Diploma Thesis

A diagnostic accuracy study of aldosterone to active renin ratio in screening for primary aldosteronism

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Zusammenfassung

Arterieller Bluthochdruck ist einer der Hauptrisikofaktoren für Erkrankungen des Herz-Kreislaufsystems, die in Österreich die häufigste Todesursache, noch vor bösartigen Neubildungen und Erkrankungen der Atmungsorgane sind.[1]

Der primäre Hyperaldosteronismus, auch bekannt als Conn-Syndrom, ist charakterisiert durch eine überschießende, weitgehend autonome Produktion des Mineralokortikoids Aldosteron und hat laut neuester Studienlage eine Prävalenz von 5-10% bei PatientInnen mit arteriellem Hypertonus.

Die Aldosteron zu Renin Ratio ist der empfohlene Screeningtest für den primären Hyperaldosteronismus, aber es gibt nur limitierte prospektive Studiendaten über die Sensitivität und Spezifität dieses Tests. Daher wurde die diagnostische Genauigkeit und somit Aussagekraft der Aldosteron zu Renin Ratio in der GECOH (The Graz Endocrine Causes of Hypertension) – Studie evaluiert. In dieser prospektiven Studie wurden 400 BluthochdruckpatientInnen eingeschlossen, die am LKH-Univ. Klinikum Graz auf endokrinen Hypertonus mittels Aldosteron zu Renin Ratio (AARR von ≥ 3.7 ng/dL/µU/mL und Plasma Aldosteron Konzentration von ≥ 9 ng/dL als Grenzwert) und Kochsalz-Belastungstest gescreent wurden.

Der primäre Studienendpunkt war die receiver operating characteristic (ROC) Kurve der Aldosteron zu Renin Ratio für die Diagnostik des primären Hyperaldosteronismus. Ausreichende Studiendaten zur Analyse des primären Endpunktes waren bei 382 PatientInnen vorhanden, wobei ein primärer Hyperaldosteronismus bei 18 PatientInnen (4,7%) diagnostiziert wurde. Die Fläche unter der ROC Kurve war 0,973 (95% Konfidenzintervall [KI]: 0,956 bis 0,990). Sensitivität und Spezifität für eine positive Aldosteron zu Renin Ratio bei der Diagnostik des primären Hyperaldosteronismus war 100% (95% KI: 81,5 bis 100) und 89,6% (95% KI: 86 bis 92,5).

Als Schlussfolgerung kann festgehalten werden, dass die Aldosteron zu Renin Ratio eine gute diagnostische Genauigkeit in der Diagnosestellung des primären Hyperaldosteronismus hat.

Abstract

Arterial hypertension is one of the main risk factors for cardiovascular diseases and the leading cause of death in Austria, followed by cancer and respiratory diseases.[1] Primary aldosteronsim, also known as Conn syndrome, is characterised by an autonomous over-production of aldosterone leading to arterial hypertension. This disease has a prevalence of 5-10% in patients with arterial hypertension.

The aldosterone to active renin ratio (AARR) is the recommended screening test for primary aldosteronism (PA), but prospective study data on its sensitivity and specificity are sparse.

Therefore, we investigated the diagnostic accuracy of the AARR for detecting PA in the Graz Endocrine Causes of Hypertension (GECOH) Study, a prospective diagnostic accuracy study. This study was conducted from February 2009 to August 2015 at the outpatient clinic of the Department of Endocrinology and Diabetology of the Medical University of Graz, Austria. We enrolled 400 patients with arterial hypertension who were referred to our tertiary care center for screening for endocrine hypertension. The study participants had a determination of the AARR (index test) and a second AARR determination followed by a saline infusion test (SIT) after 2 to 6 weeks. PA was diagnosed in individuals with any AARR of ≥ 3.7 ng/dL/µU/mL (including a plasma aldosterone concentration [PAC] of ≥ 9 ng/dL) who had a PAC of ≥ 10 ng/dL after the SIT. We did not substantially alter antihypertensive drug intake.

Primary outcome was the receiver operating characteristic (ROC) curve of the AARR in diagnosing PA. Eligible for analyses were 382 participants and PA was diagnosed in 18 patients (4.7%). The area under the ROC curve of the AARR in detecting PA was 0.973 (95% confidence interval [CI]: 0.956-0.990).

Sensitivity and specificity for a positive AARR in diagnosing PA were 100% (95% CI: 81.5-100.0) and 89.6% (95% CI: 86.0-92.5), respectively. We conclude that the AARR has a good diagnostic accuracy for detecting PA.

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Abbreviations

AARR	aldosterone to active renin ratio
ACE	angiotensin-converting enzyme
ACTH	adrenocorticotropic hormone
ADH	antidiuretic hormone
ANP	atrial natriuretic peptide
APA	aldosterone-producing adenoma
AR	angiotensin receptor
ARR	aldosterone to renin ratio
AUC	area under the curve
BP	blood pressure
CI	confidence interval
CV	coefficients of variation
EDTA	ethylene diamine tetraacetic acid
GECOH	Graz Endocrine Causes Of Hypertension
NPV	negative predictive value
NYHA	New York Heart Association
PA	primary aldosteronism
PAC	plasma aldosterone concentration
PPV	positive predictive value
ROC	Receiver Operating Characteristic
SIT	saline infusion testing
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organisation

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1 Introduction

In 2017, over 33.000 Austrians died as a result of cardiovascular diseases, making it the number one cause of death in Austria, followed by cancer and respiratory diseases.[1] According to a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines, arterial hypertension (in European guidelines commonly defined as systolic blood pressure/diastolic blood pressure ≥140/90 mm Hg) has an overall prevalence of 32%. Stratified by age, the percentage is even more impressive with a prevalence of 63% between the ages of 65 and 74 years, and 78% above the age of 75 years.[2]

As arterial hypertension is one of the main risk factors for cardiovascular diseases, its harm on the public health system and the resulting importance of targeted treatment both seem obvious. According to the severity of arterial hypertension, treatment methods vary from lifestyle modifications, such as changes in diet, physical exercise and weight loss, to antihypertensive medications such as thiazide-type diuretics, ACE-inhibitors (angiotensin-converting enzyme inhibitors), AR-blockers (angiotensin receptor blockers), calcium-channel blockers and β-blockers.[3]

In case that the recommended treatment including lifestyle changes with the optimal dosage of ≥3 drugs (like the common combination of diuretics, ACE-inhibitors/AR-blockers or calcium-channel blockers) is not able to lower systolic and diastolic blood pressure below 140/90 mmHg, the hypertension is defined as resistant. Patients who have resistant arterial hypertension are at particularly high risk of hypertension-related organ damage, chronic kidney diseases or cardiovascular events.[4]

Patients with resistant hypertension carry a risk of secondary hypertension and should therefore undergo further examinations for endocrine causes of arterial hypertension. The above-mentioned Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines lists several causes for secondary hypertension, such as renal parenchymal or vascular diseases, primary aldosteronism, obstructive sleep apnoea, drug or alcohol induced hypertension (as well as uncommon causes such as pheochromocytoma, Cushing's syndrome, hypothyroidism, hyperthyroid-

ism, aortic coarctation and other rare forms). This thesis primarily focuses on primary aldosteronism, which is not as seldom as originally thought but is rather a disease with a relatively high prevalence that is considered a public health problem.[2][5][6]

2 Physiological function of aldosterone

Gen.: Aldosterone is a steroid hormone produced in the adrenal cortex. It ensures the retention of sodium through increased renal resorption. Aldosterone is also essential for the elimination of excessive potassium.[7]

SECRETION AND SYNTHESIS

Aldosterone is a mineralocorticoid that is - as its name might imply - almost completely synthesised in the zona glomerulosa of the adrenal cortex.

For the sake of completeness, the zona glomerulosa is the outermost layer of the three cortical layers. The middle and thickest layer, the so-called zona fasciculate, produces the glucocorticoids cortisone and cortisol, as well as oestrogens and androgens. The innermost zona reticularis mainly produces androgens.[8][7]

Like other steroid hormones, aldosterone derives from the precursor hormone cholesterol (more specifically progesterone). This means that the early steps of its development and metabolism are similar to that of cortisol. From deoxycorticosterone onwards - the path's fork to either cortisol or aldosterone – the remaining biosynthesis of aldosterone is mediated by the enzyme CYP11B2 that is also called "aldosterone synthase". However, these last remaining steps of biosynthesis are normally strictly separated in the different cortical layers. As already mentioned above, cortisol is produced in the zona fasciculata and aldosterone in the zona glomerulosa. This separation of the adrenal cortex is called "functional zonation."[8]

FUNCTION AND ORIGIN

From an evolutionary perspective, living outside of the oceans required strictly regulated sodium homeostasis in order to retain water to keep intravascular volumes stable and prevent dehydration. This was necessary to make living on land possible. The lungfish was the first creature equipped with aldosterone. Nevertheless, it had gills as well.

When it comes to chronic sodium deficiency, what might not occur too often in a Western diet, but is more frequent in the highlands of New Guinea during monsoon season, plasma aldosterone levels start to increase massively to avoid losing the remaining sodium supplies.[9][10]

In order to accomplish this, aldosterone increases sodium reabsorption, which is an active process, in the collecting duct of the kidney.[11]

REGULATION

1) RENIN – ANGIOTENSIN – ALDOSTERONE - SYSTEM (RAAS)

The RAAS is the most essential regulator of aldosterone secretion. In the event of a sodium chloride deficit and the associated lack of volume and reduced circulation of the kidney, the organism reacts by stimulating the **renin** secretion in the juxtaglomerular cells of the nephrons. This is the primary activation of the renin-angiotensin-aldosterone-system.[5][12]

Renin, a protease, cleaves the **angiotensinogen** in the plasma (formed in the liver) and modifies it to **angiotensin I**.

Angiotensin I is then shortened by ACE (angiotensin I-converting-enzyme), which is also a protease and formed in the lungs, to **angiotensin II**.

Angiotensin II finally stimulates the biosynthesis of the mineralocorticoid **aldosterone** in the zona glomerulosa.[12]

Both angiotensin II and aldosterone control blood pressure, extracellular volume as well as sodium- and potassium levels:

Besides the effects of aldosterone, **angiotensin II** is a strong vasoconstrictor and can therefore stabilise or increase blood pressure. In the kidney, it immediately reduces the glomerular filtration rate through local vasoconstriction of the afferent arterioles. Furthermore, it increases the resorption of sodium in the proximal tube, triggers an appetite for sodium and thirst, and ultimately, indirectly stimulates the secretion of ADH and aldosterone, whose effects will be discussed below.[7][12][13]

Influence factors of renin production:

With RAAS kept in mind, an increase or fall of renin levels similarly influences the secretion of aldosterone. Thus, the triggers that either increase or decrease renin secretion need to be well understood. The following table gives an overview on this issue.

Table 1: Renin secretion

↑ Renin	**↓ Renin**
↓NaCl measured in the macula densa in the glomerulus**:** dietary salt restriction diuretics salt-wasting nephropathy-forms	↑ NaCl measured in the macula densa in the glomerulus: high dietary salt intake
↓ arteriolar diffusion pressure in the glomerulus: renal artery stenosis	↑ arteriolar diffusion pressure in the glomerulus: hypertension
β-receptors of the juxtaglomerular cells activated by sympathetic nervous system: ↓ blood pressure stress upright posture	↓ sympathetic activation: β-blockers
ACE-inhibitors / AT1-receptor blockers (without increase of aldosterone levels)	Angiotensin II via negative feedback
	↑ Aldosterone: primary aldosteronism
	chronic kidney disease and aging

[8][7][12][14]

2) PLASMA POTASSIUM

Serum potassium may be considered as the second most important modulator of aldosterone production. An increase in potassium leads to changes in the membrane potential of aldosterone-producing cells through plasma membrane depolarisation. Calcium-channels are activated when aldosterone is secreted. The resulting urinary potassium loss through secretion in the collecting duct is a classic feedback mechanism of potassium homeostasis.[13][15]

3) ACTH

Besides its response to stress by inducing glucocorticoid secretion, ACTH can also (albeit less significant) stimulate aldosterone production.[13]

4) ANP

To be complete, the atrial natriuretic peptide (ANP) is an antihypertensive hormone and natural antagonist of aldosterone. It inhibits the secretion of aldosterone.[5]

HORMONAL EFFECT OF ALDOSTERONE

- To counteract the loss of sodium, aldosterone stimulates sodium resorption in the kidney, more precisely, in the proximal tubule and especially the collecting duct which resembles to the function of angiotensin II mentioned above. Furthermore, aldosterone enhances sodium resorption in the colon and sweat glands and leads to saliva, tears and faeces with concentrated sodium.[12][13][10]
- Stimulation of hypovolemic thirst in the hypothalamus and appetite for sodium intake as long as isotonic liquid within a normal range in extracellular space is achieved.[7][12]
- In the kidneys, sodium resorption is linked to potassium secretion. An increased luminal influx of sodium into the cell causes a depolarisation of the luminal membrane, which is the driving force for potassium secretion through potassium channels. This describes aldosterone's function as an important potassium regulator.[12]

ALDOSTERONE ESCAPE

If the concentration of aldosterone is increased over several days (for example in the case of aldosteronism), the main effect of increased renal sodium resorption subsides. This phenomenon is called "aldosterone escape", although its origin is not understood. However, a "third factor" or natriuretic hormone, albeit not detected so far, seems to be a plausible answer for this effect.[12]

3 Hyperaldosteronism

Hyperaldosteronism, or aldosteronism in short, is an oversecretion of the mineralocorticoid hormone aldosterone by the adrenal glands. This state can either be developed through autonomous overproduction, described by the term *primary aldosteronism*, or overstimulation through the renin-angiotensin-aldosterone-system (e.g. due to renal artery stenosis etc.), summarised as *secondary aldosteronism*.[16][17]

3.1 Primary aldosteronism

Primary aldosteronism, also known as Conn's Syndrome or primary aldosteronism, is the most frequent form of secondary hypertension with a prevalence of 5 to over 10% in patients with arterial hypertension.[5][18] According to the SFE/SFHTA/AFCE primary aldosteronism consensus of 2016, this prevalence is generally estimated higher with 6 to 18% and even above when it comes to severe hypertension (systolic blood pressure ≥ 180 mmHg or diastolic blood pressure ≥ 110 mmHg), resistant hypertension and hypokalaemia associated hypertension or adrenal incidentaloma.[19]

Simply put together, there is a strong connection between primary aldosteronism prevalence and severity of hypertension. From grade 1 hypertension with a prevalence of 2%, to resistant hypertension with significantly higher prevalence of about 20%.[20] This topic will be discussed further in the chapter *Screening: Importance and who should be screened*. In addition, there is evidence of a trend towards milder forms, more in women than in men and towards bilateral cases.[21]

The main characteristics of primary aldosteronism is the autonomous and excessive secretion of aldosterone almost independently from RAAS (renin-angiotensin-aldosterone-system) or the plasma potassium concentration without an adequate suppression in response to high sodium concentrations.[22]

Thus - contrary to secondary aldosteronism - renin is supressed.

Due to the high levels of aldosterone, sodium is immoderately kept in the body, which leads to increased blood pressure and, as a last consequence, to cardiovascular damage.[23]

Other long-term effects that result from an overproduction of aldosterone include renal injury, effects on the glucose metabolism such as the development of diabetes mellitus and a higher risk for cardiovascular events such as strokes, myocardial infarctions or atrial fibrillations.[22][21]

The cardiovascular morbidity and mortality of patients with primary aldosteronism is significantly higher than that of patients with essential hypertension (matched by gender and age) with comparable levels of blood pressure. A high secretion of aldosterone has a variety of adverse cardiovascular effects, not only due to the rising blood pressure.[23]

Primary aldosteronism is common and, depending on its subtype, either well adjustable (e.g. bilateral adrenal hyperplasia) or even curable (e.g. aldosterone producing adenoma).[23] Despite the fact that the severe long-term effects, as mentioned above, could be avoided by treatment of this disease (and secondary forms of hypertension in general), this disease is still seriously under-diagnosed and often not detected.[20] This highlights the need for an earlier and more comprehensive diagnosis.[22][21]

Fortunately, the last years brought progress in the matter of primary aldosteronism and better management in healthcare such as the elaboration of guidelines and clinical protocols. In addition, the decoding and characterisation of hereditary forms of primary aldosteronism was a remarkable step into the right direction.[11]

Since the beginning of the new millennium, other new insights include the fact that primary aldosteronism also exists independently from hypokalaemia. Before, this disease was only considered when potassium levels were either acutely low or in the case of obvious hypokalaemia.[20][14]

Nevertheless, in more recent studies, the classic hypokalaemic Conn's syndrome was only detected in 24.6-37.5% of cases [24], whereas hypokalaemia more often seems to be found in the group of aldosterone-producing adenomas (48%) in comparison to 17% in the group of idiopathic aldosteronism. [23]

Other sources speak of a prevalence far below these percentages, e.g. <0.5% in hypokalemic Conn Syndrome in comparison to 5-10% of normokalemic Conn Syndrome in patients with hypertension.[5] This large gap between the prevalence estimates of different

studies might be due to different screening strategies, whether primary aldosteronism was screened routinely or if the examined patient collective had already been selected.[23]

In the majority of cases, potassium is in the normal range.[5] On the other hand, hypokalaemia without hypertension was also observed in rare cases of primary aldosteronism.[19] Concerning the further classification, primary aldosteronism can be mainly divided into two common subtypes (PAH and APA) and other rare causes.

Table 2: subtypes of primary aldosteronism

SUBTYPE	Prevalence	Subclassification
Idiopathic hyperaldosteronism	60 %	Bilateral idiopathic adrenal hyperplasia (IAH) or primary adrenal hyperplasia (PAH)
		Unilateral hyperplasia (<1%)
Aldosterone-producing adenoma (APA)	40 %	KCNJ5-gene mutation (40%)
		CTTNB1-gene mutation
Familial primary aldosteronism	<1 %	Type 1 (glucocorticoid-remediable aldosteronism)
		Type 2 (on chromosome 7p22)
		Type 3 (mutation of gene KCNJ5 for potassium-channel)
Aldosterone-producing carcinoma	rare	
Other mutations	rare	

[23][5]

3.1.1 Bilateral idiopathic adrenal hyperplasia

At around 60%, bilateral idiopathic adrenal hyperplasia is the most frequent form of primary aldosteronism. Either nodular (more common) or diffusely spread with enlargement of the zona glomerulosa. In the first case, differentiation between an adenoma can be difficult.[17]

3.1.2 Aldosterone producing adenoma

Typically, the aldosterone producing adenoma is a unilateral small node measuring less than 2 cm in diameter and weighs less than 5g. For the most part, it consists of lipid rich cells. If a detected adrenal tumour is larger than 4 cm, the rare case of an aldosterone-producing adrenal carcinoma must be considered.[23][17]

3.1.3 Familial primary aldosteronism

Inherited subclasses of primary aldosteronism, for example familial hyperaldosteronism type 1 and 3, are very rare and only make up less than 1%.[25] When primary aldosteronism is diagnosed in patients younger than 20 years of age or in patients with a family history of this disease or strokes at an age younger than 40, genetic testing for familial PA type I and type III is recommended by the Endocrine Society Clinical Practice Guidelines. Familial PA type I, also known as glucocorticoid remediable aldosteronism, is caused by a crossover between the two genes CYP11B1 and CYP11B2. The first encodes steroid 11alpha-hydroxylase and the second one aldosterone synthase. As a consequence, aldosterone secretion is not under stimulation of angiotensin II, but ACTH. Pa-

Table 3 Familial forms of primary aldosteronism (Dick et al.)

	Type I	Type III	Type III
Cause	Hybrid *CYP11B1/ CYP11B2*	Unknown	Germline *KCNJ5*
Transmission	Autosomal dominant	Autosomal dominant	Autosomal dominant
Genetic diagnosis	Long PCR	No	*KCNJ5* sequencing
Hypertension onset	Very often < 20 years	Adulthood	Very often < 10 years
Hypertension severity	Severe to resistant hypertension (normal BP is rare)	Stage 1 to resistant hypertension (normal BP is not often)	Stage 3 to resistant hypertension
Hypokalemia	Rare	Not often	Very often
Aldosterone after dexamethasone	< 4 ng/dL	> 4 ng/dL	> 4 ng/dL
Adrenal CT	Normal	Unilateral or bilateral lesions	Bilateral macronodular hyperplasia
Treatment	Dexamethasone or mineralocorticoid antagonist	Unilateral adrenalectomy or mineralocorticoid antagonist	Bilateral adrenalectomy or mineralocorticoid antagonist

BP: blood pressure. CT: computed tomography.

tients tend to have early onset of hypertension plus a high prevalence of strokes. In adults, familial PA type I is treated with dexamethasone.

Familial PA type II cannot be differentiated in sporadic forms, neither clinically nor biochemically, as the molecular basis is still unknown. Nevertheless, early studies assumed a relation between type II and the 7p22 chromosomal locus. The diagnosis is made when two first-degree family members suffer from primary aldosteronism as well.

Familial PA type III is caused by the mutation of a gene, which encodes a certain potassium channel (KCNJ5 gene). Its major characteristics are severe hypertension, even in early childhood, aldosteronism, hypokalaemia and massive bilateral hyperplasia. Mostly, bilateral adrenalectomy is the only option to get hypertension under control.[9][23]

3.1.4 Aldosterone producing carcinoma

Aldosterone producing carcinomas are rare malignant tumours, normally diagnosed through histopathological examinations. As already mentioned, especially when larger adrenal tumours are detected (>4 cm), one should keep this possibility in mind.[23][26]

3.2 Pathophysiology and long-term effects

In the chapter "physiological function of aldosterone", aldosterone's sodium-retaining properties during dietary sodium restriction has already been explained, as well as the observation, that an aldosterone excess does not necessarily lead to cardiovascular damage when sodium levels are low. The fact that only the combination of both high aldosterone and sodium levels result in cardiovascular damage should build awareness that aldosteronism is partly self-inflicted by the high intake of sodium.[9][10] In patients with primary aldosteronism, neither sodium nor aldosterone levels are low – as an aldosterone excess is no appropriate physiological response to sodium. Hypersecretion takes place in either a single or multiple foci, located in one adrenal gland (unilateral) or bilateral. As this hypersecretion is not stimulated, in other words independent from renin and angiotensin II, renin levels are usually low or even undetectable in primary aldosteronism through negative feedback from high sodium chloride levels and blood pressure, determined by the macula densa and juxtaglomerular cells. The absence of angiotensin II leads to the reduction of sodium reabsorption in the proximal tube, which is why urine with high levels of sodium reaches the aldosterone sensitive distal tube. There, sodium is resorbed by sodium-mediated channels and in exchange, hydrogen ions and potassium is excreted, which results in hypokalaemia, metabolic alkalosis and increased blood pressure (the latter due to sodium retention and volume expansion). Moreover, aldosterone secretion is not further influenced by potassium levels, so that hypokalaemia cannot suppress aldosterone either.[9][8]

While the renin-dependent aldosteronism is physiological for blood pressure homeostasis in the case of hypovolemia, the above described phenomenon of renin-independent aldosteronism is pathologic and contributes to cardiovascular damage, atrial fibrillation, diabetes, renal and metabolic disease, and a higher morbidity and mortality. These adverse cardiovascular effects of aldosterone excess do not only occur because of hypertension, for which patients with primary PA have a significantly higher risk of cardiovascular damage than essential hypertensive matched in age, sex and blood pressure.

The excessive activation of mineralocorticoid receptors in certain tissues can result in adverse effects such as oxidative stress, inflammation, hypertrophic remodelling, fibrosis, vascular remodelling and endothelial dysfunction. PA patients are more likely to have increased left ventricular and carotid intima-media thickness, and have higher rates of arrhythmias (either atrial or ventricular), myocardial infarctions, strokes and, in the end, mortality. Cardiac fibrosis, mainly localised in the left atrium, may primarily be attributed less to mechanical expansion than to high sodium intake, RAAS activation and mineralocorticoid receptor activation in macrophages, cardiomyocytes, endothelial cells and vascular smooth cells. Milliez et all., whose study compared the prevalence of adverse health consequences in PA patients and essential hypertensives, clearly confirm this statement according to their results: Strokes (12.9% vs. 3.4%), myocardial infarctions (4.0% vs. 0.6%) and atrial fibrillation (7.3% vs. 0.6%).[27]

	PA (%)	EH (%)	*p*
Cardiovascular events			
Atrial fibrilation (17)	3.9	1.1	0.001
Coronary artery disease (17)	5.7	2.8	0.03
Heart failure (17)	4.1	1.2	0.003
Nonfatal myocardial infarction (17)	4.4	1.7	0.01
Stroke (18)	7.4	3.5	0.006
Metabolic alterations			
Metabolic syndrome (19)	41.1	29.6	0.05
Abnormal glucose metabolism (20)#	22.4	16.8	0.04

#Meta analysis.

Figure 1: Cardiovascular and metabolic complications in primary aldosteronism compared to essential hypertension (Dick et al.)

Fortunately, pharmacological and surgical interventions both result in a decrease of myocardial injury, cerebral haemorrhage and renal vascular disease. Cardiac mortality and sudden cardiac death, for example, are clearly reduced through the use of spironolactone.[28][8][9][29][23][27][30]

Besides adverse cardiac events, aldosteronism is suggested to cause renal damage and, as a consequence, a loss of renal function. This was proven with rat models in which mineralocorticoid excess resulted in renal vascular and tubular inflammation and fibrosis. Patients with primary aldosteronism also show higher levels of albumin in urine than comparable primary hypertensive patients. This is probably due to glomerular hyperfiltration and, to a lesser extent, structural renal damage, as PA therapy can reverse urinary albumin elevations. Spironolactone is considered to entail the advantage on urinary protein excretion.[31][32]

As per Arlt et al., patients with primary aldosteronism show an increased co-secretion of cortisol and overall glucocorticoids which contribute to a higher metabolic risk including insulin resistance, diabetes mellitus type 2 and osteoporosis. According to their study, including 174 patients diagnosed with PA and another 162 healthy controls, glucocorticoid levels in primary aldosteronism is actually comparable to subclinical Cushing's syndrome. They concluded that the strict division into Conn's syndrome and Cushing's syndrome might be worth reconsidering. In addition, the question arises, whether mineralocorticoid receptor blockers alone are adequate to prevent related adverse effects.[25][9] Current diagnostic procedures, however, do not include cortisol levels in patients with PA.[33][8]

Whether caused by high cortisol levels or not, primary aldosteronism was identified as a risk factor for osteoporosis, low bone mass and vertebral fractures independent from the degree of blood pressure and with higher severity of fractures compared to age- and sex-matched controls. In a study by Salcuni et al., a prevalence of aldosteronism in 5.2% of patients with osteoporosis and 6.9% in patients with vertebral fractures was found and more than 20% when hypertension and hypercalcuria were included as screening criteria. As per Notsu et al., the degree of aldosterone levels, and subsequently the subtype itself, may not be relevant for the risk of fractures. The background of higher prevalence of osteoporosis in patients suffering from primary aldosteronism may be an interaction of hypercalcuria, hypocalcaemia (due to renal and faecal loss of calcium and magnesium) and counteracting secondary hyperparathyroidism, which was proven in rats and in humans. Pilz et al. came to the same conclusion in a previous publication of the GECOH-study, suggesting PA to cause secondary hyperparathyroidism. Patients with primary aldosteronism had a higher degree of PTH excess in comparison to patients with essential hypertension. After PA treatment, PTH levels normalised and subsequently resulted in a reduced risk of fractures and heart failure in patients treated by spironolactone. However, further research on this topic is required.[34][30][35]

Finally, in some patients, primary aldosteronism includes psychological effects such as depression and anxiety, episodic rage and a reduced life quality in general. According to Arlt et al. these physiological effects would also fit into the symptom complex of hyper-

cortisolism, while the reduced life quality might additionally be related to nocturia and reduced sleep. Life quality significantly improves again when treated with mineralocorticoid antagonists.[8][29][25]

3.3 Screening: Importance and who should be screened

Hypertension is the leading risk factor for premature deaths worldwide, affecting 10 to 40% of the population and is, for that reason, one of the major public health issues.[23] With a prevalence of 5 to over 10% in patients with hypertension, primary aldosteronism is important to be consequently screened. However, the criteria for hypertension with hypokalaemia is outdated and inaccurate as most PA cases are missed when using hypokalaemia as a screening tool. On the other hand, when hyperkalaemia is present, specific signs like muscle weakness, cramps and palpitations caused by arrhythmias can occur.[5][18][8]

Screening should be performed on patients with suspected primary aldosteronism:[11]

- Patients with persistent blood pressure above 150/100 mmHg
- hardly adjustable hypertension (4 drugs or more)
- spontaneous hypokalaemia or induced by diuretics in hypertensive patients. Although hypokalaemia is a quite specific symptom for excessive forms of aldosteronism, measurement of potassium alone is an insufficient screening tool
- hypertension plus adrenal incidentaloma
- hypertension plus sleep apnea
- early onset (<40a) hypertension or cerebrovascular complications in family history in patients with hypertension

3.4 Diagnostics and Measurement

3.4.1 Aldosterone to renin ratio (ARR)

Mostly undisputed and still according to latest guidelines, the aldosterone to renin ratio (ARR, AARR) is suggested as the first step of screening for primary aldosteronism.[36][37] The ARR has the highest sensitivity of all PA screening tests (with a low risk of missing a PA diagnosis)[38] and thus fulfils the purpose of a general screening tool. In addition, it outperforms plasma aldosterone, serum potassium and urinary aldosterone in terms of lower variability.[19][23] By identifying early stages of primary aldosteronism physicians are able to initiate proper treatment and avoid further cardiovascular damage[39], and as a result, save health care costs and resources.[40]

VALUES AND CUT-OFFS

As primary aldosteronism is characterised by an inadequately high level of aldosterone in relation to supressed levels of renin, the ARR is typically high in hypertensive patients of this aetiology.

The aldosterone to renin ratio is calculated from the quotient of plasma aldosterone (PAC) and either direct plasma renin (DRC) or plasma renin activity (PRA), measured by immunologic assays or the recently developed tandem mass spectrometry methodology (PRA only). Thus, the ARR is a relative value without information on absolute values.[41] Assays should be able to measure ***PRA concentrations of at least 0.2-0.3 ng/mL/h*** because the ARR is particularly sensitive to renin being the denominator of the fraction.[14][19][23][42] For ***aldosterone***, a ***minimum concentration of around 15 ng/dL or below***[33] is needed to continue further examination with regards to the respective laboratory methods and prevalence – e.g. 12.5 ng/dL[23], 6 ng/dL at the Department of Endocrinology at the Medical University of Graz[43]. Aldosterone levels of over 20 ng/dL in conjunction with renin suppression confirm a PA diagnosis[19] with either CT[23] or adrenal vein sampling[40] as further diagnostic measures for subtype classification.

When calculating the ***ratio*** with aldosterone (in ng/dL) and ***plasma renin activity*** (in ng/mL/h), the ***threshold for a positive test result is*** (or rather was) commonly **between 20 and 40**[40] in line with 30 as the cut-off according to Dick et al. 2018[23] plus the above mentioned cut-off for aldosterone.

Nowadays, the measurement of plasma renin activity is more and more replaced by the valuable, simpler and faster alternative[37][44] of the ***direct plasma renin*** measurement in commercial laboratories. Here, the recommended cut-off for the ARR is between 2.4 – 4.9, although study evidence concerning sensitivity and specificity for this range is low.[44] This is why ARR thresholds need to be revaluated for the new measurement method ***depending on the immune assay*** used.[14] However, differences in calibrations of immuno-assays limit the comparability and thus, a definition for universal cut-offs. Reference ranges established individually by laboratories and compared to method-specific cut-offs, in particularly in case of low concentrations, are suggested.[44]

In a study published in 2017 called *Novel approach to establishing an aldosterone: Renin ratio cut-off for primary aldosteronism*, population-based data instead of conversion factors of different assays was used in order to determine new cut-offs for direct renin concentrations for the first time. The primary aldosteronism prevalence of screened patients (measured by active renin concentration) and the population pool in the health care system was seen as stable and consequently adopted for calculating new cut-offs for direct renin concentrations that result in an equal detection rate. Fortunately, it was observed that the new cut-offs brought nearly equal proportions of highly probable cases and a strong overall correlation.[45]

When interpreting the results of the aldosterone to renin ratio, information does not only lie in the differentiation of an either positive or negative test result, but also quantitative one. Patients with aldosterone producing adenomas showed higher ARR (due to higher PAC) and lower potassium levels in comparison to those with bilateral adrenal hyperplasia.[40]

INFLUENCING FACTORS

Nevertheless, ARR interpretation does not always turn out to be unambiguous, because both renin and aldosterone – and consequently the ARR itself – are influenced by hormonal circles, the way of measuring and medication. Thus, drugs with strong effects on the ratio need to be stopped or at least taken into account.[36] As a consequence, it should be seen as a detection test only and repeated if results are negative, if a strong suspicion of PA remains or if sampling conditions could be further improved.[42]

STANDARDISED MEASUREMENTS

Under standardised measurement conditions, the ARR delivers reliable and reproducible results (even consistent when measured four weeks apart)[41]: The blood samples should be taken in an upright sitting position (held for 5-15 minutes in advance)[33] at least two hours after waking up.[19] When seated in an upright position, blood is translocated to the lower regions of the body which leads to an increase in plasma aldosterone. According to the GECOH study published at the Medical University Graz containing 160 hypertensive patients, all PA patients could be detected with an abnormal AARR in a seated position, whereas the number of false negative results was increased when patients were lying down.[46]

In the early morning, the stimulatory effect of aldosterone when seated is even intensified and, in addition, ACTH levels are at their peak at around 8.00. This makes an ARR elevation more likely at this time of the day due to the higher aldosterone synthesis.

Potassium levels and dietary sodium intake must be within a normal range in order to avoid aldosterone concentration values that are difficult to interpret. In particular, high sodium leads to an increase in renin levels as renin manipulates the ARR more effectively than aldosterone. On the contrary, large amounts of sodium intake can provoke false positive results.[19][47]

Furthermore, medication with a strong influence on the RAS needs to be stopped or replaced[36][19], whereas those medications with slight influence have to be taken into account for ARR interpretation and those which barely effect the ARR can, of course, be used with no need for treatment modification for the purpose of primary aldosteronism testing.

Diuretics with mineralocorticoid-receptor antagonistic effects like spironolactone, eplerenone, canrenoate, amiloride and triamterene directly inhibit aldosterone actions and, on the other hand, increase renin secretion in response to hypovolaemia. As a result, the ARR may be reduced and produces invalid values. MR-antagonists should therefore be withdrawn for at least the duration of four weeks before screening is carried out.[14][42][48]

Other antihypertensive drug classes with a possible ARR decrease are angiotensin II- antagonists, loop diuretics (a decrease in angiotensin II levels causes a fall in the feedback

inhibition of renin[49], both should therefore be paused for one week according to some literature[14]), thiazide diuretics, ACE-inhibitors and calcium antagonists. In respect to the latter, continuing this medication still allows confident ARR interpretations and therefore does not need to be stopped, as it is not in the interest of the patient to completely washout his or her antihypertensive therapy.[33][14]

An ARR increase is possible with β-blockers, but also methyldopa and clonidine. β-receptors of the juxtaglomerular cells are activated by the sympathetic nervous system. If inhibited, less renin is secreted – aldosterone as well, but not as much as other stimulating factors such as plasma potassium and ACTH. That raises the question whether β-blockers have an effect strong enough to raise the ARR above the cut-off and thus, whether they should be paused for the duration of one week before screening as well.[14][47]

AGE

In the case of the elderly, false positive ratios can be traced back to falling renin levels due to a reduction of renal function. In contrast to renin, aldosterone seems to be less effected.[47]

GENDER AND FEMALE SEX HORMONES

Plasma aldosterone and plasma renin levels are gender-specific and therefore PAC, DRC and ARR as well. Gender-specific reference intervals were established[50] in regard to the significantly higher ARR in women than in men. The reason for this might be higher levels of oestrogen in women, as the application of this hormone stimulates the production of angiotensinogen.[51] DRC falls due to negative feedback, which leads to an increased ARR. The aldosterone to renin ratio might be – even if only a little – effected by medication containing oestrogen and oral contraceptives.

Besides, progesterone and its mineralocorticoid-antagonist activity can lead to natriuresis, especially in the second half of the menstrual cycle. The loss of volume and compensatory secretion of renin and aldosterone further complicate interpretations of the ARR.[47]

3.4.2 Confirmatory tests

Once abnormally high levels of aldosterone are detected, further dynamic testing needs to be done in order to confirm an autonomous aldosterone production significantly independent from its stimulation by angiotensin II. This is mostly done by tests demonstrating continuous production of aldosterone although renin is suppressed.[52]

For this, several tests are available. Nevertheless, it should be mentioned that there is no real "gold standard" or recommendation by guidelines or consensus statements.[53] According to their own conviction and financial resources, specialised centres may use their own preferred test, which can be seen in the following example:[23]

FLUDROCORTISONE SUPPRESION TESTING

The fludrocortisone suppression test is believed to be the most reliable, according to Song et al.. Nevertheless, an accurate performance is laborious and complex.[53] Another disadvantage of this test is that inpatient admission is necessary,[36] because high oral intake of sodium as well as the application of fludrocortisone for the duration of four days in order to inhibit the production of renin and angiotensin II need to be ensured. Moreover, potassium levels in plasma (in order to adopt doses of oral KCl replacement) must be monitored. A positive result for diagnosing primary aldosteronism would be if aldosterone in plasma cannot be suppressed to lower levels than 6ng/100ml.[52] G. Kline[36] adds that this testing form might be "unsafe" for patients with hypokalaemia.

SALINE INFUSION TESTING

The saline infusion test is a commonly used test to confirm the diagnosis of primary aldosteronism. Its benefits, in comparison to the aforementioned fludrocortisone suppression testing, include its lower cost and function as outpatient-treatment. This is necessary, because the high volume of two litres infused to the patient might eventually be harmful if renal function is reduced or in case of recent heart failure or atrial fibrillation.

Considering the daytime, 0.9% saline infusion (2 litres) is given to the patient in recumbent position usually around 8.00 to 9.30 AM over four hours. Blood samples are drawn at time zero and after four hours and contain the measurement of PRA/DRC and potassium.

The best balanced results in accordance with sensitivity and specificity were found at the threshold of 6.8 ng/dL aldosterone. Nevertheless, levels of more than 10 ng/dl confirm primary aldosteronism, while less than 5 ng/dl exclude this diagnosis.[54][52][23] Another study by Y. song et al, which included 135 patients diagnosed by PA and another 101 with essential hypertonia (confirmed by FST), came to the conclusion that both saline infusion tests and captopril infusion tests are good alternatives to FST. Here, a cut-off of 8 ng (lying in the range of the above-mentioned 5 to 10 ng) was considered as the best threshold with a positive predictive value of 0.94 (95% CI, 0.88–0.97) and a negative predictive value of 0.82 (95% CI, 0.74–0.89).[53]

While the sensitivity of SIT is still seen as critical or controversial by some sources, the SIT could still surpass the FST due to its better availability and thus reach a wider patient-group because it is less time-consuming and expensive.[54]

When performed in the upright position, the saline infusion test results were shown to be more sensitive than in the recumbent position. This was demonstrated in a study performed by Michael Stowasser et al., in which 24 cases of primary aldosteronism were confirmed by furhter tests. Out of these 24 PAs 23 (96%) were also detected by SSST in comparison to 8 (33%) by RSST (in recumbant position); however, these results should be reconfirmed by a study with higher patient numbers.[52]

Finally, the question arises whether the saline infusion testing can already provide information on the PA's subtype. In patients with unilateral primary aldosteronism, PAC was measured to be significantly higher than in the patient group of bilateral PA. Thus, particularly high values of PAC seem to point out unilateral PA forms. ROC-curve analyses by K. Nanba et al. showed 100% sensitivity after an SIT of two or four hours with a cut-off of 61 and 80 pg/ml for the diagnosis of unilateral PA. The threshold of 132 pg/ml in a 4-hour PAC value brought 78% sensitivity and 95% specificity while the threshold of 119 pg/ml in a 2-hour PAC value brought 89% sensitivity and 81% specificity.[54]

CAPTOPRIL CHALLENGE TESTING

After one hour in either a sitting or standing position, the patient is orally given a dose of 50 mg captopril.

Blood samples are drawn at time zero and at one and two hours after captopril intake. Once again, PRA/DRC, plasma aldosterone and cortisol are measured in each case.

Remaining high levels of aldosterone and supressed levels of renin indicate positive test results for primary aldosteronism. Physiologically, captopril suppresses plasma aldosterone by more than 30%.[23]

3.4.3 Imaging and adrenal vein sampling

After having confirmed the diagnosis of primary aldosteronism by AARR and at least one confirmatory test, further therapy is determined depending on whether overproduction of aldosterone is bilateral or caused by the less commonly unilateral hyperplasia (or carcinoma in very rare cases). In the former event, treatment would include mineralocorticoid-receptor blockers, while in the latter case unilateral adrenalectomy could result in improvement in approximately 95% of the patients with around 50% having complete clinical remission. A lateralisation of aldosterone production can be either diagnosed by imaging (CT or MRT) or adrenal vein sampling, where the possible difference of aldosterone secretion is directly measured in both adrenal veins.[55][56][19][57][56]

COMPUTED TOMOGRAPHY

Computed tomography is far more affordable than adrenal vein sampling and usually available in most centres, though limited in sensitivity and specificity in this regard. As an adrenal carcinoma can be excluded by adrenal CT (or MRT), this procedure is recommended as the first step of subtype prediction, for example, by the Endocrine Society Clinical Practice Guidelines.[58][57] However, Dinnes et al. argue that there is still a lack of evidence whether imaging alone can differentiate malign and benign adrenal masses. Their recommended cut-off is at a density of 10 Housfield Units (HU). If an adrenal mass with the density ≤10 HU was incidentily found, this is a sign of benignancy, while densities above this threshold were sensitive (but not very specific) for malignant masses. This cut-off only applies to patients without any other cancerous dieseases.[59]
As non-aldosterone-producing adenomas become more frequent with age, also due to incidentalomas found in 5% of the CT scans,[59] adrenal vein sampling should be performed in addition to a positive imaging in patients ≥ 35 in order to avoid false positive results.[19] Otherwise, patients younger than 35-40 years (according to the study) with high PAC, spontaneous hypokalaemia (<3.5 mmol/l) and adenoma of at least 10mm clear-

ly visible in the CT do not necessarily need to be confirmed by AVS before adrenalectomy, as incidentalomas are rare in this patient group and hypokalaemia is most characteristic of aldosterone unilateral adenomas. In addition, young patients have the best chances to be cured and also profit the most from treatments measured in gained life-years.[58][60][57]

ADRENAL VEIN SAMPLING

Recent guidelines recommend adrenal vein sampling as the "gold standard" of subtyping unilateral and bilateral forms[61][57][58] with only a few exceptions. One such case would be if a patient is not fit enough for laparoscopic adrenalectomy or refuses adrenalectomy in the first place (preferring life-long MR antagonist intake) in case of unilateral diagnosis.[56][60][58] In case of the unilateral form, aldosterone excess is derived from only one adrenal gland, while the other adrenal gland usually has low aldosterone levels corrected by cortisol.[61] The AVS measures PAC directly in the adrenal vein by catheterisation, which can either be performed simultaneously in both adrenal veins or sequentially; however, values measured by the latter can result in misinterpretation, as aldosterone secretion is pulsatile and thus, time lag can produce a false gradient which actually does not exist. AVS can further be stimulated by ACTH in order to gain stronger significance. Thus, simultaneous measurement in addition to cosyntropin stimulation (applied by infusion) is recommended according to Rossi et al., while Amar et al. reject ACTH stimulation. As can be seen, measurement methods vary and consequently are hardly comparable when simply talking about "AVS".[60][58][19]

It is not necessary to completely withdraw from antihypertensive medication, but mineralocorticoid receptor antagonists should be stopped for 4 to 6 weeks before AVS. The non-dominant adrenal gland, which is normally only slightly stimulated due to low renin levels in patients with PA, can be partly activated by MR antagonists with their potential to rise renin levels, a process which falsifies the comparison between the healthy and the affected side. The diagnostic effects of other antihypertensive drug classes can be neglected as long as renin is suppressed. Potassium levels should be within a physiological range in order not to lower aldosterone secretion. If this is not the case, potassium should be supplemented.[61][60]

The selectivity index (SI), calculated by cortisol adrenal vein/cortisol peripheral vein, indicates whether catheterisation of the adrenal vein was successful. Depending on the source, this is the case when either the SI is ≥2 or >3 or when, under ACTH stimulation, the SI is ≥3 or >5.

The lateralisation index (LI), calculated by ratio of aldosterone/cortisol of the dominant vs. the non-dominant adrenal gland, indicates unilateral aldosteronism if the aldosterone concentration significantly differs between the two glands with an LI > 2 to 4, according to the source.[58]

From this follows the subsequent question: What are the arguments against AVS examination of all PA confirmed patients? First of all, "technical expertise" and its high costs are frequently mentioned in literature dealing with this topic. Moreover, AVS is an invasive procedure not suitable for all patients and is only done in very few centres of expertise; AVS is not even provided in all European countries. Finally, still success rates for cannulation depend a lot on the examiner (mostly intervential radiologists).[60][58][57]

Adrenal vein sampling is neither affordable nor necessary for all patients diagnosed with primary aldosteronism. Instead, the indication for this diagnostic method should only be given in case of strong suspicion of a unilateral form. Therefore, a specific selection policy for AVS is necessary, especially in countries with restricted health care.[60][57]

The SPARTACUS study by Dekkers et al. even appears strongly contradictory to recent guidelines, suggesting that "treatment of primary aldosteronism based on CT or AVS did not show significant differences in intensity of antihypertensive medication or clinical benefits for patients after 1 year of follow-up."[55] This point of view is related to their own study, in which 99 PA patients were assigned CT while 97 were nominated for AVS. The results were compared afterwards. Dekkers et al. found out that the primary endpoints, such as the number of patients with severe adverse events, the medication use (number of medications or daily dose) but also the mean blood pressure or the percentage reaching a target blood pressure were all similar in both groups. This finding opposes the Endocrine Society guideline, which recommends performing AVS in all PA patients in order to detect those with unilateral hyperplasia who might therefore undergo surgery. However, although the rates for adrenalectomy were similar in both groups, 50% of the patients in the group examined by AVS would have been diagnosed differently if derived

from the CT. This means that indeed the percentage of patients identified as candidates for adrenalectomy were similar, but the individual patients selected for the operations highly mismatch between the groups.[55] In a study by Blondin et al., which compared patients examined by both CT and AVS, it was shown that by reaching a diagnosis only through the means of CT and foregoing other examination such as AVS, incorrect exclusion from adrenalectomy in around 22% and performance of inaccurately indicated surgery in around 25% was the case.[62]

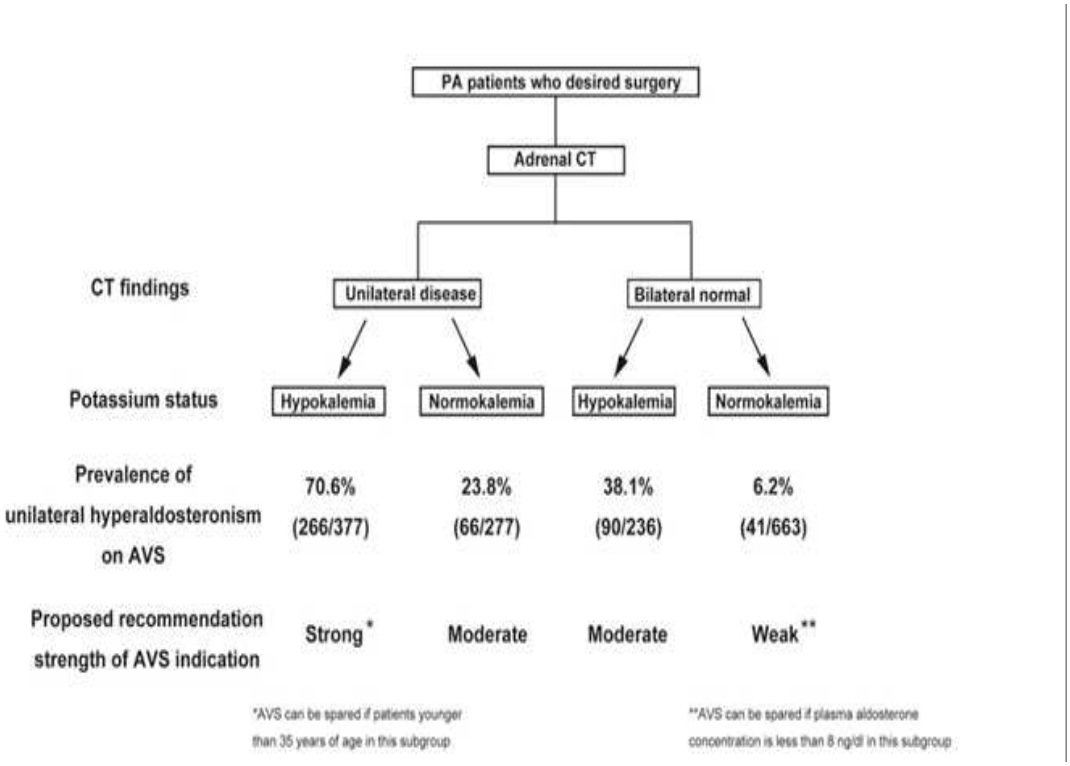

Figure 2: Prevalence of unilateral hyperaldosteronism on AVS and proposed recommendation strength of AVS indication in the subgroup categorized by CT findings and serum potassium status(Umakoshi et al.)

Consequently, it can be concluded that imaging alone cannot replace AVS, but it can—in addition to biochemical markers such as serum potassium and PAC—be a useful subtype classification tool and thus, lead to stricter AVS indication. Patients with adrenal masses shown on CT and hypokalaemia should be recommended most urgently for AVS, since they are the most likely to have unilateral aldosteronism. By contrast, patients without findings on the CT plus normokalaemia are unlikely to have the unilateral form, according to Umakoshi et al. AVS could be spared in the latter case as well as in patients under the age of 35 showing clear symptoms of aldosteronism, as was mentioned before.[61][57]

Guidelines and Treatment

Options to treat primary aldosteronism include surgery or medication, whereby both ways of treatment reduce cardiovascular morbidity caused by aldosterone excess.[33] No matter whether surgical or pharmacological treatment is chosen, patients should additionally cut down on their salt intake.[10] It was shown through the means of a large case-control study that by both adrenalectomy or treatment through medication mortality rates match those of patients with primary hypertension.[63]

ADRENALECTOMY AND RADIOFREQUENCY ABLATION

In the case of unilateral aldosteronism (around 30%) diagnosed by clear lateralisation in AVS (overproduction of the one/suppression of the other adrenal gland), unilateral adrenalectomy is recommended, whereby normokalemia and a reduction in blood pressure can be achieved in nearly all patients and complete cure of hypertension in 50 to 60% without the aid of antihypertensive drugs. These estimates should be interpreted and viewed with caution: as reported by Williams et al., there is still no standard criteria according to which surgery outcomes and persistence rates of potential cures can be measured. As their study using uniform criteria indicates, long-term cure of hypertension can be achieved in 17 to 62% of patients with the best chances for complete remission in younger patients and especially females, who profit the most from adrenalectomy. One plausible explanation might be the vasoprotective role of premenopausal oestrogens preventing vascular damage.[64][25]

Another advantage of the surgical treatment is that, in the remaining cases, less antihypertensive drugs are needed in order to control blood pressure, which results in a better quality of life in comparison to medical treatment only. Moreover, adrenalectomy seems superior due to its ability to reduce carotid intima-media thickness, arterial stiffness and left ventricular mass (leading to a better diastolic function) to a greater extent. One-sided incidentalomas without suspicion of malignancy and excessive aldosterone secretion are not indicated for resection.[8][65][33][66]

Adrenalectomy can either be done by open technique or laparoscopic surgery. The latter variety is now preferred, as it is associated with fewer complications and on average shorter hospital stays. Open surgery is mainly favoured in very obese patients or in case of unusual anatomy. Furthermore, some patients undergo partial adrenalectomy, alt-

hough this option is regarded critically by some sources, because AVS can only detect the hypersecreting adrenal gland, but not the exact location of the affected tissue. Thus, in order to avoid persistent hypertension, total adrenalectomy is generally recommended with some exceptions.[33][8] This viewpoint is opposed by Fu et al., pursuant to their study including 212 patients with a follow up of 96 months of retroperitoneoscopic partial adrenalectomy, which had similar therapeutic results compared to total adrenalectomy. In all of their patients, hypertension was ameliorated with both plasma renin activity and aldosterone normalisation. As most aldosterone-producing adenomas are benign, the small, superficial laparoscopic method is especially feasible and safe.[67]

In order to ensure a trouble-free operation, patients should be treated with aldosterone antagonists or amilorid preoperatively for a few months. This reduces the risks of hypokalemia and uncontrolled blood pressure. A stability in blood pressure and plasma potassium is necessary despite the fact that surgery might be postponed for that reason. Especially patients with hypertensive heart disease should be treated pharmacologically before undergoing surgery in order to reduce risks of operation.

During surgery and the 24-hour post-op window, potassium is not supplemented as long as plasma levels are ≥3 mM. This is due to the fact that the remaining gland might be suppressed by the other, which is removed, and consequently is not able to lower potassium concentration by aldosterone secretion. In addition, potassium sparing diuretics are contraindicated during operation as well. Postoperative saline infusions (around two litres per 24h) are recommended.

For the first two days after unilateral adrenalectomy, plasma potassium levels should be measured ≥2 times a day and at least one time a day during the remaining hospital stay (four days on average). Hyperkalaemia may occur when glomerular filtration rates were reduced preoperatively and in the case of increased levels of serum creatinine. After surgery, plasma renin activity and aldosterone should be measured as well in order to assess treatment success in terms of the respective hormonal response. Antihypertensive medication can still be given after surgery, but can be slowly reduced over the following months. In around 5% of the patients aldosterone needs to be replaced by fludrocortisone if hypoaldosteronism persists.[8][33]

Radiofrequency ablation is an upcoming cheaper alternative for laparoscopic adrenalectomy. In a study conducted by Liu et al., treatment by radiofrequency ablation was related to shorter operation time, hospital stay and faster recovery in comparison to laparoscopic adrenalectomy, whereas morbidity was equal in both methods. Nevertheless, adrenalectomy had better outcomes regarding reduction of hypertension and cure of aldosteronism. This might be due to incomplete adenoma resection, which is why RFA needs to be repeated. Radiofrequency ablation is contraindicated in adrenal regions close to the lungs, kidneys and giant vessels; it is also not recommended in malignant or larger lesions. In conclusion, radiofrequency ablation is still less reliable than the surgical alternatives, but it might become more improved in the future.[68]

MEDICATION

Bilateral adrenal hyperplasia is usually treated pharmacologically, as bilateral adrenalectomy would mean lifelong supplementation of glucocorticoids and mineralocorticoids. Moreover, unilateral adrenalectomy only cures 15% of patients suffering from bilateral adrenal hyperplasia. Still, the latter option might rarely be appropriate e.g. if medication is hardly tolerated. Contrary to this, Vaidya et al. discuss more and more data, suggesting that removing the source has better impact on the patient than just blocking its action, while a study by Muth et al. opposes this point of view by showing comparable values of blood pressure, serum potassium, and renal function cardiovascular damage, among others, in both ways of treatment.[69][8][9][65] Furthermore, treatment by medication is indicated in patients who are unfit for surgery, unwilling to undergo adrenalectomy, or already refuse further investigation (such as CT or AVS) after aldosteronism has been diagnosed.[33]

The aim of medical treatment, therefore, should be to block excessive aldosterone action (without removing the cause) in order to normalise blood pressure as well as potassium and reduce the cardiovascular risks by enabling pathologic mineralocorticoid receptor action. Generally, these medications should be given with caution and in well-adjusted dosages regarding adverse effects due to overdose, such as hyperkalemia, prerenal failure and high creatinine levels.

As aldosterone-inhibiting agents take a long time (weeks or months) to achieve their full effect, antihypertensive therapy may be started in addition and withdrawn afterwards.[9][8][29]

Spironolactone as the first mineralocorticoid antagonist is used as potassium-sparing diuretic for treatment of primary aldosteronism, as well as, for example, as an add-on resisting hypertension (albeit not as 1st or 2nd line treatment as it was not tested to be superior in treatment in patients with primary hypertension), oedemas, or heart failure. In the group of patients with heart failure, spironolactone and eplerenone have shown survival benefits by reduction of cardiovascular events when given in addition to ACE inhibitors and β-bockers. In comparison, in patients with renal disease, glomerular injury could be improved. Besides, its antifibrotic and anti-inflammatory quality was proven in experimental studies. Its usual starting dose is at least 25 mg per day, which can be increased to a maximum of 100 - 400 mg per day, always in consideration of dose-related gynecomastia. Studies show that there is no further antihypertensive effect by giving more than 150 mg per day. Treatment with spironolactone has shown reductions in both systolic and diastolic blood pressure of about 20 mm Hg.
The active substance binds to the mineralocorticoid receptor and, thus, inhibits aldosterone in a competitive way without MR turning into the active configuration. Due to its unselective binding on steroid-receptors, its most problematic and quite common side effect is gynecomastia. Besides, it can provoke breast pain, menstrual irregularities, erectile dysfunction and elevated HbA1c and cortisol levels.[70][8][71][33][72][73]

In the early 2000s, eplerenone, a spironolactone-derivate which is similar in its mechanisms, was introduced with the positive characteristic of provoking 5 times less gynecomastia. This is due to the fact that eplerenone is far more selective in terms of mineralocorticoid receptors and only slightly sensitive to androgen and progesterone receptors. The initial dose is 25mg per day (according to Deinum et al. 25mg twice a day), which can be continuously increased to 50 mg per day within the duration of 4 weeks (according to Deinum et al. the maximum dose is 200mg). The dosage should further be adjusted to potassium levels. Even though it is much less potent than its precursor spironolactone in terms of decreasing blood pressure, according to Deinum et al., measuring the percent-

age of patients reaching a diastolic blood pressure of < 90 mmHg or with a reduction of >10 mmHg, eplerone (47%) is less effective than spironolactone (68.1%). Moreover, eplerenone is still the more expensive option, which is the reason why spironolactone is primarily prescribed in patients with PA and only changed to eplerenone in case of intolerable side effects.[70][8][71][33][72][73]

Third-generation mineralocorticoid receptor antagonists combining the high potency of spironolactone with the better selectivity and safety of eplerenone are seen as today's desirable goal.[70][8][71][33][72][73]

Amilorid, triamterene, and potassium-sparing diuretics can be used as an alternative to MRAs. Their function lies in the effects on epithelial sodium channels. Amilorid is less potent than the aforementioned MRAs and, therefore, suitable for patients with milder forms of hypertension. As amilorid does not block the mineralocorticoid receptors, its cardiovascular and renal benefits might not be as strong as those of MRAs.[8]

4 GECOH study - Introduction

The following section outlines the rationale for the current data analysis of the GECOH study which is the core part of this thesis. Primary aldosteronism (PA) affects approximately 5% to 10% of patients with arterial hypertension and is associated with an excess risk of morbidity and mortality when compared to essential hypertension [33][9][24]. Therefore, the Endocrine Society Clinical Practice Guideline for the management of PA: case detection, diagnosis and treatment, recommends a wide screening for PA in approximately 50% of patients with arterial hypertension including e.g. patients with resistant hypertension or hypokalemia [33]. “Real-life” data do, however, show that screening for PA is rarely performed so that PA remains an under-diagnosed and under-treated disease [24].

The recommended screening test for PA is the determination of the aldosterone to renin ratio as it reflects the degree of aldosterone synthesis that is autonomous with reference to its principle trophin renin [33][8]. Measuring and interpreting the aldosterone to renin ratio is, however, a challenge because several factors impact on aldosterone and renin concentrations [39][52]. Moreover, prospective studies on the diagnostic accuracy of the aldosterone to renin ratio in detecting PA are sparse [37][41][53][38][74][75][44][76]. Several previous studies in this field were limited by e.g. a missing pre-specified statistical analysis plan, by restricting confirmatory tests for PA to participants with a positive screening test and thus risk of verification bias, or by incomplete adherence to guidelines for study reporting [37][41][53][38][74][75][44][76]. In many of those studies, common antihypertensive drugs were discontinued for the purpose of PA diagnostics, an approach that may limit the implementation of such procedures into routine clinical care. Hence, there exists still a need for diagnostic accuracy studies on the aldosterone renin ratio in detecting PA.

In this work we present the results of the prospective Graz Endocrine Causes of Hypertension (GECOH) study, a diagnostic accuracy study with the primary aim to evaluate the sensitivity and specificity of the aldosterone to active renin ratio (AARR) in detecting PA [18][46].

4.1 Methods

4.1.1 Design

The GECOH study is a single-center, prospective diagnostic accuracy study of the AARR in detecting PA. Details of the study design and methods including sample size calculation or statistical analysis plan have been published previously, and we adhere to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) statement and the Declaration of Helsinki [18][46][77][78]. We obtained ethical approval by the Ethics Committee of the Medical University of Graz, Austria and all study participants gave written informed consent.

According to our published study protocol patients were scheduled to have two determinations of the AARR 2 to 6 weeks apart [18]. A saline infusion test (SIT) in the recumbent position with infusion of 2 liters 0.9% saline i.v. over 4 hours was performed on the day of the second AARR determination in the first consecutive 200 study participants, and afterwards exclusively in those participants with a positive (pathologic) AARR [18]. PA was diagnosed in individuals with any AARR of ≥ 3.7 ng/dL/µU/mL (including a plasma aldosterone concentration [PAC] of ≥ 9 ng/dL) who had a PAC of ≥ 10 ng/dL after the SIT [79][14].

4.1.2 Participants

We enrolled 400 patients aged ≥ 18 years with arterial hypertension who were routinely referred to our department for screening for endocrine hypertension. As certain medications have a significant effect on aldosterone and renin concentrations and according to the Endocrine Society guideline, we did not include participants who received spironolactone, canrenoate, eplerenone, amilorid and/or triamterene within 4 weeks before study inclusion [33][18]. Other exclusion criteria were a glomerular filtration rate below 30 ml/min/1.73m², hepatic failure with Child-Pugh class B or C, severe heart failure with NYHA class 3 or 4, acute coronary syndrome within the last two weeks, immunosuppressive therapy, glucocorticoid therapy, ongoing chemotherapy, pregnancy and any other disease with an estimated life expectancy below 1 year.

Study participants were informed about the study and recruited from the outpatient clinic by the principle investigators of the GECOH study (SP and AT). Therefore, our study popu-

lation is a convenience sample because subjects were enrolled when the principle investigators were present in the outpatient clinic and had enough time for this investigation. The entire GECOH study was performed from 3rd February 2009 to 10th August 2015 in the outpatient clinic of the Department of Endocrinology and Diabetology at the Medical University of Graz, Austria. Data entry was finished in December 2018.

4.1.3 Outcome Measure

The primary outcome measure is the receiver operating characteristic (ROC) curve for the first AARR in detecting PA. The secondary outcome is the ROC curve for the SIT in detecting PA.

4.1.4 Measurements

Details of laboratory measurements and study procedures have been published elsewhere [18][46]. In brief, all blood samplings for this study were performed after an overnight fast in the morning (8:00 to 11:00) after the patients have been seated for 10 minutes. Participants were advised to avoid smoking and intake of their antihypertensive drugs in the morning before the blood collection.

Active renin concentration measurements were performed in EDTA plasma by a "RENIN III GENERATION" (GEN. III) RIA assay (Renin IRMA RIA-4541, DRG Instruments GmbH, Marburg, Germany), that has been calibrated against a WHO standard [80]. Intra-assay and inter-assay coefficients of variation (CV) of this assay are 0.6 to 4.5% and 2.7 to 14.5%, respectively. PAC was measured by RIA (Active Aldosterone RIA DSL-8600, Diagnostic Systems Laboratories, Inc., Webster, Texas, USA) with an intra-assay and inter-assay CV of 3.3. to 4.5% and 5.9 to 9.8%, respectively [79].

4.1.5 Data analysis

Continuous data following a normal distribution are shown as means with standard deviations, while parameters with a skewed distribution are shown as medians with interquartile ranges. Categorical data are presented as percentages. Where appropriate, skewed variables were log(e) transformed before they were used in parametric analyses. Group differences were calculated by student´s t-test, Qui square test, or Fisher´s exact test, as appropriate. We calculated ROC curves with area under the curve (AUC) and respective 95% confidence intervals (CI) for the first AARR and for the SIT in detecting PA. Moreover, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios for both tests were also calculated [81]. A p-value <0.05 was considered statistically significant. Statistical analyses were performed by using SPSS Version 23.0 (IBM SPSS Inc., Chicago, IL, USA).

4.2 Results

Table 4: Baseline characteristics of the GECOH Study population

Variable	All study participants	No primary aldosteronism	Primary aldosteronism	p[a]
Numbers	382	364	18	
Age (years)	50.3 ± 14.9	50.3 ± 15.1	48.9 ± 9.3	0.543
Females (%)	56.3	56.6	50.0	0.582
BMI (kg/m²)	28.7 ± 5.7	28.7 ± 5.7	28.7 ± 6.1	0.984
Systolic blood pressure (mm Hg)	155 ± 22	154 ± 22	177 ± 22	<0.001
Diastolic blood pressure (mm Hg)	96 ± 33	96 ± 34	107 ± 13	0.235
Aldosterone (ng/dL)	16.1 (11.9-23.3)	15.7 (11.6-22.0)	41.6 (25.9-59.8)	<0.001
Renin (µU/mL)	13.4 (6.6-30.6)	14.9 (7.4-33.0)	3.2 (2.5-5.8)	<0.001
Baseline AARR (ng/dL/µU/mL)	1.15 (0.47-2.39)	1.06 (0.45-2.12)	9.35 (6.24-15.95)	<0.001
Second AARR (ng/dL/µU/mL)	1.47 (0.64-3.17)	1.38 (0.57-2.57)	10.48 (6.97-13.58)	<0.001
Aldosterone after the SIT (ng/dL)	5.6 (4.2-7.9)	5.3 (4.0-7.2)	20.7 (14.8-43.0)	<0.001
Serum potassium (mmol/L)	3.9 ± 0.4	3.9 ± 0.4	3.1 ± 0.4	<0.001
Serum potassium <3.5 mmol/L (%)	12.3	9.1	77.8	<0.001
Serum sodium (mmol/L)	141 ± 2	141 ± 2	144 ± 2	<0.001
Creatinine (mg/dL)	0.92 ± 0.51	0.92 ± 0.52	0.92 ± 0.23	0.989
GFR-MDRD (ml/min/1.73m²)	80.4 ± 18.0	80.4 ± 18.0	80.5 ± 18.4	0.984
Parathyroid hormone (pg/mL)	48.6 ± 20.7	47.2 ± 19.2	75.4 ± 31.6	0.002
Fasting glucose (mg/dL)	90 (84-100)	90 (83-99)	90 (86-109)	0.386
HbA1c (mmol/mol)	36 (33-39)	36 (33-39)	34 (34-40)	0.926
HDL-cholesterol (mg/dL)	61 ± 19	61 ± 19	58 ± 23	0.492
LDL-cholesterol (mg/dL)	119 ± 31	119 ± 31	113 ± 19	0.258
Triglycerides (mg/dL)	103 (74-146)	103 (73-146)	125 (78-154)	0.678
C-reactive protein (mg/dL)	1.8 (1.0-3.9)	1.8 (1.0-3.9)	2.8 (1.1-5.3)	0.299
Number of antihypertensive drugs	2 (1-3)	2 (1-3)	4 (2-4)	0.002
ACE-inhibitors (%)	34.6	35.2	22.2	0.318
Angiotensin-II-receptor blockers (%)	24.9	24.2	38.9	0.159
Calcium channel blockers (%)	35.9	34.1	72.2	0.001
Beta-blockers (%)	53.7	52.7	72.2	0.106
Diuretics (%)	27.5	27.2	33.3	0.569
Thiazide diuretics (%)	32.7	32.4	38.9	0.568
Loop diuretics (%)	1.8	1.9	0	1.000
NSAID (%)	11.5	11.8	5.6	0.707

BMI: body mass index; AARR: aldosterone to active renin ratio; SIT: saline infusion test; GFR-MDRD: glomerular filtration rate-Modification of Diet in Renal Disease; NSAID: non steroidal anti-inflammatory drugs

Continuous data are presented as means ± standard deviation or as medians with interquartile ranges and categorical data are presented as percentages

[a] p-value for paired student`s t-test for continuous variables and for Fisher´s exact test for categorical variables comparing patients with primary aldosteronism versus no primary aldosteronism

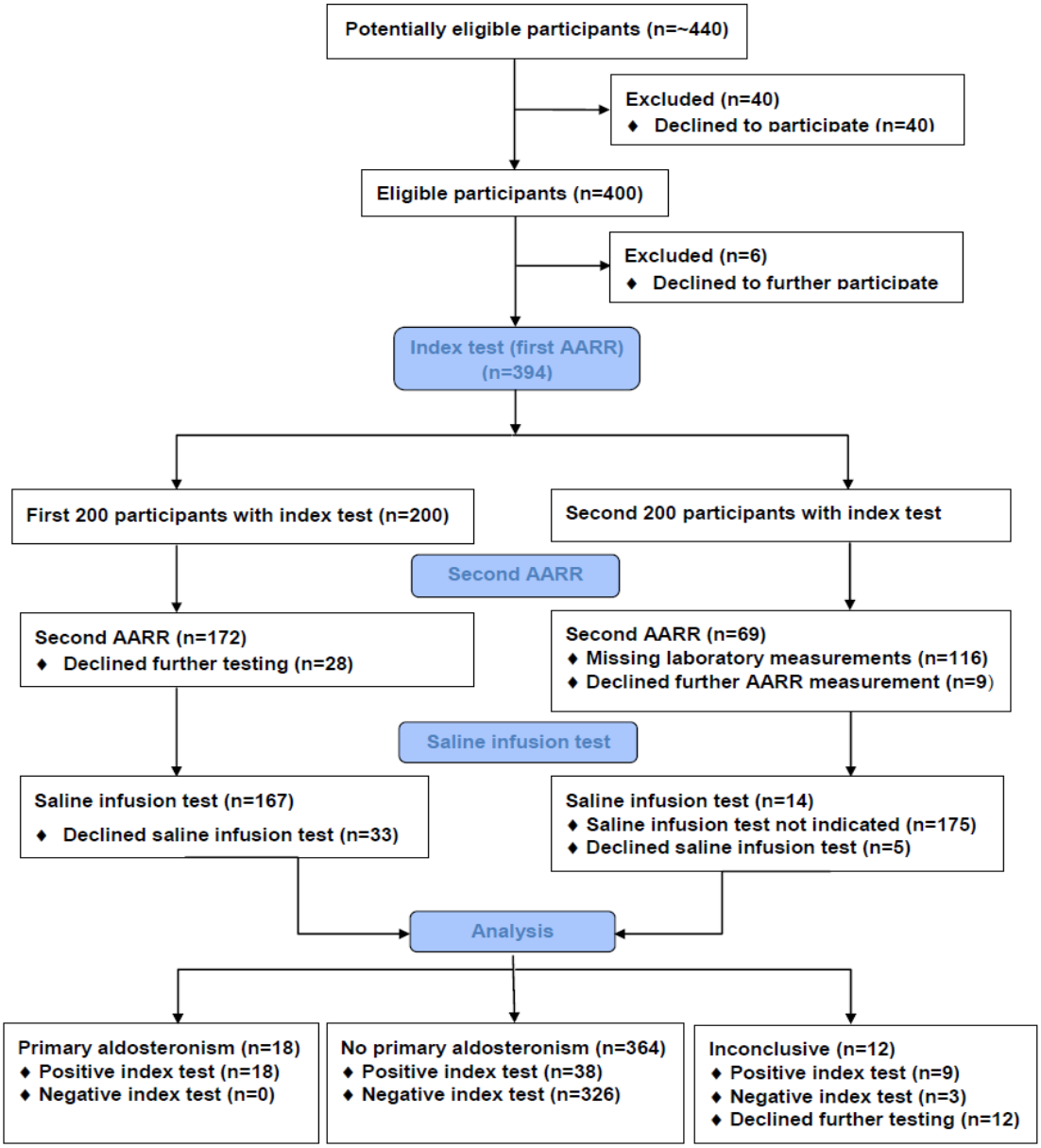

Figure 3: Participant flow-chart

The participant flow-chart of the GECOH study is shown in Figure 3. In brief, of approximately 440 patients who were asked for study inclusion, 400 agreed to participate in the GECOH study. The SIT was performed regardless of AARR values in 167 out of the first 200 study participants, while 33 of the first 200 study participants, did not want to undergo the SIT. Afterwards, the SIT was exclusively performed in participants with a positive AARR, i.e. an AARR of ≥ 3.7 ng/dL/µU/mL including a PAC of ≥ 9 ng/dL. Due to insufficient funding for this study, a second AARR determination was only routinely performed in the first consecutive 268 participants (resulting in 231 available values) and was thereafter

not measured except in those participants who had undergone a SIT. In the entire study, two determinations of the AARR with a median (interquartile) time interval in between of 30 days (22-38) were available in 241 participants and the SIT was performed in 181 participants. We excluded 18 participants who refused to undergo further testing to exclude or confirm PA. Therefore, 382 were eligible for analyses of the present investigation as they had sufficient data to exclude or confirm the diagnosis of PA with either consistently negative (normal) AARR or any positive AARR plus the result of the SIT.

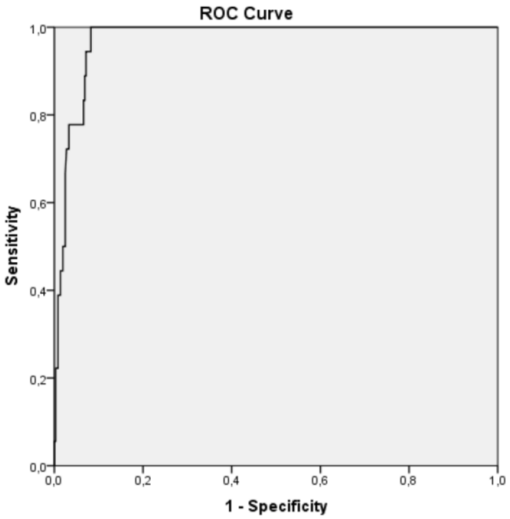

Figure 4: ROC curve for the AARR

Baseline characteristics of all eligible study participants and stratified according to the presence or absence of PA are shown in Table 4.

PA was diagnosed in 18 out of the 382 participants (4.7%). Besides, two patients were diagnosed with pheochromocytoma and two with hypercortisolism, respectively.

The contingency table for the diagnostic accuracy of a positive AARR at the first study visit (at baseline), and for a positive SIT result, i.e. a PAC of ≥ 10 ng/dL after the SIT is shown in Table 5.

Table 5: Contingency table for the diagnostic accuracy of the AARR and the SIT in detecting PA

	No PA	PA	Total
AARR			
Positive	38	18	56
Negative	326	0	326
Total	364	18	382
SIT			
Positive	8	18	26
Negative	155	0	155
Total	163	18	181

AARR: aldosteron to active renin ratio; SIT: saline infusion test; PA: primary aldosteronism

The area under the ROC curve for the AARR at the first study visit in detecting PA was 0.973 (95% CI: 0.956-0.990) (see Figure 4). The sensitivity, specificity, PPV, NPV and positive and negative likelihood ratio (with 95% CIs) of a positive AARR in detecting PA was 100% (81.5-100.0), 89.6% (86.0-92.5), 32.1% (26.0-39.0), 100%, 9.58 (7.09-12.94) and 0, respectively.

The respective area under the ROC curve for the PAC concentration after the SIT was 0.998 (95% CI: 0.994 to 1.000). The sensitivity, specificity, PPV, NPV and positive and negative likelihood ratio (with 95% CIs) of a positive SIT for detecting PA was 100% (81.5-100.0), 96.1% (90.6-97.9), 69.2% (53.4-81.6), 100%, 20.4 (10.4-40.1), and 0, respectively. Characteristics of patients without PA who had either a positive AARR at the first study visit or a positive SIT result are shown in Table 5. Of note, among participants without PA, the PAC after the SIT (median with interquartile ranges) was not significantly different in those with (n=38) and without (n=125) a positive baseline AARR (5.7 [4.6-7.4] versus 5.2 [3.8-7.0] ng/dL; p=0.071).

Some of the pre-specified secondary outcomes could not be analysed as we did not have funding for measurements of plasma renin activity, PAC by liquid chromatography mass spectrometry or 24 hours urine aldosterone concentrations with subsequent comparisons of the ROC curves using these parameters with the ROC curve of the AARR in detecting PA. Furthermore, we did not compare the ROC curves before (first AARR determination) and after (second AARR determination) discontinuation of beta-blockers because the study participants were largely unwilling to alter their antihypertensive treatment with only ten patients on beta-blocker therapy stopping this treatment before the second visit. Importantly, in all participants of the GECOH study with two available AARR measurements (n=241) there was no significant difference between the values at the first versus the second AARR measurement (1.58 [0.67-3.51] versus 1.54 [0.66-3.38] ng/dL/µU/mL; p=0.685; Spearman correlation coefficient: 0.841; p<0.001).

Although not pre-specified in the study protocol, we want to note that according to previously defined criteria, the 6 APA patients of the GECOH study who were subsequently treated by unilateral adrenalectomy showed all a complete biochemical success with a complete and partial clinical success in 4 and 2 patients, respectively (data not shown) [23]. Follow-up data for the remainder 12 PA patients who were treated with MR blocker therapy are not reported as there are no clear criteria for medical treatment success of PA.

Table 6: Characteristics of participants without primary aldosteronism who had either a positive AARR at baseline or a positive SIT

Variable	Positive AARR and no primary aldosteronism	Positive SIT and no primary aldosteronism
Numbers	38	8
Age (years)	58.6 ± 11.6	42.9 ± 13.6
Females (%)	60.5	25.0
BMI (kg/m²)	27.3 ± 4.1	31.1 ± 6.4
Systolic blood pressure (mm Hg)	162 ± 25	153 ± 27
Diastolic blood pressure (mm Hg)	96 ± 12	95 ± 11
Aldosterone (ng/dL)	19.5 (15.3-24.6)	26.1 (17.4-32.5)
Renin (µU/mL)	3.4 (2.3-4.2)	17.7 (7.2-41.0)
Baseline AARR (ng/dL/µU/mL)	5.63 (4.53-7.42)	1.42 (0.66-2.59)
Second AARR (ng/dL/µU/mL)	5.59 (3.06-9.23)	1.01 (0.31-2.04)
Aldosterone after the SIT (ng/dL)	5.7 (4.6-7.4)	11.2 (10.2-11.9)
Serum potassium (mmol/L)	3.8 ± 0.3	3.8 ± 0.5
Serum potassium <3.5 mmol/L (%)	10.5	25.0
Serum sodium (mmol/L)	142 ± 2	142 ± 3
Creatinine (mg/dL)	0.93 ± 0.21	0.94 ± 0.16
GFR-MDRD (ml/min/1.73m²)	73.8 ± 15.7	82.8 ± 14.4
Parathyroid hormone (pg/mL)	48.2 ± 14.8	50.2 ± 11.4
Fasting glucose (mg/dL)	90 (82-106)	91 (84-104)
HbA1c (mmol/mol)	37 (35-38)	34 (31-47)
HDL-cholesterol (mg/dL)	59 ± 13	56 ± 26
LDL-cholesterol (mg/dL)	122 ± 30	111 ± 30
Triglycerides (mg/dL)	103 (81-159)	121 (81-165)
C-reactive protein (mg/dL)	1.7 (1.0-3.1)	3.4 (1.3-26.4)
Number of antihypertensive drugs	3 (1-4)	3 (1-4)
ACE-inhibitors (%)	23.7	37.5
Angiotensin-II-receptor blockers (%)	34.2	25.0
Calcium channel blockers (%)	52.6	50.0
Beta-blockers (%)	68.4	37.5
Diuretics (%)	34.2	62.5
Thiazide diuretics (%)	39.5	50.0
Loop diuretics (%)	0	12.5
NSAID (%)	18.4	0

BMI: body mass index; AARR: aldosterone to active renin ratio; SIT: saline infusion test; GFR-MDRD: glomerular filtration rate-Modification of Diet in Renal Disease; NSAID: non steroidal anti-inflammatory drugs

Continuous data are presented as means±standard deviation or as medians with interquartile ranges and categorical data as percentages

[a] p-value for paired student`s t-test for continuous variables and for Fisher´s exact test for categorical variables

4.3 Discussion

In this prospective diagnostic accuracy study in hypertensive patients we have shown that the area under the ROC curve for the AARR at the first study visit in detecting PA was 0.973 (95% CI: 0.956-0.990). There was also a good diagnostic accuracy of the SIT and we reported a good intra-individual reproducibility of the AARR.

The results from the GECOH study significantly add to the rare knowledge on prospective studies on the diagnostic accuracy of the AARR in detecting PA. Our findings are roughly in line with previous investigations with a meta-analysis in 974 patients from 9 studies reporting a sensitivity, specificity and AUC of the AARR in detecting PA of 0.89 (95% CI: 0.84-0.93), 0.96 (95% CI: 0.95-0.98) and 0.985, respectively [76]. Methodological differences and limitations are, however, inherent in the existing literature on the diagnostic accuracy of the AARR and account for a relatively high heterogeneity [76]. Notably, Vorselaars et al. performed a prospective study in 233 patients with difficult to control hypertension who were all referred to a SIT [10]. In that study, the PA prevalence was 6.9% and the sensitivity, specificity, PPV and NPV for the aldosterone to renin activity ratio in detecting PA was 100%, 86.7%, 35.6% and 100%, respectively [38]. These findings are, despite using different laboratory methods, very similar compared to our GECOH study [38].

One major difference between our study and the existing literature is that, in contrast to previous investigations in this field, we performed PA diagnostics under ongoing antihypertensive treatment without substantially altering antihypertensive drug intake. We are well aware that several antihypertensive drugs may alter renin concentrations and PAC, but we have shown that AARR measurements performed under standardized conditions as in the GECOH study are excellently reproducible with a very low intra-individual variability [46]. In this context, it bears mentioning that in our study, the second AARR determination did not lead to the diagnosis of any additional PA case that would have been overlooked by just a single AARR determination. Whether ongoing drug intake might have contributed to missing diagnoses of PA cannot be excluded, but within the group of individuals without PA, the PAC after the SIT was not significantly different in those with and without a positive baseline AARR. Furthermore, only 8 participants with a positive SIT had a negative AARR and were thus not classified as having PA. These participants were, however, likely to suffer from secondary aldosteronism with relatively high renin concentra-

tions and only slightly elevated post SIT PAC. Apart from this, it has to be acknowledged that diagnostic studies on PA are, in general, prone to verification bias and are limited as there is no universally accepted gold standard for the confirmation or exclusion of PA, although there are excellent approaches for standardization [64]. This diagnostic challenge may be attributed to the fact that there exists a continuum of the AARR regarding its association to blood pressure and cardiovascular risk [82]. In this context, a recent trial has shown that the blood pressure lowering effects of spironolactone is in a linear fashion associated with the prevailing AARR that was measured, as in the GECOH study, under ongoing antihypertensive treatment [83]. While it may therefore appear arbitrary to choose an AARR cut-off for PA diagnostics, it has to be stressed that correct diagnosis of PA is pivotal due to highly effective treatment of this disease with either unilateral adrenalectomy or mineralocorticoid receptor (MR) blocker therapy resulting in significantly improved overall health outcome [84]. Of note, the 6 APA patients undergoing unilateral adrenalectomy showed excellent responses to treatment with 4 of these patients reaching normotensive BP values without the aid of antihypertensive drugs.

Our data are limited because we studied a cohort of patients referred to a tertiary care center, and can therefore not uncritically generalize our results to other populations. In addition, there are no gold standard criteria for the diagnosis of PA, and although we strictly adhered to published criteria in terms of assay cut-offs and guideline recommended case confirmation by the SIT, we cannot rule out some misclassifications. We have to acknowledge that we could, mainly due to missing funding, not analyze some prespecified secondary outcomes. Furthermore, our participants were not willing to stop beta-blocker intake suggesting that at least in our study setting, but probably also in other populations, it may hardly be possible to perform AARR measurements without potentially interfering drugs like e.g. beta-blockers or ACE-inhibitors. Therefore, we consider it as a main strength of our study that we provide data on the diagnostic accuracy of the AARR under ongoing antihypertensive treatment, an approach that is supported by the Endocrine Society Clinical Practice Guideline for the management of PA: case detection, diagnosis and treatment [33]. Finally, our results confirm some previously published characteristics of PA patients such as the high parathyroid hormone concentrations [35].

In conclusion, we have documented in the GECOH study that even without substantially altering antihypertensive drug treatment, the AARR has a good diagnostic accuracy in

detecting PA as does the SIT. These findings may hopefully contribute to a broader implementation of PA diagnostics, as unfortunately screening guidelines and current clinical practice still widely diverge.

As explained in the first part of this diploma thesis, the accurate diagnostic steps for the final diagnosis "primary aldosteronism" are time-consuming and not without costs. Though, the conjunction of the first literature part and the second one presenting the GECOH-study are, from my point of view, a good illustration, that by the alone measuring of the aldosterone-to-renin ratio we already gain a lot of information about the hormonal background of someone's hypertension. In case of a high ratio, at least the implantation of a mineralocorticoid-receptor blocker can be justified, even if further testing is refused by the patient so that the diagnosis PA cannot be made with certainty. But anyways, one should be aware of the fact that the diagnosis "primary aldosteronism" does not have a clear cut-off written in stone but is a continuous misbalance between interacting hormones.

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