BP measured on the right arm after immediate standing up on stabilometric force platform after 1 and 3 minutes. Orthostatic hypotension (OH) was defined by the American Autonomic Society and the American Academy of Neurology(21) as a systolic blood pressure decrease

of at least 20 mm Hg or a diastolic blood pressure decrease of at least 10 mm Hg within three minutes of standing.

5.3.2.2 Electroneurography (ENG) evaluation of lower limbs

For electroneurography (ENG) assessment patients were investigated first time within medical washout from antihypertensive drugs which alter the RAAS, then for the second time under MR antagonists and for third time after adrenalectomy if PA cure is fully satisfy the gold diagnostic standard. All tests were performed with Nicolet^{TM} EDX-VIKING with Software version 20 or Newer, and with one licensed neurophysiologist. The ENG record was performed using stimulating, and recording superficial electrodes, and with disposable standard concentric electrodes. The motor fibers of the peroneal nerve, and sensory fibers of the sural nerve was selected for the stimulation. Selected electroneurographic parameters were analyzed in detail: stimulated nerve latency (Lat), stimulated nerve amplitude, and nerve conduction velocity (CV). Sensory nerve action potential (SNAP) parameters were also evaluated: the latency (Lat), sensory nerve conduction velocity (SCV), and its amplitude. The neurophysiologist performing nerve conduction assessment was blinded to the patients clinical state to reduce interpretation bias.

Electrode placement during ENG

The CMAP was record active electrode was placed on the belly of the extensor digitorum brevis muscle, and the indifferent electrode on the distal tendon of the recorded muscle. The peroneal nerve was stimulated over the dorsum of the foot near the ankle, at 7 centimeters from the recording electrodes (Figure 4). The stimulus intensity was gradually increased until the maximal CMAP was obtained(22).
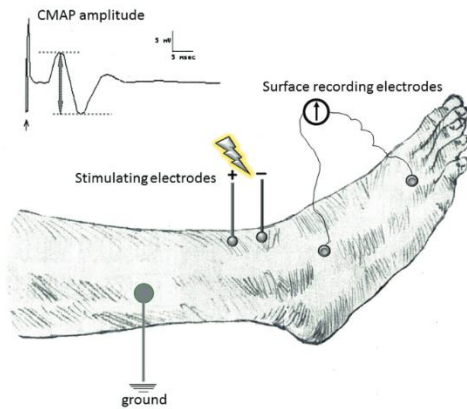
Figure 4. Schematic electrode placement during CMAP recording of the lower limbs.

The sural nerve with surface electrodes were done with antidromic stimulation as described earlier(23). The recording site was behind the lateral malleolus, and the stimulation electrode was placed 13 cm proximally, lateral to the edge of the Achilles tendon. A dorsal sural nerve was done with antidromic stimulation. Recording site was the mid-portion of the fifth meta-tarsal bone just lateral to the extensor digitorum longus tendon of the fifth toe with the reference electrode 2 cm distally. Stimulation site was posterior to the lateral malleolus with the cathode placed 12 cm proximal from the recording electrode (Figure 5). Since there are no international recommendations for the exact measurements, and amplitude of the SNAP can be measured in several different way, we decided to measure sural SNAP as a following: from the baseline to the last positive peak(23) (Figure 5).
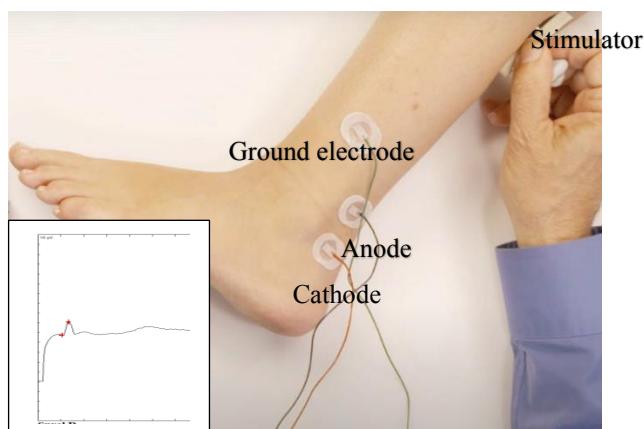
Figure 5. Electrode placement during the SNAP measurement of sural nerve.

To avoid the negative effect of a low temperature on conduction parameters the room temperature always maintain at same degree during each measurement.

Statistical analysis

Statistical analysis performed with SPSS (Version 29.0 for Windows, SPSS Inc., Chicago, IL) and GraphPad Prism 9. Continuous variables: results are expressed as mean and standard deviation (SD), and as frequencies (percentage) for categorical variables. Differences across three clinical state in demographic, biochemical characteristics, postural balance and nerve conduction were analyzed by a Chi-Squared test for categorical variables, and either Mann-Whitney U or paired t-tests, as appropriate. The association between postural balance/nerve conduction was analyzed with stepwise linear regression analysis.

5.4 Results

A major difficulty that occurred during this study was the development of the COVID-19 pandemics, which stopped and delayed for surgery including adrenalectomy from March 2020 till February 2021. Therefore, this current study comprised 19 patients, and 2 patients refused to participate, so we include 17 patients in their washout state, from which 8 patients reassessed under MRA treatment, and 6 patients who had adrenalectomy studied again when they had full recovery of PA under gold diagnostic standard as mentioned in Chapter 3.

Demographic characteristics is reported in Table 1. Mean age of patients was 52 ± 10.6, female patients were 23.5%, mean BMI of whole cohort was 26.9 ± 3. On 1 and 3 minutes after immediate standing up on stabilometric platform was measured BP, and there was no significant difference between measurements (p=0.3).

Table 1. Demographic characteristics of the patients (n=17) enrolled in the study.

Variables	Washout (n=17)	MR antagonist (n=8)	Follow-up (n=6)
Gender male/female, % (range)	14/3 (82.3)	8/0 (100)	5/1 (20)
Age (years), whole cohort	52±10.6		
Systolic BP (mm Hg)	143.4±18.48	132.9±11.23	130.3±23.66
Weight (Kg)	169.6±8		
Height (cm)	77.7±12.4		
BMI (Kg*m^{-2}), whole cohort	26.9±3		

Abbreviations: BMI, body mass index; BP, blood pressure; MR, mineralocorticoid.

None of patients did not show orthostatic hypotension (OH) following the American Autonomic Society and the American Academy of Neurology definition(21). To distinguish possible effect of antihypertensive drugs on postural balance daily defined dose of antihypertensive drugs calculated in three different state, and we did not found differences (p=0.09). Biochemical characteristics, postural balance and ENG results reported in the Table 2.

Table 2. Biochemical characteristics, postural balance and ENG. Comparison of washout, MR antagonists and follow-up state.

Variable	Washout (n=17) (mean±SD)	MR antagonist (n=8) (mean±SD)	Follow-up (n=6) (mean±SD)	p value
Biochemical data				
PAC (ng/dL)	34.6±22.6		7.5±2.6	0.04 (WO vs FU)
DRC (mIU/L)	4.5±0.7		14.2±14.6	n/s
ARR (ng/dL/mIU/L)	164.3±157.4		0.3±0.5	0.05 (WO vs FU)
s K^+ (mmol/L)	3.5±0.4		4.5±0.6	0.04 (WO vs FU)
Postural balance open eyes single task				
CoPv OE (mm/sec)	10.7±2.7	12.0±5.1	7.9±1.9	n/s
SA OE (cm^2)	16.0±8.9	18.8±7.2	8.0±3.8	n/s
AP sway OE (cm)	20.9±6.1	24.8±6.0	18.5±6.2	n/s
ML sway OE (cm)	19.6±5.2	20.5±5.6	12.5±4.1	0.01 (WO vs FU)
Postural balance closed eyes single task				
CoPv CE (mm/sec)	15.3±4.7	13.5±3.5	7.9±1.9	0.002 (WO vs FU) 0.03 (MR vs PO)
SA CE (cm^2)	23.5±10.7	25.2±17.8	8.0±3.8	n/s
AP sway CE (cm)	25.9±5.6	26.7±8.3	29.8±12.5	n/s
ML sway CE (cm)	23.1±6.2	23.6±5.6	18.1±5.5	n/s
Postural balance open eyes head extended				
CoPv OE-HE (mm/sec)	15.6±4.9	13.0±4.4	9.1±2.4	n/s
SA OE-HE (cm^2)	26.6±14.4	19.7±10.5	13.2±5.9	n/s
AP sway OE-HE (cm)	25.6±6.2	21.2±3.4	24.6±4.8	n/s
ML sway OE-HE (cm)	25.7±7.9	20.4±6.5	14.6±4.2	0.01 (WO vs FU)
Postural balance open eyes dual task				
CoPv OE DT (mm/sec)	14.7±5	13.4±5.8	11.7±3.8	n/s
SA OE DT (cm^2)	22.3±15.1	21.4±20.2	17.8±14.0	n/s
AP sway OE DT (cm)	22.8±6.5	21.4±6.3	23.7±16.0	n/s
ML sway OE DT (cm)	20.5±7.4	20.2±9.2	16.3±6.1	n/s
Electroneuromyography				
Peroneal Ankle latency (ms)	3.7±0.6	2.4±3.2	2.7±2.7	n/s
Peroneal Fibula latency (ms)	10.6±1.0	10.06±1.7	10.6±1.2	n/s
Peroneal Ankle amplitude (mV)	5.3±1.4	6.0±1.1	5.2±1.9	0.005 (WO vs MRA)
Peroneal Fibula amplitude (ms)	4.9±1.4	5.3±0.7	4.9±1.9	0.01 (WO vs MRA)
Peroneal Fibula CV (m/S)	48.3±3.8	46.9±3.1	46.8±5.9	n/s
Sural latency (ms)	3.2±0.5	3.2±0.4	3.4±0.2	0.005 (WO vs FU)
Sural amplitude (mV)	9.9±5.1	11.0±7.3	7.7±3.0	n/s
Sural CV (m/S)	47.4±6.0	44.6±2.5	45.5±3.7	n/s

Abbreviations: AP, antero-posterior; ARR, aldosterone-to-renin ratio; CE, closed eyes; CoPv, center of pressure velocity; CV, conduction velocity; DRC, direct renin concentration; DT, dual task; direct renin concentration; HE, head-extended; ML, medio-lateral; s K^+, serum potassium level; SA, sway area;

There was significant reduction of ML sway in eyes open and eyes open head-extended state with p value of 0.01 for each measurement, between washout and follow-up state. Although, CoPv in closed eyes condition was significantly higher in wash-out state (Figure 6), meaning

that patients affected by PA may have higher postural sway specially without vision control in compare to the same patients who were biochemically cured from PA. However, none of patients at each time point did not report falling history in the one year of period. Surprisingly, when we did paired t-test analysis of nerve conduction there was no differences between wash-out and follow-up state except sural nerve latency (p=0.03 WO vs PO), moreover, we found peroneal nerve ankle amplitude higher in wash-out in compare to MR antagonist state (p=0.005) as well as a peroneal fibula amplitude (p=0.01). From above reported analysis we found that postural balance is associated with PA and PA cured status, since it was needed to perform regression analysis. When we run the linear regression analysis with stepwise adjustment CoPv correlated not only with sural nerve amplitude and also correlated with serum potassium level with significance of p=0.04 and p=0.02 respectively.

Center of Pressure Closed eyes
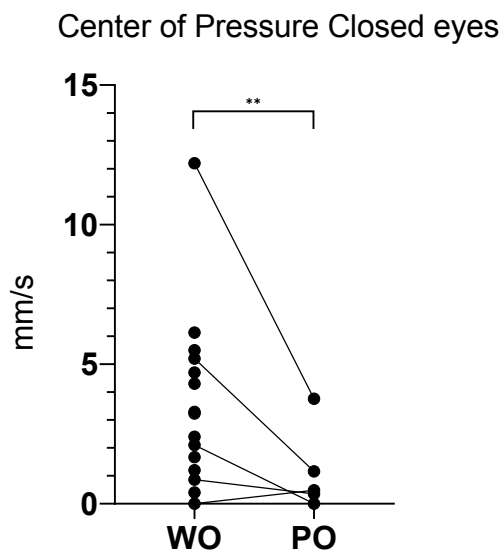
Abbreviations: PO, post-operative; WO, wash-out

Figure 6. Different distribution of CoPv before and after adrenalectomy.

5.5 Discussion

This pilot clinically driven protocol, has prompted the design of larger study unselected hypertensive patients, undergoing standardized biochemical assessment for the exclusion of secondary causes, for which ethics approval is ongoing.

We hypothesized that patients with PA will have reduction of action potential of sensory and motor nerve due to chronic potassium depletion, and systemic influence of high BP, as a consequence postural balance will be worse leading to the higher risk of fall. However, in this study patients did not report history of fall. When we performed postural balance assessment we found significant reduction of body sway in particular CoPv and ML sway in closed eyes condition in patients who were cured from PA in comparison the same patients who had PA. In the previous studies reported negative correlation between HT and postural balance performance, and worse postural balance likely associated with age(18,24). To best of our knowledge this study is the first study for the assessment of postural balance in PA patients, and from HT point of view current study had similar results with previous studies, where found positive association between high BP and postural balance in hypertensive patients in compare to the non-HT patients moreover, results were same when compared patients with controlled HT and uncontrolled HT(25,26). Shen.S et.al studied postural balance in hypertensive patients with and without orthostatic hypotension (OH) and they found patients with hypertension but without OH have better postural balance performance(25), however, in this study we did not see relation between OH and postural balance.

HT could have an additional negative effect on postural balance affecting large and small cerebral arteries which supply specific functional areas of the brain, moreover, it is the main risk factors for diffuse (WML)(27). However, we aimed to focus more on peripheral mechanism of postural balance. The association between peripheral neuropathy and postural

balance is described in few studies nevertheless, their results demonstrate that as well as disease deteriorating the postural balance becomes worse meaning that deficits of sensory feedback lead to the increase of postural sway including CoPv(28).

HT has been identified as an independent risk factor for the development of neuropathy, possibly due to generalized endothelial dysfunction(29,30), results of our study demonstrated the increase of sural nerve latency in wash-out condition and in MR antagonists condition, which might be explained by systemic effect of hypertension, however regression analysis did not show significance.

From the clinical standpoint the importance of this study is that the mean sural nerve amplitude in three different conditions regardless of PA cure was comparable to the patients with diabetic neuropathy(31), moreover, regarding to that point that during this study not one patient developed conduction block and/or reversible conduction failure, this cohort of patients might have sub clinical sensory neuropathy due to demyelination of peripheral nerve. The reason why sural nerve amplitude did not improved after biochemical cure of PA may related to remyelination of peripheral nerves which is highly dependent from the degree and location of the nerve injury and patient related factors(32), moreover, may it is related to the tissue potassium deposition.

In conclusion, this study demonstrate that PA patients have worse postural balance performance in compare to same patients who were cured from PA, especially in closed eyes condition.

Predictors for postural balance improvement after adrenalectomy were sural nerve amplitude and serum potassium level, however, there was no significant difference of sural nerve amplitude between PA patients before, and after adrenalectomy. The limitation of current study is that we did not performed neuroimaging study to the same cohort, moreover study

cohort is small to drive conclusion for clinical and neurophysiological stand point, therefore, additional prospective studies with larger sample size is required, to understand better risk factors of worse postural balance performance and sensory nerve conduction in patients with PA.

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6. CHAPTER 5. THE PREVALENCE OF PRIMARY ALDOSTERONISM AMONG THE MONGOLIAN POPULATION (PAMP STUDY)

6.1 INTRODUCTION

By WHO report, mortality rate due noncommunicable diseases in Mongolia highest across Asia Pacific countries, moreover in 2018 six from ten deaths were associated with cardiovascular diseases (34.4%)(1).

With ratio of 1:1 ischemic, and hemorrhagic stroke are leading cardiovascular (CV) diseases among Mongolian population, furthermore the high blood pressure (BP) is leading cause of hemorrhagic stroke (2015 Stroke guideline, Mongolia). The mortality from stroke is 120 per 100.0 population which is highest in the world. WHO STEPS-4 survey showed nearly one of two person (49.4%) is overweight, one of five people developed CV disease in recent ten years, and values of triglycerides increased among population in compare to 2013. Moreover, this survey in 2019 showed that usage of dietary salt exceed WHO recommendations 2 fold in rural people, and newly diagnosed hypertension (HT) cases more frequent in younger population [2019 STEPS Country Report Mongolia (brief summary)].

Most common and curable form of hypertension (HT) is primary aldosteronism (PA), PA diagnosis rely on aldosterone-to-renin ratio (ARR). ARR is firstly described Kuniwata et.al in 1982, however low-middle income countries still in lack to measure ARR. Recent guidelines suggest to screen for PA in certain category of patients, however in countries where not able to measure ARR to diagnose PA is difficult.

Therefore, due to lack of adequate screening PA, is commonly under screened and, therefore under detected. This is the tragedy because many patients with undetected PA develop stage III and/or drug resistant hypertension, moreover, dead full conditions leading to cardiovascular events and deaths. Thus far screening programs for PA were never conducted in Mongolia for this reason we aimed to investigate the prevalence of PA in adult population of Ulaanbaatar Mongolia.

6.2 Methods

6.2.1 Patient Selection

Newly diagnosed hypertensive patients with serum $K^+ < 4.0$ mmoL/l will do a measurement of plasma aldosterone concentration (PAC) and direct renin concentration (DRC) while patients with serum $K^+ > 4.0$ mmol/l will serve as a control group. Following the 2020 Italian Society of Arterial Hypertension Practical Guideline for the management of PA (2) we also will include patients with below listed conditions as additional conditions for patients who are referred to the Mongolian-Japanese teaching hospital of Mongolian National University of Medical Sciences in Ulaanbaatar Mongolia from September of 2023 till December of 2023 (Table 1). Although prevalence of OSA in hypertensive patients never conducted in Mongolia, since we will also assess all newly diagnosed hypertensive with Epworth sleepiness scale with following polysomnography.

Table 1. PAMP study inclusion criteria

Condition	Description
Severe hypertension	Hypertension stage 3, i.e. SBP ≥180 mmHg and/or DBP ≥110 mmHg
Resistant hypertension	BP values remain above goal in spite of concurrent use of three antihypertensive agents of different classes. If tolerated, one of the three agents should be a diuretic, and all agents should be prescribed at maximum recommended antihypertensive doses.
Patients with hypertension associated with (permanent or intermittent) spontaneous or diuretic-induced hypokalemia	Serum potassium (K^+) < 3.5 mmol/l in absence of other potential causes of hypokalemia.
Hypertension or hypokalemia associated with adrenal incidentaloma	Hypertensive patients with an adrenal mass detected on imaging.
Normal potassium levels (≥3.5 to ≤5.0 mmol/L) associated with another of the above mentioned indications	
When HMOD and cardiovascular or renal morbidity are more severe than expected from the level and duration of hypertension	HMOD such as microalbuminuria, renal disease, hypertensive retinopathy, left ventricular hypertrophy and diastolic dysfunction, etc.
Hypertension and sleep apnea	AHI > 5
Hypertension and a family history of early onset hypertension and/or cerebrovascular accident at a young age (<40 years) and of first-degree relatives with PA	
Newly presenting patients with hypertension and a high chance of cure with adrenalectomy, as, for example, young, women, with short duration of hypertension	

Abbreviations: AHI, apnea-hypopnea index, BP, blood pressure; DBP, diastolic blood pressure; PA, primary aldosteronism; SBP, systolic blood pressure;

Patients with any known prior diagnosis of secondary hypertension, renal artery stenosis, renal insufficiency, and patients who are receiving blood pressure influencing drugs like oral contraceptives will be excluded from the study (Table 2).

Table 2. PAMP study exclusion criteria

Condition	Description
Secondary Hypertension	RAS, PKD, renal insufficiency (GFR<15ml/min/1.73m²)
Heart failure by ACC/AHA Guidelines[10]	**Stage A** identifies the patient who is at high risk for developing HF but has no structural disorder of the heart; **Stage B** refers to a patient with a structural disorder of the heart but who has never developed symptoms of HF; **Stage C** denotes the patient with past or current symptoms of HF associated with underlying structural heart disease; and **Stage D** designates the patient with end-stage disease who requires specialized treatment strategies such as mechanical circulatory support, continuous inotropic infusions, cardiac transplantation, or hospice care
Pregnant women and women who receive oral contraceptives	
Malignant disease	All patients with malignant disease except who has an aldosterone producing carcinoma.

Abbreviations: HF, heart failure; GRF, glomerular filtration rate; PKD, polycystic kidney disease; RAS, renal artery stenosis;

6.2.2 Laboratory measurements

All patients will be instructed to have an overnight fast of 10 hours. In the presence hypokalemia the serum K^+ levels potassium supplementations will be prescribed. Before venous blood sampling, patients will rest in a supine position quietly for at least for 1.5 hours. Blood sampling for measurement of PAC, DRC, and measurement of plasma electrolytes and 25 OH Vit D3 will be withdrawn between 8 to 10 am. 24h urinary Na^+ and K^+ excretion will be measured with a collection of urine samples with beforehand instruction to the patients. All laboratory measurements except PAC, and DRC will be analyzed in the central clinical laboratory of the Mongolian-Japanese teaching hospital. PAC and DRC will be measured using the Diasorin Liason direct renin and aldosterone assay with chemiluminescent immunoassay (CLIA) at the clinical laboratory of the Ulaanbaatar Songdo hospital in Ulaanbaatar Mongolia. Collected samples for PAC and DRC measurement will be stored, and transported from the Mongolian-Japanese teaching hospital to Ulaanbaatar Songdo hospital at 20ºC for no longer than 6 hours after sampling. Aldosterone CLIA kit has inter-assay and intra-assay coefficient of variation is < 10% and < 4.2% respectively

with reliable dilution for samples greater than 100 ng/dL. The direct renin CLIA kit has functional and analytical sensitivity correspondingly <1.96 μIU/mL and < 0.53 μIU/mL and utilizes monoclonal antibodies direct against specific epitopes of the renin molecule allowing its direct quantification.

To prevent untoward effects of antihypertensive drugs, patients will be discontinued medications which interfere the RAAS like potassium wasting diuretics, B-adrenergic blockers, ACE inhibitors, dopaminergic and antihistaminergic medications, and selective serotonin reuptake inhibitor antidepressants at least for 4 weeks. To control HT calcium channel blockers (CCB) and/or α-adrenergic blockers will be prescribed. Since in Mongolia CCB and α-adrenergic blockers not have big choice in case of need MRA will be administered, since MRA like canrenone shown not to alter the ARR as well serum potassium following the EMIRA study(3).

ARR will be calculated with the Smartphone ARR application calculating also PA probability in percentage(4). The cut-off for a positive screening ARR will be defined as more than 2.5 ng/dl per μIU/mL (20.5 ng/mIU) together with PAC of >12 ng/dl and DRC of <12 μIU/mL in case of not markedly elevated ARR (<20.6 but < 45ng/mIU) the author suggests to repeat the ARR under better-standardized condition including dietary salt intake, serum K^+ level and current drug therapy.

BP measurement will be taken thrice in the right arm in a seated position after 5 minutes of rest before vein sampling. The mean of the last two readings will be considered. Heart rate also will be recorded during the blood pressure measurement. Hypertension will be defined as Systolic Blood Pressure (SBP) greater than or equal to 140 or Diastolic Blood Pressure (DBP) greater than or equal to 90 mmHg referring to the European Society of Hypertension guidelines(5). The classification of BP will be done in 3 different hypertension grades: grade

1, SBP 140 to 159 and/or DBP 90; grade 2, SBP 160 to 179 and/or DBP 100 to 109; and grade 3, SBP ≥180 and/or DBP ≥ 110 mm Hg. RH will be defined as blood pressure that remains above 140/90 mmHg in spite of the concurrent use of three antihypertensive agents of different classes, among which is diuretics, at pharmacologically effective doses or controlled BP on four or more antihypertensive agents. Since PA screening never conducted before in Mongolia, might some of patients will present also with hypokaliemic periodic palsy. The diagnosis of hypokalemic periodic palsy will be established on the previous history of an acute attack of flaccid paralysis associated with documented hypokalemia with serum potassium level less than 3.5 mmol/l and recovery of limb muscle weakness after potassium control.

As discusses in the Chapter 3, there is no diagnostic gain of captopril challenge test (CCT) and saline infusion test (SIT) over carefully performed baseline ARR, and based on quantitative information of ARR increases diagnostic accuracy(6) in this study we will not use any confirmatory tests.

All patients with elevated ARR will underwent contrast-enhanced abdominal Computed Tomography (CT) scan of the adrenal glands using 3 mm thick slices to exclude aldosterone producing carcinoma.

Since an adrenal vein sampling (AVS) is not available in Mongolia, patients will receive long-term treatment with MRA except for younger patients who <35 years old with history spontaneous hypokalemia, marked aldosterone excess, and radiologically proven unilateral adrenal lesion, with a diameter of nodule ≥ 10 mm with a normally appearing contralateral adrenal will undergo laparoscopic adrenalectomy with post-surgery histopathological confirmation (Figure 1). On diagnostic workup, an Adjudication Committee (G.P.R.,

T.M.S.) will be responsible for distinguishing whether patients had PA or not using web-based platforms.

All procedures planned in the study are in accordance with the ethical standards of the institutional and/or national research committee and the study will start after approval of the Ethics Committee of the Mongolian National University of Medical Science.
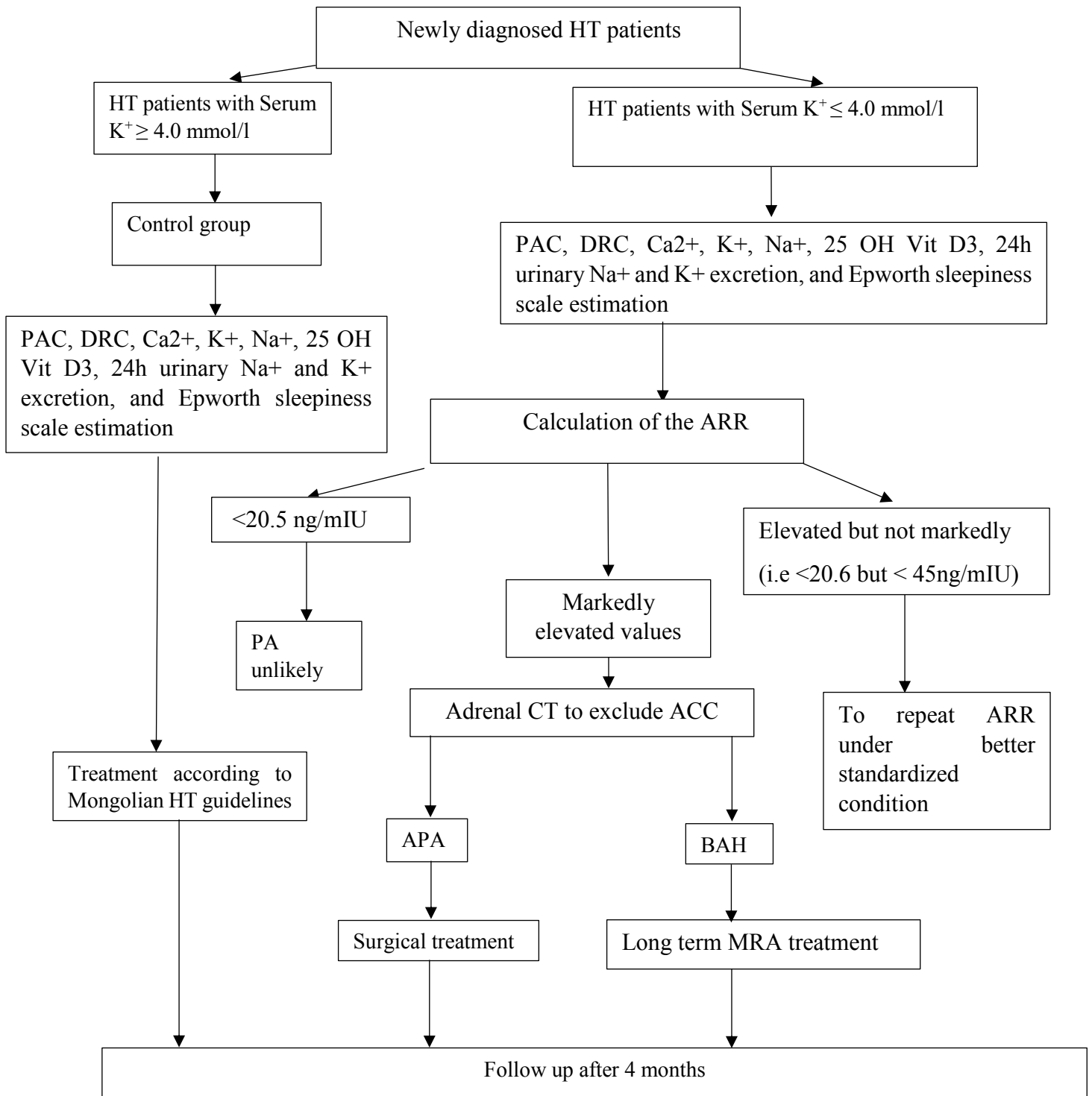
Newly diagnosed HT patients

HT patients with Serum $K^+ \geq 4.0$ mmol/l

HT patients with Serum $K^+ \leq 4.0$ mmol/l

Control group

PAC, DRC, Ca2+, K+, Na+, 25 OH Vit D3, 24h urinary Na+ and K+ excretion, and Epworth sleepiness scale estimation

PAC, DRC, Ca2+, K+, Na+, 25 OH Vit D3, 24h urinary Na+ and K+ excretion, and Epworth sleepiness scale estimation

Calculation of the ARR

<20.5 ng/mIU

Elevated but not markedly (i.e <20.6 but < 45ng/mIU)

Markedly elevated values

PA unlikely

Adrenal CT to exclude ACC

To repeat ARR under better standardized condition

Treatment according to Mongolian HT guidelines

APA

BAH

Surgical treatment

Long term MRA treatment

Follow up after 4 months

Figure 1. PAMP study flowchart. Abbreviations: ACC, aldosterone producing carcinoma; APA, aldosterone producing adenoma; ARR, aldosterone to renin ratio; BAH, bilateral adrenal hyperplasia; CT, computed tomography; DCR, direct renin concentration; HT, hypertension; MRA, mineralocorticoid antagonists; PA, primary aldosteronism; PAC, plasma aldosterone concentration;

Sample size calculation

Among the Mongolian population prevalence of arterial HT is 27.5% without a difference between urban and rural residents. Depending on that the current population in Ulaanbaatar is 1.4 million we calculated 400 hypertensive patients will allow detection of around 50 cases of PA referring to a 11% prevalence(7) but to insure diagnostic sensitivity we decide to enlarge the study by including all newly diagnosed hypertensive patients with serum K^+ <4.0 mmol/l who referred to Mongolian-Japanese teaching hospital.

Sample size is calculated using following formula:

Sample size = $Z^2 \times P \times$ (1−P)/M2 with 95% of Confidence Interval (CI).

For adjusted sample size = (S)/(1+(S−1)/Population)

Where,

- S = sample size for infinite population
- Z = Z score
- P = population proportion (0.5)
- M = Margin of error

Statistical analysis

Results will be expressed as mean ± SD or median and interquartile range. Student t-tests will be used for the within-patient comparison of quantitative variables as appropriate in the PA and HT sub-cohorts. The sensitivity, specificity, and accuracy of tests including fall in plasma potassium concentration, the ratio of urinary potassium excretion to serum potassium and PAC will be assessed by the area (AUC) under the receiver operating characteristic (ROC) curve, to determine Mongolian people specific ARR cutoff value. The distribution

of categorical variables will be investigated by chi-square analysis. Significance will be set at p<0.05. SPSS version 26 (IBM) will be used for data analysis.

6.2.3 Patients treatment and follow up

All patients with BAH, and who not agreed to have surgical treatment will receive treatment with MR antagonists. Spironolactone, which is more commonly used in Mongolia than canrenone, will be prescribed as a first-line medication. All patients receiving pharmacological and surgical treatment will be assessed for hormonal and serum ion measurements after at least 120 days. Patients who had surgical treatment, PA cure will be validated with gold diagnostic standard comprising a PAC value of 2-9 ng/dL, serum potassium >3.8 mEq/L, and DRC >6 mU/L with an ARR <20 ng/mU as stated in Chapters 1 and 3.

6.3 Primary endpoint

The primary endpoint of this study will be the rate of patients diagnosed with PA.

Secondary endpoints will be

- Rate of surgically cured patients

- Rate of patients with an achieved BP target after MRA treatment alone in PA patients or on top of the ongoing treatment in those without PA

- Rate of OSA patients in newly diagnosed hypertensive patients

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8. CHAPTER 6. NEPAL STUDY DISCUSSION AND SUMMARY

Using the predefined diagnostic work-up, we investigated the prevalence of secondary forms of hypertension (HT) in 462 consecutive hypertensive patients in **Chapter 1**. After careful investigation of secondary causes around 56% of patients had secondary causes, moreover, when we applied suggestive symptoms/signs to each case with secondary hypertension (SH) regarding the current guideline(1) we were at risk to underdiagnose 9% of the patients. As initially expected primary aldosteronism (PA) was the most common cause of SH prevailing at 15% of patients which is in line with previous studies(2), moreover 27% of those patients had unilateral primary aldosteronism (uPA) referring to the gold diagnostic standard. However, the prevalence of PA varies according to the population examined and applied diagnostic criteria, moreover, it is more common in patients with III stage HT and resistant hypertension (RHT). In the current study, 14% of PA patients had resistant hypertension by ESC/ESH guideline(1), which is in kin to previous results(2).

Computed tomography (CT) is one of the well-established diagnostic approach for the uPA is, however, 46% of uPA patients were CT negative regarding multicentral international study(3) since in the current study patients with increased plasma aldosterone concentration (PAC), decreased direct renin concentration (DRC) accordingly high aldosterone to renin ratio (ARR) with normal urinary sodium excretion for 24h, who was under proper pharmacological washout were grouped as PA patients, which may lead to the overestimation of real PA rate. However, quantitative information increases the diagnostic accuracy of the baseline ARR for the identification of uPA(4), which was applied to this group of patients. Therefore, for the initial screening of PA, we used slightly high baseline ARR, which provides higher specificity rather than sensitivity, since might some of the patients missed for PA diagnosis. Along with high urinary sodium blunts renin production, on top of hypokalemia decreases aldosterone might that patients with overlapped reasons for decreasing renin were also misclassified as primary hypertensives with low renin levels, if

there was no any other secondary causes of HT, however, those patients received an instruction to follow a reduced sodium diet for further RAA system screening.

The second major cause of SH was Obstructive sleep apnea (OSA). At ≥5 events/h apnea-hypopnea index (AHI) in general population, the prevalence of OSA ranged from 9% to 38% and higher in men, and it increases with increasing age(5). For OSA diagnosis we referred to AHI, and an improvement of BP level after CPAP or oropharyngeal surgical reconstruction with the same or less antihypertensive drugs, patients with daily sleepiness with high Epworth scale, snoring and apnea without PSNG results were regarded for other HT groups, and, this may the explanation of why the prevalence of OSA is lower in comparison to the other studies(6), however, the prevalence of OSA is slightly dependent from conducted age group.

The isolated systolic hypertension (iSHT) is due to the reduced elasticity of arteries, in which a crucial role plays increased deposition of calcium in the arterial wall causing increased arterial wall thickness, as a result, stiffened arteries lead to an increase in pulse pressure and pulse wave velocity, causing an elevation of SBP. The iSHT is common in elderly population, however Saladini et.al reported about possible central genesis of increased systolic blood pressure (SBP) since younger male population with iSHT were found increased levels of norepinephrine. In our study, patients with iSHT were older as expected and they had higher SBP in comparison to the other groups. Previous studies reported a higher proportion of females gender, high BMI, and current smokers in iSHT group(8,9) in iSHT patients we did not see any kind of picture, however, a higher level of HDL in comparison to the other groups was similar to other studies(9,10).

The relation between high BP and hypothyroidism exists in different opinions(11,12). However, hypothyroidism patients have low renin levels which lead to increased salt sensitivity and as a consequence renal sodium reabsorption following the expansion of blood

volume. In the current cohort, 7.3% of patients had hypothyroidism, which is in comparison to other reports(13) quite small.

With premises of increasing, CKD counted for 10–15% of the population worldwide(14). HT is both a cause and effect of CKD and contributes to its progression since, distinguishing if high BP leads to kidney damage, or if kidney damage leads to high BP is difficult. The prevalence of CKD among prehypertensive and undiagnosed hypertensive patients is at 17.3% and 22.0%, respectively, along with 27.5% in diagnosed hypertension and 13.4% with normal BP(15). In the current cohort, 2.4% of patients had CKD this might be because we used too strict criteria for CKD with an estimated glomerular filtration rate [eGFR] < 59 mL/min/1.73 m2 and/or proteinuria for ≥ 3 months duration, on the basis of the exact cause of kidney damage except for HT.

RAS prevalence is different in different age groups and gender, in the young female population common cause for RAS is FMD, it is most often due to medial dysplasia of mid and distal segments of the renal artery, in contrast, atherosclerotic RAS typically involves the proximal segment of the renal artery, and represents >90% of all RAS among elderly patients. The prevalence of RAS estimates in hypertensive patients is from 1 to 5%(16), which is comparable to our results.

People who overuse licorice which induces the AME much more common than it is in genetic forms(17). To the best of our knowledge, there are no reports which conducted prevalence of licorice-induced AME, in the current cohort iAME prevailed in 5.6 %.

In conclusion, this study emphasizes that rate of secondary hypertension is twofold higher than the rate of essential hypertension, suggesting that most of the patients with hypertension have underdiagnosed secondary cause.

In Chapter 2 We meta-analyzed eligible studies for diagnostic gain and accuracy of exclusion tests for PA over baseline aldosterone to renin ratio. Current guidelines (18,19) recommend exclusion tests to exclude patients from invasive subtyping. Nevertheless, studies that unambiguously prove the accuracy and diagnostic gain of exclusion tests over the ARR are lacking, thus we conceived a meta-analysis aimed at determining if the systematic use of the exclusion tests in the clinical decision-making is justified in the work-up of PA. We selected eligible studies applying predefined strict inclusion criteria, moreover, since case-control studies might exaggerate diagnostic accuracy we analyzed only cohort studies.

The key issue in PA is to identify unilateral surgically curable forms, due to we meta-analyzed 31 datasets that used, as a reference, the "gold" diagnosis of unilateral PA, as confirmed by biochemical cure after surgery, and also a less certain (golden) diagnosis, entailing results of adrenal vein sampling (AVS), pathology, and BP fall after surgery.

Based on these diagnoses involving different levels of certainty, our meta-analysis involved two phases: an exploratory phase that used a golden standard, as a reference, and a validation phase that involved studies that used a gold standard as a reference.

The first and most important findings were that when carefully performed in a standardized way the ARR, provided high accuracy in identifying uPA, neither captopril challenge test (CCT) nor saline infusion test (SIT) furnished an additional diagnostic gain. In the largest study that examined prospectively with a similar standardized protocol more than two thousand newly-diagnosed referred hypertensive patients, 4% of whom received a diagnosis of uPA by the gold criterion, reported identical results(20). In a prospective study of 102 patients with an elevated ARR (>20 ng/dL/ng/mL/h), where the accuracy of the ARR for discriminating uPA from PH and IHA patients was compared to CCT, SIT, and furosemide upright test (FUT)(21), also reached these conclusions. However, Fries et al. using a golden

(not a gold) reference standard, reported that a post-SIT PAC cut-off value, of 83 pmol/L (3.0 ng/dL), measured PAC by liquid chromatography-tandem mass spectrometry (LC-MS/MS), which corresponded to the lowest limit of detection for reliable PAC measurement by their assay, carried the highest accuracy, i.e. sensitivity of 97% and a specificity of 92% in 104 consecutive patients with suspected PA(22). In the PAPY study that used the optimal cut-off formally determined by YI analysis, which was 6.75 ng/dL for the PAC radioimmunoassay (RIA)(2) close to 5 ng/dL found by Burello et al(23). Thus, owing to the lack of antibody cross-reactivity with other steroids(24) LC-MS/MS can give lower values than the immunoassays. In our experience using a gold reference standard, with a low cutoff, the overlap of post-SIT PAC values between uPA patients and primary hypertension (PH) and/or bilateral PA patients is huge(25,26).

Furthermore, while all uPA patients are likely identified, thus accounting for the very high sensitivity, the ARR false positives are not.

There are, several plausible reasons for heterogeneity across studies: one could be the biochemical methods for measuring aldosterone. Currently, three methods available for measuring aldosterone: LC-MS/MS, RIA, and chemiluminescence (CLIA), although expected to provide similar results, in reality, exhibit significant inter-assay variabilities and generate different results. Regarding that the ARR can be performed using renin measured as PRA (by RIA), or as direct renin concentration by CLIA or by LC-MS/MS, which provides results in different units of measure, to homogenize expressing units conversion can be made by an available APP(27).

To add a two-week withdrawal of interfering drugs that blunt and raise renin levels is too short, particularly for β-blockers and RAS inhibitors.

The thresholds also differed among studies: 45% of the investigators used arbitrarily chosen cutoff values and only 55% of them determined their cutoffs by receiver operating curve (ROC) curve and Youden index (YI) analysis. A further source of heterogeneity for the CCT was the choice of the readout of this test, i.e. PAC or the ARR. Finally, the use of PA or uPA diagnosis, as a reference, added an important confounder.

Factors such as race, serum K^+ levels, and salt intake at testing, might have also contributed to heterogeneity, although they did not emerge at meta-regression.

A preselection bias was evident, at least in some studies, as the ARR was not done in consecutive hypertensive patients, in contrast, the exclusion tests were performed in patients with positive ARR. In fact, the uPA/controls ratio differed by 26.5-fold (from 2.8% to 73.5%, 32.94 ± 24.20%), and by 5.9-fold (from 11.8% to 69.7%, 37.65 ± 20.16%) for SIT.

The fludrocortisone suppression test (FST) has been proposed as the most reliable exclusion test for PA (25) but, surprisingly, its use is supported by only one study(28). The same applies to the FUT. Thus, our meta-analysis could not evaluate these tests.

Notwithstanding all the aforementioned limitations, this meta-analysis has major strengths: a painstaking selection of the eligible studies based on a novel quantitative analysis of their quality, the evaluation of the performance of each test using uPA as a reference, and the fact that data were examined in two-phases according to the level of diagnostic certainty.

In conclusion, the under-detection of PA is a major issue in the field of arterial hypertension and the complexity of the diagnostic work-up contributes substantially to it. This meta-analysis of the studies that met the tightest quality criteria revealed that, albeit seemingly highly sensitive and specific, the exclusion tests provided no diagnostic gain over a well performed ARR. These tests are time-consuming, and not free of risks because of the need to keep patients on the "switch" antihypertensive treatment, and because of precluding cure

to patients with angiotensin II-responsive adenoma. They also increase the costs and complexity of the diagnostic work-up of PA. Therefore, their usefulness should be proven in a large outcome-based prospective study comparing head-to-head strategies "with and without exclusion tests" before their systematic use can be recommended.

Hypothesizing that patients with PA will have a worse postural balance due to a reduction of the amplitude of sensory and motor nerves because of chronic potassium depletion and the systemic influence of high BP on peripheral nerves in **Chapter 3** we assessed 17 PA patients before and after MR antagonist treatment and/or surgical cure. After adrenalectomy PA patients had a reduction of body sway, in particular, the center of pressure velocity (CoPv) and media lateral (ML) sway in closed eyes condition. To the best of our knowledge, this is the first study of postural balance in PA patients. HT patients have worse postural balance in comparison to non-HT patients, moreover, results were similar in patients with controlled and uncontrolled HT(29,30). The worse postural balance is related not only to HT it is related also to age(31). However, in this study, we did not found any association of age, and BP level in relation to postural balance. Interestingly, patients with hypertension but without orthostatic hypotension (OH) had better postural balance(29), applying the American Autonomic Society and the American Academy of Neurology(32) in the current study was no difference of BP after standing up on stabilometric platform within 1 to 3 minutes.

In our study sural nerve amplitude and serum potassium level were predictors of CoPv diminishing after adrenalectomy, meaning that sensory feedback affect postural sway including CoPv(33), which is similar to the previous studies that reported as peripheral neuropathy deteriorates the postural balance becomes worse.

MR antagonists induce amelioration of nerve myelination in animal models (34), in contrast in our study we found an increase of sural nerve latency under MR antagonists treatment, which may be explained by the systemic effect of hypertension.

There were no changes of the sural nerve amplitude regarding the treatment type of PA, however mean amplitude of PA is similar to the patients with diabetic neuropathy with less than median duration of diabetes(35).

In conclusion, PA patients have worse postural balance in closed eyes condition, however, these changes are reversible after adrenalectomy within achievement of biochemical cure. Moreover, sural nerve amplitude is reduced regardless of PA treatment with comparable mean value to the patients with diabetic neuropathy, however, the reason why sural nerve amplitude did not improve after the biochemical cure of PA may be related to the remyelination of peripheral nerves taking a more long time in PA in comparison to the remyelination process after acute nerve injury, and moreover, may it is related to the tissue potassium deposition.

Nonetheless, that sural nerve amplitude is comparable to the patients with diabetic neuropathy meaning that patients with PA suffer from subclinical sensory neuropathy, moreover, those changes are not reversible after the PA cure. The limitation of the current study is that we did not perform a neuroimaging study on the same cohort and therefore, additional prospective studies with a larger sample size are required, to understand risk factors of worse postural balance performance and sensory nerve conduction in patients with PA.

PA patients develop stage III and/or drug resistant hypertension, leading to cardiovascular events thus far screening programs for PA were never conducted in Mongolia, in **Chapter 4** we aimed to investigate the prevalence of PA in the adult population of Ulaanbaatar,

Mongolia. Among the Mongolian population, prevalence of arterial hypertension is 25.6% without a significant difference between urban and rural residents(36). Depending on that the current population in Ulaanbaatar is 1.4 million we expect that in 430 hypertensive patients will allow detection of nearly 50 cases of PA referring to an 11% prevalence but to insure diagnostic sensitivity we decide to include all newly diagnosed HT patients with stage I, II, II, and serum K+ <4.0 mmol/l who referred to Mongolian-Japanese teaching hospital.

SUMMARY

Chapter 1 provides the prevalence of secondary causes of hypertension (HT). The screening for secondary causes of arterial hypertension (HT) is crucial because of i) the identification of the underlying cause of HT will lead to the target treatment of HT leading to the cure of HT or improvement of blood pressure (BP) control and iii) better prevention of target-organ damage and cardiovascular events by a specific pharmacological treatment. Applying the suggestive signs/symptoms for the screening of secondary hypertension regarding the guidelines, we were at risk to underdiagnose 9% of patients with secondary hypertension (SH). This study emphasizes that rate of secondary hypertension is higher than primary hypertension (HT) suggesting that most of the patients with hypertension have underdiagnosed secondary cause.

Chapter 2. Provides diagnostic gain and accuracy of exclusion tests over baseline aldosterone renin ratio (ARR), this meta-analysis of the studies that met the tightest quality criteria revealed that, albeit seemingly highly sensitive and specific, the exclusion tests provided no diagnostic gain over a well performed ARR. These tests are time-consuming, and not free of risks because of the need to keep patients on the "switch" antihypertensive treatment, and because of precluding cure to patients with angiotensin II-responsive adenoma. They also increase the costs and complexity of the diagnostic work-up of primary aldosteronism (PA). Therefore, their usefulness should be proven in a large outcome-based prospective study comparing head-to-head strategies "with and without exclusion tests" before their systematic use can be recommended.

Chapter 3 provides the postural balance of PA patients in relation to peripheral nerve conduction. Results of our study shows that PA patients have worse postural balance in compare to same patients who were cured of PA, especially in closed eyes condition.

Predictors for the improvement of postural balance were sural nerve amplitude and serum potassium level, however, in our study there was no significant difference of sural nerve amplitude in PA before and after adrenalectomy. Nonetheless, that sural nerve mean amplitude was comparable to the patients with diabetic neuropathy meaning that patients with PA might suffer from subclinical sensory neuropathy, moreover, those changes not reversible after biochemical cure of PA.

In Chapter 4 we present the study protocol of PA screening among the population. We aimed to screen hypertensive patients among Mongolian population, since the most common cause of hemorrhagic stroke among current population is high BP, moreover screening for PA is never programmed in Mongolia. Study will be conducted since September 2022 in the Japanese-Mongolian teaching hospital of Mongolian National University of Medical Sciences. Consecutive 430 HT patients with defined serum potassium levels without known secondary causes of HT will be included in this prospective study. We will collect blood samples, including blood count, glycemia, lipid profile, plasma aldosterone concentration (PAC), direct renin (DRC), serum creatinine, serum ions (Na^+, K^+, Ca^{2+}), and 24-h urinary K^+ excretion on top of data medical history, and current drug therapy. Before measurement of PAC and DRC, all patients will have a drug washout at least for 4 weeks and switch antihypertensive drugs will be prescribed if needed. This study will provide the rate of PA among Mongolian population first time and patients who will have the diagnosis of PA will receive proper pharmacological and surgical treatment.

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