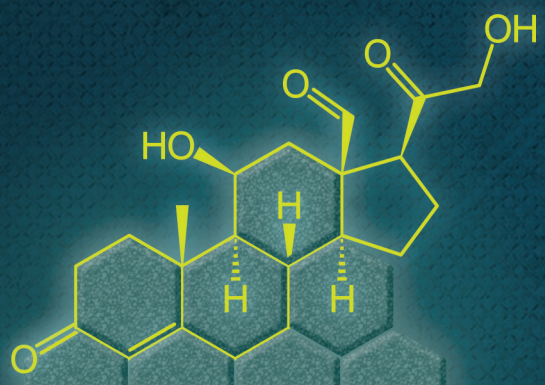
CHALLENGING ASPECTS OF PRIMARY ALDOSTERONISM



Tanja Dekkers

CHALLENGING ASPECTS OF PRIMARY ALDOSTERONISM

The work presented in this thesis was carried out within the Radboud Institute for Health Sciences.

Financial support for this thesis was provided by ZonMW DoelmatigheidsOnderzoek 2010–2012 E&K (171002102)

COLOFON

Author:	Tanja Dekkers
Cover design en lay-out:	Miranda Dood, Mirakels Ontwerp
Printing:	Gildeprint - The Netherlands
ISBN:	978-90-9032373-2

CHALLENGING ASPECTS OF PRIMARY ALDOSTERONISM

Proefschrift

Ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van rector magnificus prof. dr. J.H.J.M van Krieken,
volgens besluit van het college van decanen
in het openbaar te verdedigen op dinsdag 26 november 2019
om 14.30 uur

door

Tanja Dekkers
geboren 18 september 1984
te Eindhoven

PROMOTOREN

Prof. dr. J.W.M. Lenders

Prof. dr. G.J. van der Wilt

Prof. dr. L.J. Schultze Kool

COPROMOTOR

Dr. J. Deinum

MANUSCRIPTCOMMISSIE

Prof. dr. J.F.M. Wetzels (voorzitter)

Prof. dr. M.M. Rovers

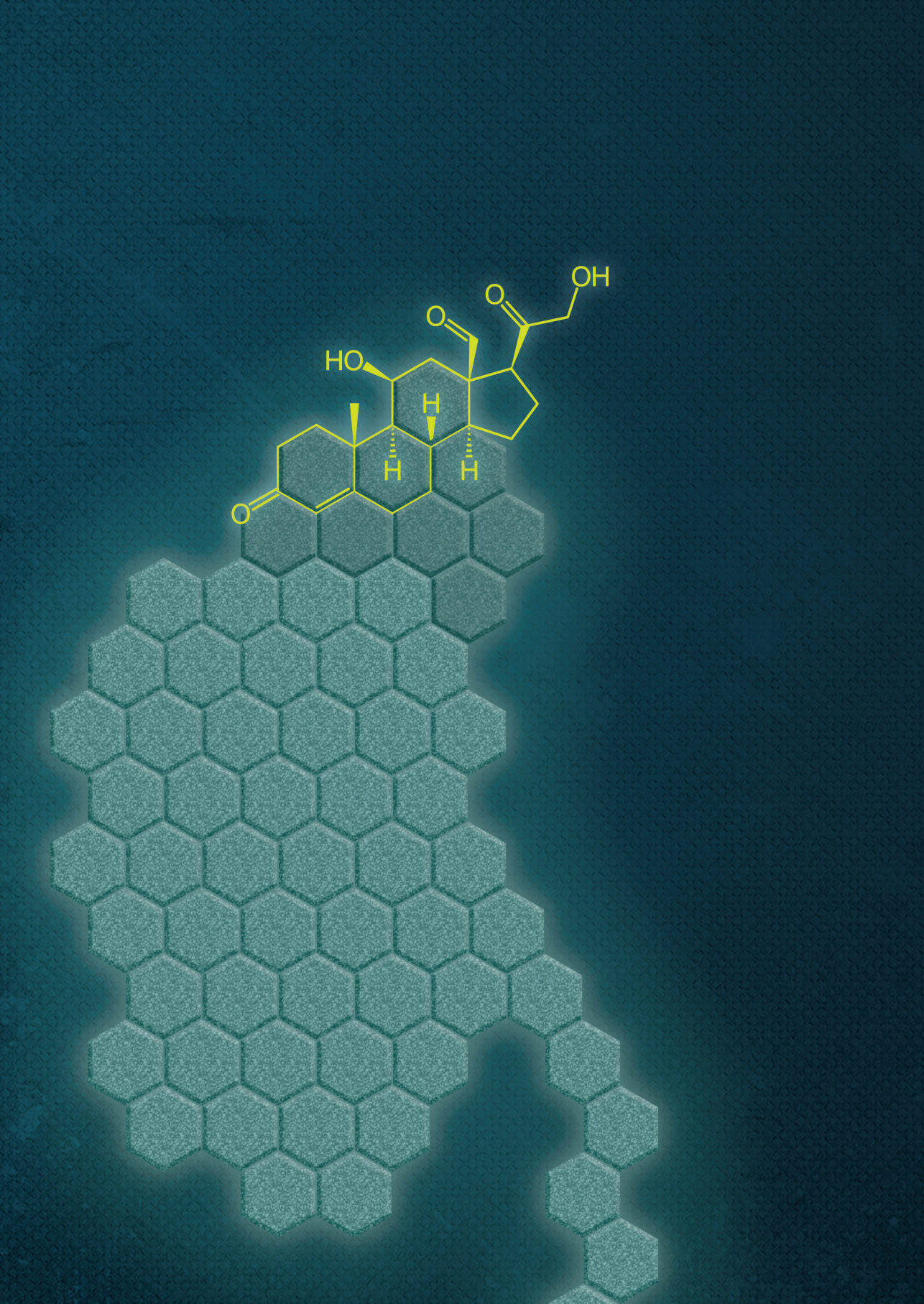
Prof. dr. A.M. Pereira Arias (Leids Universitair Medisch Centrum)

Daglicht

Uit chaos van lakens en
voorgevoel opgestaan, gordijnen
open, de radio aan, was
plotseling Scarlatti
heel helder te verstaan:
Nu alles is zoals het is geworden,
nu alles is zoals het is
komt het, hoewel, misschien
hoewel, tenslotte nog in orde.

Judith Herzberg. Zeepost

TABLE OF CONTENTS


OH
O
O
HO
H
H
H
O

Introduction and outline of the thesis

1

THE LEGACY OF DR. CONN

A 48 years old, male patient visits his physician because of complaints of fatigue and muscle weakness. Upon examination, a blood pressure of 150/94 mmHg is found. The hypertension persists despite the prescription of 25 mg of hydrochlorothiazide. Laboratory results show a decreased plasma potassium level of 3.1 mmol/l. When confronted with such a patient a clinician has to choose whether or not to initiate the diagnostic work-up for secondary hypertension. Many clinicians probably will not do so because they attribute the hypokalemia to the use of hydrochlorothiazide. They may stop the diuretic, start an angiotensin-converting-enzyme-inhibitor and might add a calcium antagonist later. Indeed, the patient's treatment was converted to 10 mg lisinopril. However, when the patient returned a few weeks later there was persistent hypertension and persistent hypokalemia (3.4 mmol/l). The question is: should these findings ring a bell? In 1955, similar but more severe findings did ring a bell for Dr. Jerome Conn when he was confronted with a young women who sought his consultation after seven years of complaints of muscle weakness, spasms and cramps of her hands.[2] On examination she had severe hypertension (174/106 mmHg), a severely decreased serum potassium (1.6 to 2.5 mmol/L) and an alkalosis (pH 7.62).

Jerome Conn was an American endocrinologist and dedicated scientist, investigating salt loss by perspiration in World War II soldiers.[1,3,4] Focusing their research on the human body's ability to adjust its salt loss in case of high environmental temperatures and high humidity of the Pacific, Dr. Conn and his team discovered that increased environmental temperature resulted in a decrease in sodium excretion through perspiration, urine and saliva. This sodium retention seemed to be the result of an increased secretion of an unknown salt-retaining corticosteroid. It was only two

Figure 1. Jerome Conn (1907-1994)[1]

years later that Simpson and Tait isolated and later characterized this steroid as aldosterone.[5] Further research showed that this hormone was elevated in patients with cardiac failure and decompensated hepatic cirrhosis. Dr. Conn hypothesized that this was a consequence of the pathophysiology of oedematous and hypocirculatory conditions and classified the condition of these patients as "secondary aldosteronism". This term suggested that there might also be a disease called "primary aldosteronism". Indeed, with the case of the young woman, Dr Conn found his first patient with what is still known as "Conn's disease". Using a bioassay for urinary corticoid-activity Dr. Conn found an elevated aldosterone level in her urine. At surgical exploration a unilateral benign adrenal tumour was found. The patient was cured after removal of the affected adrenal gland, thus confirming that an aldosterone producing adrenal tumour must have been the source of the elevated urinary aldosterone levels.

In the 1960s and 1970s Dr. Conn and contemporary researchers laid the basis for our current knowledge of the prevalence, diagnostics and treatment of primary aldosteronism (PA). With his patient Dr. Conn faced the same challenges as we still do today. One of the first problems was the discrimination between PA and secondary aldosteronism. In 1964 Dr. Conn discovered that suppressed plasma renin activity indicated PA.[6] Once he had a diagnostic tool for the detection of PA, Dr. Conn identified several patients with PA without severe hypokalemia. This "normokalemic primary aldosteronism" is still a prevalent finding in clinical practice and can be present in up to 70% of the PA patients.[7,8] With this finding Dr. Conn realized that the prevalence of PA could be much higher than previously estimated. Based on autopsy and laboratory data he calculated a PA prevalence of 10 to 20% of the hypertensive population.[9] In the last fifty years the debate on the prevalence of PA still hasn't been settled with reported prevalences ranging from less than 1% in the 1980s to 5-15% nowadays.[7,8,10-13]

While PA was first regarded as a relatively benign disease, later it was recognized as a serious condition with the potential to cause severe cardiovascular complications.[14-16] With that, the need for proper treatment became obvious. Dr. Conn assumed that all PA patients might be cured by an operation. Although he used surgical exploration to detect the adrenal lesions in his first patient, he acknowledged the importance of preoperative detection of adrenal anomalies as source of excessive unilateral or bilateral aldosterone secretion. Techniques such as adrenal venography and ^{131}iodine-labeled 19-iodocholesterol adrenal scanning were introduced in the 1970s to select those who might benefit from surgery.[17,18]

Also adrenal vein sampling (AVS) was introduced in the 1970s, but fell into disuse after the introduction of the non-invasive CT-scan to detect adrenal nodules.[19-21] However, in the last two decades, AVS made its comeback as we will discuss later. Nowadays, we are still struggling to find the best technique to distinguish unilateral from bilateral disease.

When Dr. Conn first discovered PA, a new field of research expanded rapidly. Despite more than sixty years of research PA is still an enigmatic entity, challenging researchers worldwide. Considerable progress has been made in the last decades but there are still several aspects in the diagnostic work-up and treatment of PA that have not been elucidated satisfactorily. This thesis attempts to shed light on some of these aspects. The next paragraph will first provide a short overview on the current knowledge on PA and this will be followed by a discussion on the uncertainties and controversies in this field.

PRIMARY ALDOSTERONISM

PATHOPHYSIOLOGY OF PA

ALDOSTERONE

Aldosterone is produced in the adrenal cortex as one of the major end products of the renin-angiotensin-aldosterone system. This system is one of the most powerful blood pressure regulating systems in the human body (Figure 2). In case of low blood pressure or hypovolemia, the increased production of renin by the renal juxtaglomerular cells induces a cascade of reactions resulting in elevation in angiotensin II. This causes both widespread vasoconstriction and promotes the adrenal cortex to secrete aldosterone. Aldosterone binds to the mineralocorticoid receptors in the kidney, causing an increase in the number of Na-K-ATPase pumps in the basolateral membrane and of sodium channels in the apical membranes of the cortical collecting tubules and distal tubules. This causes an increase in sodium reabsorption, with concomitant potassium secretion driven by the electric gradient created. Sodium with concomitant water reabsorption causes volume expansion which ultimately results in a rise in blood pressure. Through negative feedback this volume expansion suppresses the activity of the renin-angiotensin-aldosterone system, resulting in normalization of renin and aldosterone secretion.

In PA the physiological regulation of the renin-angiotensin-aldosterone system is overruled by excessive autonomous aldosterone secretion by one or both diseased adrenal glands. In most cases autonomous aldosterone secretion is caused by either a unilateral aldosterone producing adenoma (APA) or bilateral adrenal hyperplasia (BAH).

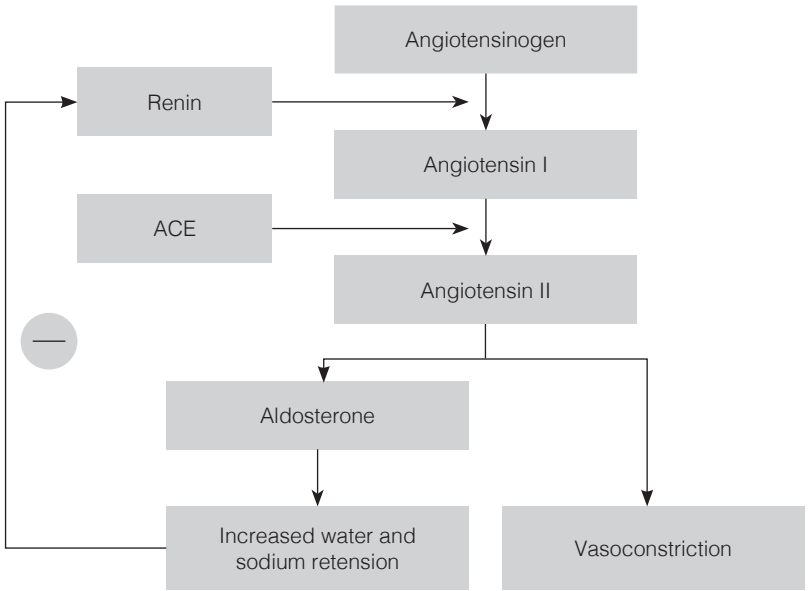


Figure 2. Renin-angiotensin-aldosterone system.

Low blood pressure or hypovolemia is detected by the renal juxtaglomerular cells. In response renin is produced which enhances the conversion of angiotensin into angiotensine I. Subsequently angiotensin I is converted into Angiotensin II bij angiotensin converting enzyme (ACE). Angiotensin II causes vasoconstriction and increased water and sodium resorption in the kidneys.

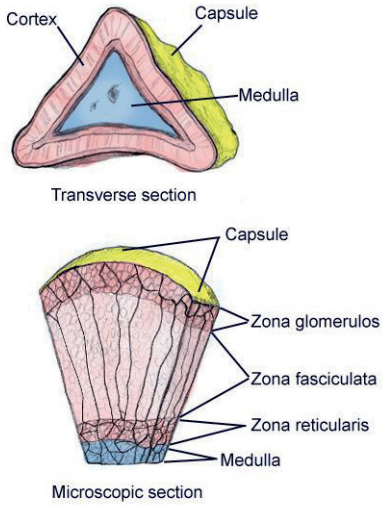


Figure 3. Cross-section of the adrenal gland.

Zona glomerulosa: production of aldosterone; Zona fasciculata: production of cortisol; Zona reticularis: production of androgens. Image reproduced with permission from Medscape Drugs & Diseases (https://emedicine.medscape.com/), Suprarenal (Adrenal) Gland Anatomy, 2016, available at: https://emedicine.medscape.com/article/1898785-overview.

ALDOSTERONE SYNTHESIS

Aldosterone is synthesised from cholesterol in the outermost layer, the zona glomerulosa, of the adrenal cortex (Figure 3).[22,23] In the cortical cells cholesterol is transported to the inner mitochondrial membrane by STaR (steroidogenic acute regulatory protein) where it is converted to pregnenolone (Figure 4). In multiple steps pregnenolone is converted to aldosterone. The final steps of aldosterone synthesis are mediated by aldosterone synthase (p450C18, encoded by *CYP11B2*). Pregnenolone is also the precursor in cortisol synthesis, which requires hydroxylation by 17α-hydroxylase (*CYP17*). The final step in the cortisol synthesis is mediated by cortisol synthase (11β-hydroxylase, p450C11 encoded by *CYP11B1*).[23] In normal adrenal glands aldosterone synthase (*CYP11B2*) is only expressed in the zona glomerulosa, confining aldosterone synthesis to this adrenal layer. Cortisol synthase (*CYP11B1*) and 17α-hydroxylase (*CYP17*) are only expressed in the zona fasciculata and zona reticularis facilitating cortisol synthesis in these layers.[22]

Aldosterone synthesis can be regulated instantly (within minutes) by affecting STAR or pregnenolone production or slowly (within hours to days) by altering CYP-enzyme

expression. The most important stimuli for aldosterone synthesis are angiotensin II, potassium and (to a lesser extent) adrenocorticotropic hormone (ACTH). Adrenal glomerulosa cells have a highly negative membrane potential due to a high resting potassium conductance. High extracellular potassium or closure of the potassium channels by angiotensin II causes membrane depolarization which activates voltage-gated calcium channels. The following increase in intracellular calcium provides a signal for augmented expression of the enzymes required for (both instant and slow) aldosterone synthesis (Figure 5A and 5B). [22]

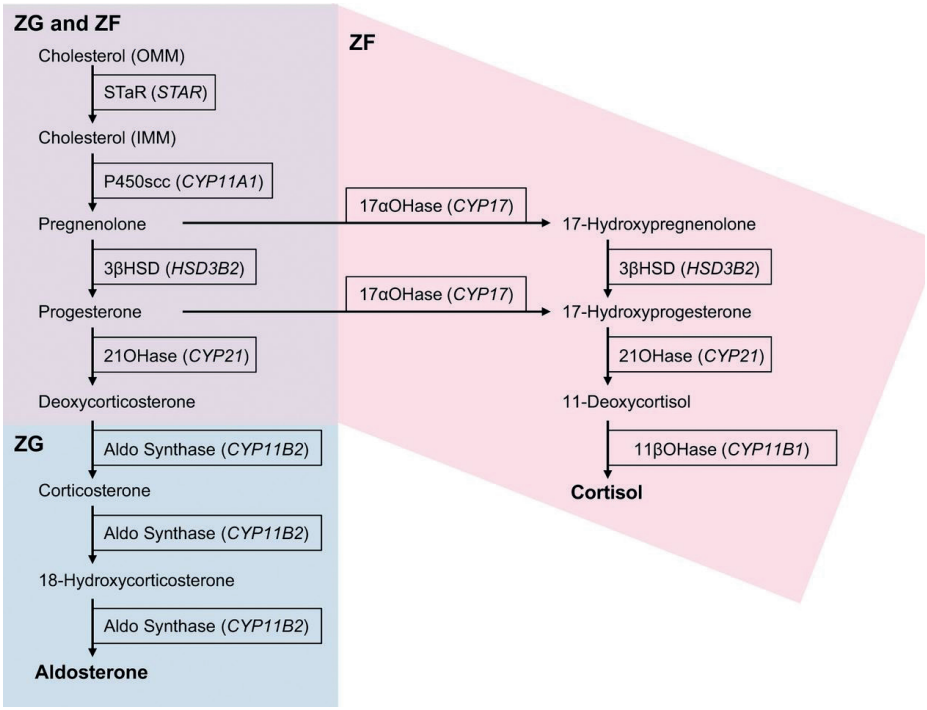


Figure 4. Aldosterone synthesis.

Biosynthetic pathways of aldosterone and cortisol formation. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; STaR, steroidogenic acute regulatory protein; p450scc, cholesterol side chain cleavage enzyme; 3HSD, 3-hydroxysteroid dehydrogenase; 21OHase, 21-hydroxylase; aldo synthase, aldosterone synthase; 17OHase, 17-hydroxylase; 11OHase, 11-hydroxylase. The genes encoding these enzymes are shown in parentheses.

From: Stowasser and Gordon; Primary aldosteronism: Changing definitions and new concepts of physiology and pathophysiology both inside and outside the kidney. Physiol Rev 96: 1327–1384, 2016. Copied with consent of the publisher.

SOMATIC MUTATIONS

In recent years, several somatic mutations involved in the mechanisms described above have been discovered in PA. *KCNJ5* mutations are the most frequent mutations found, being present in about 43% of the APAs.[24] Mutations in *KCNJ5*, which encodes for an inward rectifying potassium channel, result in sodium entry via the potassium channel leading to chronic depolarization in which calcium influx causes aldosterone production (Figure 5C).[25] Other, less frequently mutated, genes are *ATP1A1* (encoding for the α-subunit of Na^+-K^+ATPase), *ATP2B3* (encoding a Ca^{2+}-ATPase calcium pump), *CACNA1D* (encoding a voltage-gated calcium channel subunit), *CTNNB1* (encoding for Catenin-β) and CNCL2 (encoding for voltage-gated chloride channel expressed in adrenal glomerulosa).[26-30] Like KCNJ5, these mutations cause a calcium influx leading to increased aldosterone production.[22] The discovery of these mutations has contributed profoundly to the understanding of the pathogenesis of PA.

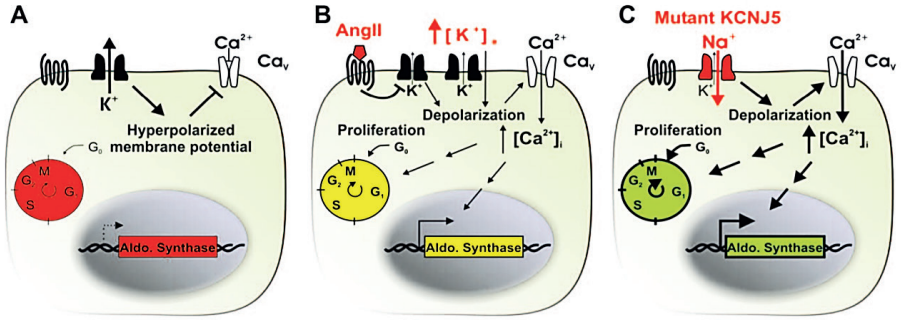


Figure 5. Proposed mechanism underlying aldosterone-producing adenoma.

(A) Adrenal glomerulosa cells have a high resting potassium (K+) conductance, which produces a highly negative membrane potential. (B) Membrane depolarization by either elevation of extracellular K+ or closure of K+ channels by angiotensin II activates voltage-gated calcium (Ca2+) channels, increasing intracellular Ca2+ levels. This provides signals for increased expression of enzymes required for aldosterone biosynthesis, such as aldosterone synthase, and for increased cell proliferation. (C) Channels containing KCNJ5 with G151R, T158A, or L168R mutations conduct sodium (Na+), resulting in Na+ entry, chronic depolarization, constitutive aldosterone production, and cell proliferation.

From: Choi M, Scholl UI, Yue P, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. Science (New York, NY) 2011;331:768-7225. Copied with consent of the publisher

DIAGNOSIS OF PRIMARY ALDOSTERONISM

IMPORTANCE OF DETECTION AND TREATMENT

In recent years the awareness has grown that PA is not a rare disease. Nowadays PA is considered to be the most frequent form of secondary hypertension.[7,8,10,31,32] However, the actual PA prevalence is a fervently discussed topic. An incidence of hypertension of more than 160 000 patients per year in the Dutch population would imply 8000 new PA cases each year when presumed that in 5% of the patients with hypertension it can be attributed to PA.[33] Awareness of the magnitude of this problem is highly relevant as PA patients have a higher risk for cardiovascular morbidity and mortality than age-, sex- and blood pressure-matched controls with essential hypertension[34-42]. As specific treatment by adrenalectomy (ADX) or mineralocorticoid receptor antagonists (MRA) is effective in reducing these cardiovascular complications, early detection and treatment of PA is of utmost importance.[36,41-43] Screening for PA is based on measurement of plasma aldosterone and renin concentrations. The ratio between the two, the aldosterone-to-renin-ratio (ARR), is regarded as the most reliable biomarker for PA screening.[33] Subsequently, the diagnosis is confirmed by an aldosterone suppression test.[44] Patients who fail to suppress the plasma aldosterone levels after intravascular volume expansion by administration of saline are diagnosed as PA.

DIFFERENTIATING UNILATERAL FROM BILATERAL PA

Once the diagnosis of PA is established it is important to differentiate unilateral from bilateral disease as this determines the choice of treatment. Unilateral disease is best treated by laparoscopic ADX which is potentially curative, while lifelong medical treatment with MRA is recommended for patients with bilateral disease. Therefore, to justify surgical treatment a diagnostic technique is required that correctly diagnoses unilateral disease.

CT-SCAN

Computed tomography scanning (CT) has been widely used to differentiate between a unilateral or bilateral cause of PA. Imaging showing a unilateral lesion with a normal contralateral gland reflects unilateral disease and thus ADX is indicated (Figure 6). In case of bilateral lesions or symmetric normal adrenal glands treatment with MRA is recommended.[44]

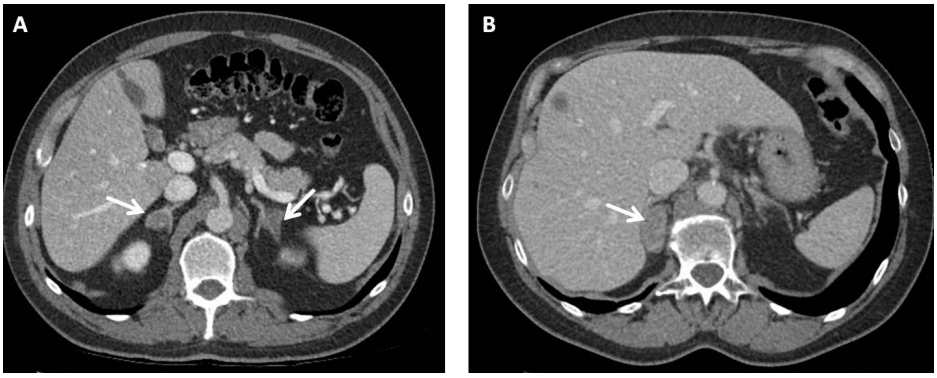


Figure 6. A. Adrenal CT-scan with bilateral hyperplasia. **B.** Adrenal CT-scan with an adenoma of the right adrenal gland

ADRENAL VEIN SAMPLING

For AVS blood from the veins draining the adrenal glands is collected by catheterization (Figure 7).[45] The catheter is inserted in the iliac vein in the groin and moved towards the adrenal veins. The adrenal veins are localized using digital subtraction imaging. Blood samples from both adrenal veins and from a peripheral vein are collected. Cortisol measurements in all samples are needed for establishing the correct catheter position and to correct for non-adrenal venous blood contamination. Cortisol secretion can be enhanced and stabilized by the use of cosyntropin. A high cortisol ratio between the adrenal venous sample and the peripheral venous sample documents correct catheter placement.[44] The aldosterone levels corrected for cortisol in both adrenal veins are compared and an asymmetrical aldosterone production is indicative of unilateral PA (Figure 8).[44]

CT-SCAN VERSUS AVS

Adrenal CT-scan has the advantage that it is non-invasive, relatively cheap and available in all hospitals. However, it has several potential pitfalls. The diagnostic sensitivity of the CT scan is limited due to failure to detect small adenomas. The diagnostic specificity is also compromised as the CT scan cannot differentiate between aldosterone-producing adenomas and non-functioning adenomas. The last being frequently found beyond 40 years of age.[44] AVS has the advantage that it may find small adenomas that are missed because of the CT detection limit and that it may prove CT-identified adenomas to be non-functional.[46-49] Therefore, AVS has emerged as the 'reference standard' to differentiate unilateral from bilateral disease. However, AVS has the disadvantages that it is invasive, expensive and inconvenient for the patient. Moreover, AVS demands great skills and has a high failure

rate in inexperienced hands.[44,50] In the Endocrine Society guideline AVS is considered to be essential to direct appropriate treatment with the only role for CT-scan to detect potential malignant adrenal lesions.[44]

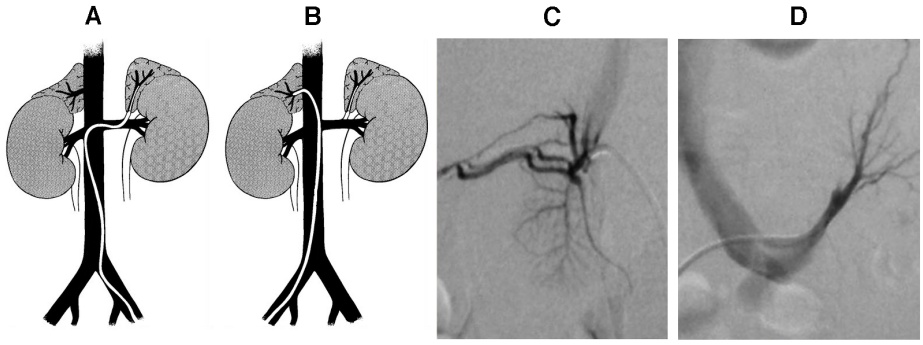


Figure 7. Adrenal vein sampling.

A. Catheterization of the left adrenal vein. B. Catheterization of the right adrenal vein. C. Digital subtraction angiography (DSA) of the right adrenal vein. D. DSA of the left adrenal vein.

From: Melby JC, Spark RF, Dale SL, Egdahl RH, Kahn PC. Diagnosis and localization of aldosterone-producing adenomas by adrenal-vein catheterization. The New England journal of medicine 1967;277:1050-6, Copied with consent of the publisher.[20]

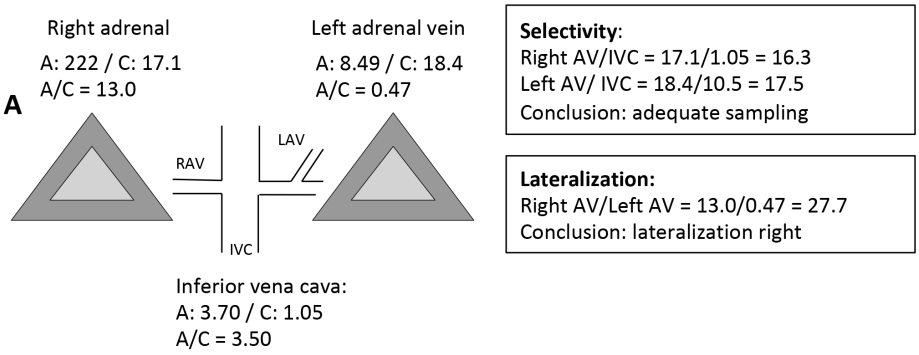


Figure 8. Example of the results of AVS with calculation of selectivity and lateralization of aldosterone production.

Selectivity represents correctness of catheter placement and is calculated as the cortisol ratio between the adrenal vein (AV) and the inferior vena cava (IVC). In this example a ratio (selectivity index) ≥ 3.0 indicates correct catheter placement. Lateralization of aldosterone production is determined by calculation of the aldosterone to cortisol ratio of one (dominant) adrenal gland compared to the aldosterone to cortisol ratio of the other (non-dominant) adrenal gland. In this example a gradient of ≥4.0 indicates unilateral aldosterone production. Moreover, some centres require an additional suppression of the non-dominant gland, calculated as the aldosterone to cortisol ratio of the gland's adrenal vein compared to the aldosterone to cortisol ratio of the IVC (cut-off < 1.0). In this example: Left AV/VCI = 0.47/3.5 = 0.13. RAV = right adrenal vein; LAV = left adrenal vein; IVC = inferior vena cava; A = aldosterone, C= cortisol, A/C = aldosterone/ cortisol ratio

TREATMENT

As stated above, APA is best treated by laparoscopic ADX.[44] Nevertheless, even after biochemical remission hypertension persists to some extent in about two thirds of ADX cases, but most patients need fewer antihypertensive drugs.[51] Medical management with MRA is recommended for BAH patients and this therapy is usually highly effective although additional antihypertensive drugs are often needed. Spironolactone is the best-known MRA but may cause side effects. Eplerenone is a newer alternative with minimal side-effects at the expense of a lower potency.[52]

CHALLENGING ASPECTS OF PRIMARY ALDOSTERONISM

Although PA is nowadays a well-known clinical entity, there are several aspects of the diagnostic workup and management that are debatable and controversial. The reason to question some of these generally accepted concepts is the realization that convincing evidence is lacking. Recommendations from international guidelines are mainly based on retrospective studies and on expert opinion. Therefore there is a need for more thorough, prospective research to obtain a more solid base for development of clinical guidelines. In this thesis there are three aspects we would like to address.

1. PREVALENCE

The first aspect we want to address is the prevalence of PA. For health care planning and allocation of resources, realistic estimation of the prevalence of PA is necessary. In primary care centres, reported prevalences vary from 6% to 13%; in secondary care centres, prevalences of 23% to almost 30% have been reported. [39,44,53,54] However, many clinicians have the impression that they encounter only a few patients with PA in their entire career. The reason for this discrepancy may be threefold. First, clinicians may not consider the possibility of PA as a cause of hypertension. Secondly, our screening and detection methods are not infallible. Thirdly, PA is just not as prevalent as suggested by most prevalence studies. Reported prevalences of PA are highly variable. This might be due to study heterogeneity. We tried to identify and explain the sources of heterogeneity in studies that aimed to establish the prevalence of PA in hypertensive patients.

2. SUBTYPING: SELECTING THOSE PATIENTS THAT WILL BENEFIT FROM SURGERY

The second aspect discussed in this thesis is the presumed superiority of AVS over CT-scan for selecting those patients that will benefit from unilateral ADX. The current Endocrine Society guideline advises to treat patients with unilateral disease with adrenalectomy and those with bilateral disease with MRA.[44] Selecting those who can benefit from adrenal surgery is very relevant. Therefore, it is important to find the optimal technique. As mentioned before, the most commonly used techniques are either adrenal CT scan or AVS. It is striking that when applied in the same patient population these techniques show a high discordance concerning their final conclusion. In a systematic review of 38 articles *Kempers et al.* showed a discordance of 37.8% between CT/MRI results and AVS.[55] The key question is which of these techniques, AVS or CT is superior.

CT-SCAN VERSUS AVS

The current clinical guideline recommends AVS as the reference standard for distinguishing unilateral from bilateral PA.[44] However, this recommendation is primarily based on observational or retrospective studies.[56-58] In the studies of *Young et al* .[56] and *Nwariaku et al.* [57] AVS is used to determine further treatment and applied as a gold standard to assess CT accuracy. Although both studies report (AVS-based) post-operative clinical parameters, no clear criteria for post-operative treatment outcome were defined. Due to these study designs the presumed AVS superiority is based on circular reasoning. The third study referred to by the guideline is a chapter in a hypertension study book. As the chapter describes no original research or systematic literature analysis it is merely an expert opinion.[58] A fourth study brought by the guideline to underscore AVS superiority is the systematic review of *Kempers et al.* mentioned above.[55] In this study, however, due to the lack of follow-up data in many of the included studies, it was not possible to assess whether the diagnosis of unilateral aldosterone excess was correct. In conclusion, these articles provide no convincing support for AVS superiority.

There are more reasons that potentially generate equipoise for the decision to perform AVS.[59] First, there is a lack of standardization of the AVS technique and protocol.[60] This includes sequential versus simultaneous sampling[61], and use of cosyntropin stimulation to minimize stress induced cortisol fluctuations. Another issue is the validity of cut-off values for selectivity and lateralization.[62-65] Where some centres regard a non-cosyntropin-stimulated cortisol

ratio between the adrenal gland and the peripheral blood of 1.1 as selective, others require a non-stimulated ratio of 4.0 or even a stimulated ratio of 10.0.[60] For lateralization indices this ranges across studies from 2.0 to 5.0. *Mulatero et al.* found that, in patients who had undergone AVS twice, the concordance among the conclusions of the two procedures was only 35%. When applying three different diagnostic criteria for lateralization, concordance among the conclusions concerning the diagnoses was 32%.[64] *Lethielleux et al.* found similar results applying different diagnostic criteria in 500 AVS procedures. They found that AVS procedures were classified as unsuccessful five times more often when applying the most strict criteria compared to the least strict criteria. Moreover, two times more AVS procedures lateralized using the least stringent criteria compared to the most stringent criteria.[63] Two recently published expert consensus statements have tried to overcome these differences.[66,67] However, as thorough underlying evidence is lacking many issues remain unresolved and with that inconsistencies persist.

If neither CT-scan nor AVS can be regarded to be the reference standard, then what can we do? In the absence of a gold standard there are several options available as shown in table 1.[68,69] Considering these different approaches, the validation of the index test results is the most favourable option in the case of PA. For subtyping PA, thorough patient follow-up of blood pressure and biochemical test results may provide a test for adequacy of diagnosis.

Table 1. Options for a reference standard in absence of a gold standard[69]

Approach	Main Characteristic
Composite reference standard	Combing results of different imperfect reference tests
Differential verification	A different reference standard is used in different patient subgroups
Discrepant analysis	Patients with a discordant result between the index test and imperfect reference test are retested with an additional reference test.
Panel or consensus diagnosis	A group of experts determines presence or absence of target condition
Latent class analysis	A statistical model combines different pieces of patient information to construct a reference standard.
Validate index test results	The index test results are related to future clinical events and outcomes.

If we decide that the reference standard to assess diagnostic accuracy in PA should be based on prospective follow-up and treatment outcome as suggested in the last option of Table 1, this strategy could also be applied to the comparison of AVS to CT-scan. Unfortunately, prospective randomized outcome studies that have compared both techniques and that support the superiority of AVS over CT scan are not available. In this thesis we describe a diagnostic outcome-based randomized trial to compare patients diagnosed by either CT-scan or AVS.

THE NEED FOR PA SUBTYPING

Because of the difficulties in PA subtyping described above some clinicians abstain from subtyping and treat all patients, regardless of unilateral or bilateral disease, with MRA only. Although the guideline recomments treatment with MRA only in case of bilateral disease, it can also reduce blood pressure in PA patients with unilateral disease.[70-72] The question is whether this justifies abstinence from subtyping.

A recent review of the literature, comparing treatment outcome after adrenalectomy and MRA treatment, showed comparable effects on blood pressure, medication use and hypokalemia in six studies, and better results after surgery in another six studies.[73] Unfortunately, the quality of the studies is not discussed. Moreover, achievement of blood pressure targets might not be the best outcome measure, but the amount of medication needed to achieve these targets might be a better outcome. *Rossi et al.* showed that, although they all reach the same blood pressure, adrenalectomised PA patients require less medication than matched patients with MRA-treated PA or patients with primary hypertension. [74] However, the ultimate goal in hypertension treatment is prevention of (subclinical) organ damage and cardiovascular events. After adrenalectomy for unilateral PA the risk of a cardiovascular event equals that of matched patients with essential hypertension.[38,43,72] Whether the protective cardiovascular effect is also present in patients treated with MRA is somewhat controversial. Some studies report similar beneficial effects for treatment with mineralocorticoid antagonists as others show persistence of the increased cardiovascular risk despite treatment.[36,38,42,72,74] *Reincke et al*. showed that adrenalectomy (as compared with MRA treatment) was associated with reduced all-cause mortality.[75] A possible explanation for these controversies may be provided by a recent study of *Hundemer et al.* showing a decrease in cardiovascular risk in only those patients whose renin levels are no longer suppressed (indicating effective antagonism of aldosterone effects) after MRA treatment.[41,43]

For patients both the amount of medication and organ damage can have a serious impact on health related quality of life. Especially the use of spironolactone is associated with side-effects, like gynaecomastia and impotence in male patients. Differences in quality of life between surgical and medical treatment have been assessed in several studies. *Ahmed et al.* showed that patients with unilateral PA treated surgically had a faster and more complete recovery of quality of life than those treated medically.[76] Two other studies showed a reduced quality of life only in female patients treated with MRA compared to those treated with surgery.[77,78] Beside patient-related factors also societal factors may be important. *Kline et al.* showed that after adrenalectomy follow-up time is shorter and clinical visits are fewer, resulting in a more cost-effective treatment. [79] Based on this information subtyping of PA, and with that the question on AVS or CT superiority, seems highly relevant.

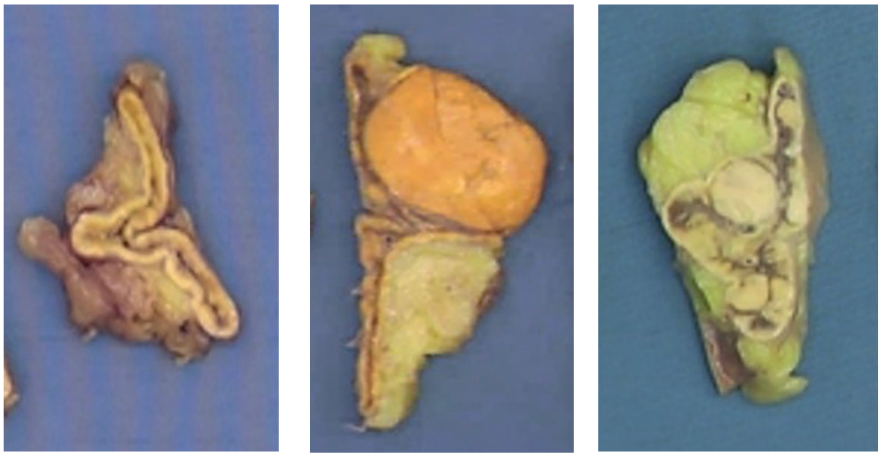


Figure 9. Adrenal macroscopic anatomy.

Normal adrenal gland (upper panel), Solitary adenoma (middle panel), multinodular hyperplasia (lower panel). Published with the approval of E. van de Wiel, J. Langenhuijsen, B. Kusters and J. Deinum (Dept of Urology, Pathology and Internal Medicine, Radboud University Medical center)

3. HISTOLOGICAL DICHOTOMY OF APA AND BAH

The third aspect we want to consider is the histological dichotomy between unilateral and bilateral adrenal disease in PA. The diagnostic and treatment strategy in PA is based on the assumption that PA is caused by one of two entities: either a unilateral aldosterone producing adenoma (APA) or bilateral adrenal hyperplasia (BAH) (Figure 9). However, multiple studies

show that adrenals with a suspected single adenoma show various patterns of macronodular or micronodular hyperplasia. [80-84]

There are many other features in the adrenal histopathology of PA that indicate a more complex underlying pathology than the commonly assumed dichotomy. For example, many adrenal nodules do not seem to originate from the aldosterone-producing zona glomerulosa, but from the cortisol-producing zona fasciculata.[80,85,86] Also the often found adrenal zona glomerulosa thickening, where atrophy would be expected, is a remarkable finding.[86,87] Moreover, immunostaining using monoclonal antibodies has provided further insights in the histopathology of PA. Monoclonal antibodies against *p450C11* (CYP11B1) and *p450C18* (CYP11B2) can be used to indicate the areas in the resected gland that produce cortisol and aldosterone, respectively.[86,88] Different studies show that some adrenal nodules seem capable of producing both aldosterone and cortisol.[84,86,89] This raises also the question which other steroids are produced by adrenal nodules. Another noteworthy feature discovered with the use of immunostaining is the presence of extranodular cell clusters in the zona glomerulosa capable of aldosterone production (aldosterone producing cell clusters, APCCs). The real function of these APCCs is still unknown.[86,90,91]

OUTLINE OF THE THESIS

This thesis focuses on three different aspects of PA.

The first aspect, the prevalence of PA, is investigated in **chapter 2**. The actual prevalence of PA is a matter of continuing debate as prevalence reported in literature varies widely. In a systematic review we assess the factors determining the wide variety of prevalences found in previously published studies.

In **chapter 3** we describe a prospective, randomized, diagnostic trial on the value of AVS and CT-scan for the subtyping of PA. As advocated in the introduction above, this study used treatment outcome as a reference standard to assess diagnostic accuracy.

In the two following chapters two aspects of the AVS procedure that could be improved to increase the accuracy and efficiency are discussed. **Chapter 4** comprises a small study regarding AVS cost minimalisation by the use of single instead of duplicate blood samples during the AVS procedure. In **chapter 5** we discuss the use of metanephrine, another hormonal metabolite, instead of cortisol to determine selectivity in AVS.

The third aspect addressed in this thesis is discussed in **chapter 6**. This chapter describes the histopathological and genetic findings in adrenal glands removed because of suspicion of a unilateral adenoma. It questions the dichotomy of unilateral APA and BAH as many adrenal glands seem to be multinodular.

In **chapter 7** we describe a case of a patient with PA in whom there were no adrenal anomalies on CT-scan and a bilateral suppression of adrenal aldosterone production on AVS. This case is illustrative in showing the pitfalls of both CT-scan and AVS.

We conclude this thesis with **chapter 8** where we discuss the implications of our findings for clinical practice and for future research.

REFERENCES

1. Herder Wd. Jerome W. Conn (1907-1981). Endocrinologie 2010:23-5.
2. Conn JW. Presidential address. I. Painting background. II. Primary aldosteronism, a new clinical syndrome. The Journal of laboratory and clinical medicine 1955;45:3-17.
3. Conn JW. Electrolyte composition of sweat; clinical implications as an index of adrenal cortical function. Archives of internal medicine (Chicago, Ill : 1908) 1949;83:416-28.
4. Schteingart DE. The 50th anniversary of the identification of primary aldosteronism: a retrospective of the work of Jerome W. Conn. The Journal of laboratory and clinical medicine 2005;145:12-6.
5. Simpson SA, Tait JF, Bush IE. Secretion of a salt-retaining hormone by the mammalian adrenal cortex. Lancet (London, England) 1952;2:226-8.
6. Conn JW, Cohen EL, Rovner DR. SUPPRESSION OF PLASMA RENIN ACTIVITY IN PRIMARY ALDOSTERONISM. Jama 1964;190:213-21.
7. Rossi GP, Bernini G, Caliumi C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. Journal of the American College of Cardiology 2006;48:2293-300.
8. Mulatero P, Stowasser M, Loh KC, et al. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. The Journal of clinical endocrinology and metabolism 2004;89:1045-50.
9. Conn JW. The evolution of primary aldosteronism: 1954-1967. Harvey lectures 1966;62:257-91.
10. Hannemann A, Wallaschofski H. Prevalence of primary aldosteronism in patient's cohorts and in population-based studies--a review of the current literature. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2012;44:157-62.
11. Gordon RD, Stowasser M, Tunny TJ, Klemm SA, Rutherford JC. High incidence of primary aldosteronism in 199 patients referred with hypertension. Clinical and experimental pharmacology & physiology 1994;21:315-8.
12. Swales JD. Primary aldosteronism: how hard should we look? British medical journal (Clinical research ed) 1983;287:702-3.
13. Berglund G, Andersson O, Wilhelmsen L. Prevalence of primary and secondary hypertension: studies in a random population sample. British medical journal 1976;2:554-6.
14. Clarke D, Wilkinson R, Johnston ID, Hacking PM, Haggith JW. Severe hypertension in primary aldosteronism and good response to surgery. Lancet (London, England) 1979;1:482-5.
15. Kaplan NM. PRIMARY ALDOSTERONISM WITH MALIGNANT HYPERTENSION. The New England journal of medicine 1963;269:1282-6.

16. Pringle SD, Macfarlane PW, Isles CG, et al. Regression of electrocardiographic left ventricular hypertrophy following treatment of primary hyperaldosteronism. Journal of human hypertension 1988;2:157-9.
17. Cerny JC, Nesbit RM, Conn JW, et al. Preoperative tumor localization by adrenal venography in patients with primary aldosteronism: a comparison with operative findings. The Journal of urology 1970;103:521-8.
18. Conn JW, Beierwaltes WH, Lieberman LM, et al. Primary aldosteronism: preoperative tumor visualization by scintillation scanning. The Journal of clinical endocrinology and metabolism 1971;33:713-6.
19. Espiner EA, Jameson JB, Perry EG, Miles K. Adrenal venography and sampling in the diagnosis and treatment of primary aldosteronism. The New Zealand medical journal 1976;83:313-6.
20. Melby JC, Spark RF, Dale SL, Egdahl RH, Kahn PC. Diagnosis and localization of aldosterone-producing adenomas by adrenal-vein cateterization. The New England journal of medicine 1967;277:1050-6.
21. Linde R, Coulam C, Battino R, Rhamy R, Gerlock J, Hollifield J. Localization of aldosterone-producing adenoma by computed tomography. The Journal of clinical endocrinology and metabolism 1979;49:642-5.
22. Stowasser M, Gordon RD. Primary Aldosteronism: Changing Definitions and New Concepts of Physiology and Pathophysiology Both Inside and Outside the Kidney. Physiological reviews 2016;96:1327-84.
23. Moors M, Williams TA, Deinum J, Eisenhofer G, Reincke M, Lenders JW. Steroid Hormone Production in Patients with Aldosterone Producing Adenomas. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2015;47:967-72.
24. Lenzini L, Rossitto G, Maiolino G, Letizia C, Funder JW, Rossi GP. A Meta-Analysis of Somatic KCNJ5 K(+) Channel Mutations In 1636 Patients With an Aldosterone-Producing Adenoma. The Journal of clinical endocrinology and metabolism 2015;100:E1089-95.
25. Choi M, Scholl UI, Yue P, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. Science (New York, NY) 2011;331:768-72.
26. Beuschlein F, Boulkroun S, Osswald A, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. Nature genetics 2013;45:440-4, 4e1-2.
27. Azizan EA, Poulsen H, Tuluc P, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. Nature genetics 2013;45:1055-60.

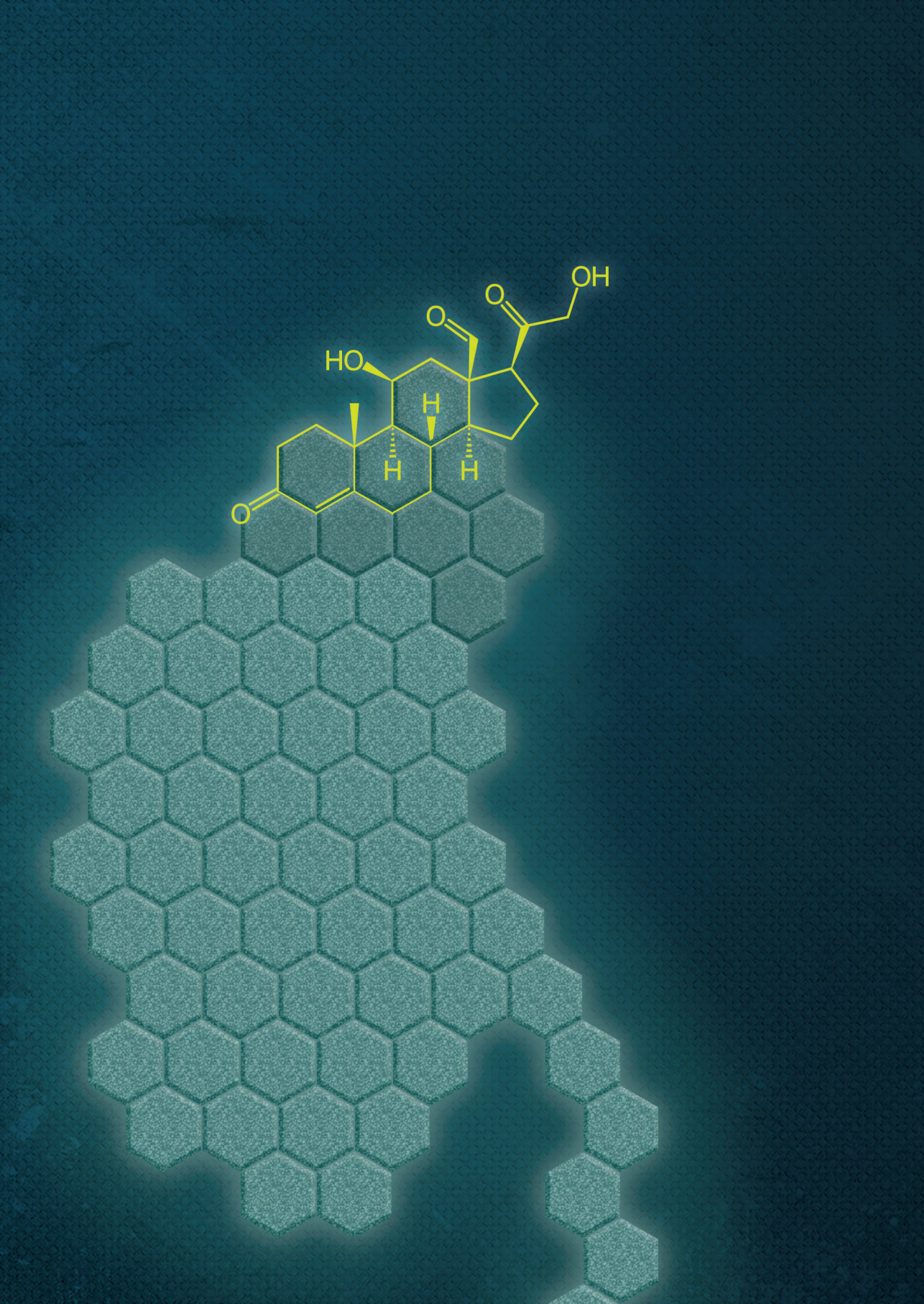
28. Teo AE, Garg S, Shaikh LH, et al. Pregnancy, Primary Aldosteronism, and Adrenal CTNNB1 Mutations. The New England journal of medicine 2015;373:1429-36.
29. Fernandes-Rosa FL, Daniil G, Orozco IJ, et al. A gain-of-function mutation in the CLCN2 chloride channel gene causes primary aldosteronism. Nature genetics 2018;50:355-61.
30. Scholl UI, Stolting G, Schewe J, et al. CLCN2 chloride channel mutations in familial hyperaldosteronism type II. Nature genetics 2018;50:349-54.
31. Jansen PM, Boomsma F, van den Meiracker AH. Aldosterone-to-renin ratio as a screening test for primary aldosteronism--the Dutch ARRAT Study. The Netherlands journal of medicine 2008;66:220-8.
32. Douma S, Petidis K, Doumas M, et al. Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. Lancet (London, England) 2008;371:1921-6.
33. Bots MLB, J.; Dis van, I. ; Vaartjes, I.; Visseren, F.L.J. Hart- en vaatziekten in Nederland, 2016. Hartstichting 2016.
34. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. Journal of the American College of Cardiology 2005;45:1243-8.
35. Born-Frontsberg E, Reincke M, Rump LC, et al. Cardiovascular and cerebrovascular comorbidities of hypokalemic and normokalemic primary aldosteronism: results of the German Conn's Registry. The Journal of clinical endocrinology and metabolism 2009;94:1125-30.
36. Catena C, Colussi G, Nadalini E, et al. Cardiovascular outcomes in patients with primary aldosteronism after treatment. Archives of internal medicine 2008;168:80-5.
37. Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular complications associated with primary aldosteronism: a controlled cross-sectional study. Hypertension 2013;62:331-6.
38. Mulatero P, Monticone S, Bertello C, et al. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. The Journal of clinical endocrinology and metabolism 2013;98:4826-33.
39. Monticone S, Burrello J, Tizzani D, et al. Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice. Journal of the American College of Cardiology 2017;69:1811-20.
40. Monticone S, D'Ascenzo F, Moretti C, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. The lancet Diabetes & endocrinology 2018;6:41-50.
41. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Incidence of Atrial Fibrillation and Mineralocorticoid Receptor Activity in Patients With Medically and Surgically Treated Primary Aldosteronism. JAMA cardiology 2018;3:768-74.

42. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Renal Outcomes in Medically and Surgically Treated Primary Aldosteronism. Hypertension 2018;72:658-66.
43. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. The lancet Diabetes & endocrinology 2018;6:51-9.
44. Funder JW, Carey RM, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. The Journal of clinical endocrinology and metabolism 2016;101:1889-916.
45. Daunt N. Adrenal vein sampling: how to make it quick, easy, and successful. Radiographics : a review publication of the Radiological Society of North America, Inc 2005;25 Suppl 1:S143-58.
46. Lim V, Guo Q, Grant CS, et al. Accuracy of adrenal imaging and adrenal venous sampling in predicting surgical cure of primary aldosteronism. The Journal of clinical endocrinology and metabolism 2014;99:2712-9.
47. Mulatero P, Bertello C, Rossato D, et al. Roles of clinical criteria, computed tomography scan, and adrenal vein sampling in differential diagnosis of primary aldosteronism subtypes. The Journal of clinical endocrinology and metabolism 2008;93:1366-71.
48. Raman SP, Lessne M, Kawamoto S, et al. Diagnostic performance of multidetector computed tomography in distinguishing unilateral from bilateral abnormalities in primary hyperaldosteronism: comparison of multidetector computed tomography with adrenal vein sampling. Journal of computer assisted tomography 2015;39:414-8.
49. Mathur A, Kemp CD, Dutta U, et al. Consequences of adrenal venous sampling in primary hyperaldosteronism and predictors of unilateral adrenal disease. Journal of the American College of Surgeons 2010;211:384-90.
50. Vonend O, Ockenfels N, Gao X, et al. Adrenal venous sampling: evaluation of the German Conn's registry. Hypertension 2011;57:990-5.
51. Williams TA, Lenders JWM, Mulatero P, et al. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. The lancet Diabetes & endocrinology 2017;5:689-99.
52. Deinum J, Riksen NP, Lenders JW. Pharmacological treatment of aldosterone excess. Pharmacology & therapeutics 2015;154:120-33.
53. Eide IK, Torjesen PA, Drolsum A, Babovic A, Lilledahl NP. Low-renin status in therapy-resistant hypertension: a clue to efficient treatment. Journal of hypertension 2004;22:2217-26.
54. Calhoun DA, Nishizaka MK, Zaman MA, Harding SM. Aldosterone excretion among subjects with resistant hypertension and symptoms of sleep apnea. Chest 2004;125:112-7.

55. Kempers MJ, Lenders JW, van Outheusden L, et al. Systematic review: diagnostic procedures to differentiate unilateral from bilateral adrenal abnormality in primary aldosteronism. Annals of internal medicine 2009;151:329-37.
56. Young WF, Stanson AW, Thompson GB, Grant CS, Farley DR, van Heerden JA. Role for adrenal venous sampling in primary aldosteronism. Surgery 2004;136:1227-35.
57. Nwariaku FE, Miller BS, Auchus R, et al. Primary hyperaldosteronism: effect of adrenal vein sampling on surgical outcome. Archives of surgery (Chicago, Ill : 1960) 2006;141:497-502; discussion -3.
58. Gordon RD. Diagnostic investigations in primary aldosteronism. In: A Z, ed. Clinical medicine series on hypertension Maidenhead: McGraw-Hill International; 2001:101-11.
59. Stewart PM, Allolio B. Adrenal vein sampling for Primary Aldosteronism: time for a reality check. Clinical endocrinology 2010;72:146-8.
60. Rossi GP, Barisa M, Allolio B, et al. The Adrenal Vein Sampling International Study (AVIS) for identifying the major subtypes of primary aldosteronism. The Journal of clinical endocrinology and metabolism 2012;97:1606-14.
61. Carr CE, Cope C, Cohen DL, Fraker DL, Trerotola SO. Comparison of sequential versus simultaneous methods of adrenal venous sampling. Journal of vascular and interventional radiology : JVIR 2004;15:1245-50.
62. Kline GA, Harvey A, Jones C, et al. Adrenal vein sampling may not be a gold-standard diagnostic test in primary aldosteronism: final diagnosis depends upon which interpretation rule is used. Variable interpretation of adrenal vein sampling. International urology and nephrology 2008;40:1035-43.
63. Lethielleux G, Amar L, Raynaud A, Plouin PF, Steichen O. Influence of diagnostic criteria on the interpretation of adrenal vein sampling. Hypertension 2015;65:849-54.
64. Mulatero P, Bertello C, Sukor N, et al. Impact of different diagnostic criteria during adrenal vein sampling on reproducibility of subtype diagnosis in patients with primary aldosteronism. Hypertension 2010;55:667-73.
65. Wolley MJ, Gordon RD, Ahmed AH, Stowasser M. Does contralateral suppression at adrenal venous sampling predict outcome following unilateral adrenalectomy for primary aldosteronism? A retrospective study. The Journal of clinical endocrinology and metabolism 2015;100:1477-84.
66. Rossi GP, Auchus RJ, Brown M, et al. An expert consensus statement on use of adrenal vein sampling for the subtyping of primary aldosteronism. Hypertension 2014;63:151-60.
67. Monticone S, Viola A, Rossato D, et al. Adrenal vein sampling in primary aldosteronism: towards a standardised protocol. The lancet Diabetes & endocrinology 2015;3:296-303.

68. Reitsma JB, Rutjes AW, Khan KS, Coomarasamy A, Bossuyt PM. A review of solutions for diagnostic accuracy studies with an imperfect or missing reference standard. Journal of clinical epidemiology 2009;62:797-806.
69. Rutjes AW, Reitsma JB, Coomarasamy A, Khan KS, Bossuyt PM. Evaluation of diagnostic tests when there is no gold standard. A review of methods. Health technology assessment (Winchester, England) 2007;11:iii, ix-51.
70. Sechi LA, Colussi GL, Novello M, Uzzau A, Catena C. Mineralocorticoid Receptor Antagonists and Clinical Outcomes in Primary Aldosteronism: As Good as Surgery? Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2015;47:1000-6.
71. Ghose RP, Hall PM, Bravo EL. Medical management of aldosterone-producing adenomas. Annals of internal medicine 1999;131:105-8.
72. Catena C, Colussi G, Lapenna R, et al. Long-term cardiac effects of adrenalectomy or mineralocorticoid antagonists in patients with primary aldosteronism. Hypertension 2007;50:911-8.
73. Muth A, Ragnarsson O, Johannsson G, Wangberg B. Systematic review of surgery and outcomes in patients with primary aldosteronism. The British journal of surgery 2015;102:307-17.
74. Rossi GP, Cesari M, Cuspidi C, et al. Long-term control of arterial hypertension and regression of left ventricular hypertrophy with treatment of primary aldosteronism. Hypertension 2013;62:62-9.
75. Reincke M, Fischer E, Gerum S, et al. Observational study mortality in treated primary aldosteronism: the German Conn's registry. Hypertension 2012;60:618-24.
76. Ahmed AH, Gordon RD, Sukor N, Pimenta E, Stowasser M. Quality of life in patients with bilateral primary aldosteronism before and during treatment with spironolactone and/or amiloride, including a comparison with our previously published results in those with unilateral disease treated surgically. The Journal of clinical endocrinology and metabolism 2011;96:2904-11.
77. Apostolopoulou K, Kunzel HE, Gerum S, et al. Gender differences in anxiety and depressive symptoms in patients with primary hyperaldosteronism: a cross-sectional study. The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry 2014;15:26-35.
78. Kunzel HE, Apostolopoulou K, Pallauf A, et al. Quality of life in patients with primary aldosteronism: gender differences in untreated and long-term treated patients and associations with treatment and aldosterone. Journal of psychiatric research 2012;46:1650-4.
79. Kline GA, Pasieka JL, Harvey A, So B, Dias VC. Medical or surgical therapy for primary aldosteronism: post-treatment follow-up as a surrogate measure of comparative outcomes. Annals of surgical oncology 2013;20:2274-8.

80. Enberg U, Volpe C, Hoog A, et al. Postoperative differentiation between unilateral adrenal adenoma and bilateral adrenal hyperplasia in primary aldosteronism by mRNA expression of the gene CYP11B2. European journal of endocrinology / European Federation of Endocrine Societies 2004;151:73-85.
81. Murashima M, Trerotola SO, Fraker DL, Han D, Townsend RR, Cohen DL. Adrenal venous sampling for primary aldosteronism and clinical outcomes after unilateral adrenalectomy: a single-center experience. Journal of clinical hypertension (Greenwich, Conn) 2009;11:316-23.
82. Tresallet C, Salepcioglu H, Godiris-Petit G, Hoang C, Girerd X, Menegaux F. Clinical outcome after laparoscopic adrenalectomy for primary hyperaldosteronism: the role of pathology. Surgery 2010;148:129-34.
83. Weisbrod AB, Webb RC, Mathur A, et al. Adrenal histologic findings show no difference in clinical presentation and outcome in primary hyperaldosteronism. Annals of surgical oncology 2013;20:753-8.
84. Nanba K, Tsuiki M, Sawai K, et al. Histopathological diagnosis of primary aldosteronism using CYP11B2 immunohistochemistry. The Journal of clinical endocrinology and metabolism 2013;98:1567-74.
85. Ganguly A. Cellular origin of aldosteronomas. The Clinical investigator 1992;70:392-5.
86. Nishimoto K, Nakagawa K, Li D, et al. Adrenocortical zonation in humans under normal and pathological conditions. The Journal of clinical endocrinology and metabolism 2010;95:2296-305.
87. Boulkroun S, Samson-Couterie B, Dzib JF, et al. Adrenal cortex remodeling and functional zona glomerulosa hyperplasia in primary aldosteronism. Hypertension 2010;56:885-92.
88. Gomez-Sanchez CE, Qi X, Velarde-Miranda C, et al. Development of monoclonal antibodies against human CYP11B1 and CYP11B2. Mol Cell Endocrinol 2014;383:111-7.
89. Fallo F, Pezzi V, Barzon L, et al. Quantitative assessment of CYP11B1 and CYP11B2 expression in aldosterone-producing adenomas. European journal of endocrinology / European Federation of Endocrine Societies 2002;147:795-802.
90. Nanba K, Tsuiki M, Sawai K, et al. Histopathological Diagnosis of Primary Aldosteronism Using CYP11B2 Immunohistochemistry. The Journal of clinical endocrinology and metabolism 2013.
91. Nishimoto K, Seki T, Hayashi Y, et al. Human Adrenocortical Remodeling Leading to Aldosterone-Producing Cell Cluster Generation. International journal of endocrinology 2016;2016:7834356.


OH
O
O
HO
H
H
H
O

Study heterogeneity and estimation of prevalence of primary aldosteronism: a systematic review and meta-regression analysis

Tanja Dekkers[2] and Sabine C. Käyser[1*] | Hans J. Groenewoud[3]
Gert Jan van der Wilt[3] | J. Carel Bakx[†1] | Mark C. van der Wel[1]
Ad R. Hermus[2] | Jacques W. Lenders[2,4] | Jaap Deinum[2]

* Both authors contributed equally to the work. † Dr. C. Bakx passed away during the study.

[1] Department of Primary and Community Care, Radboud University Medical Center, Nijmegen, The Netherlands
[2] Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands
[3] Department for Health Evidence, Radboud University Medical Center, Nijmegen, The Netherlands
[4] Department of Internal Medicine III, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany

J Clin Endocrinol Metab. 2016 Jul;101(7):2826-35.

2

ABSTRACT

Context: For health care planning and allocation of resources, realistic estimation of the prevalence of primary aldosteronism is necessary. Reported prevalences of primary aldosteronism are highly variable, possibly due to study heterogeneity.

Objective: Our objective was to identify and explain heterogeneity in studies that aimed to establish the prevalence of primary aldosteronism in hypertensive patients.

Data Sources: PubMed, EMBASE, Web of Science, Cochrane Library, and reference lists from January 1, 1990, to January 31, 2015, were used as data sources.

Study Selection: Description of an adult hypertensive patient population with confirmed diagnosis of primary aldosteronism was included in this study.

Data Extraction: Dual extraction and quality assessment were the forms of data extraction.

Data Synthesis: Thirty-nine studies provided data on 42 510 patients (nine studies, 5896 patients from primary care). Prevalence estimates varied from 3.2% to 12.7% in primary care and from 1% to 29.8% in referral centres. Heterogeneity was too high to establish point estimates (I^2 = 57.6% in primary care; 97.1% in referral centres). Meta-regression analysis showed higher prevalences in studies 1) published after 2000, 2) from Australia, 3) aimed at assessing prevalence of secondary hypertension, 4) that were retrospective, 5) that selected consecutive patients, and 6) not using a screening test. All studies had minor or major flaws.

Conclusions: This study demonstrates that it is pointless to claim low or high prevalence of primary aldosteronism based on published reports. Because of the significant impact of a diagnosis of primary aldosteronism on health care resources and the necessary facilities, our findings urge for a prevalence study whose design takes into account the factors identified in the meta-regression analysis.

INTRODUCTION

Primary aldosteronism (PA) is assumed to be the most frequent form of secondary hypertension; however, the actual prevalence of PA is a matter of continuing debate. Clarity regarding the prevalence of PA is highly relevant because it has strong implications for future policy decisions concerning screening strategies for PA.

Identifying PA as the underlying cause of (therapy-resistant) hypertension is considered important for two reasons. First, PA is associated with an increased rate of cardiovascular complications.[1-3] Second, specific treatment by mineralocorticoid receptor antagonists or adrenalectomy is effective in reducing these cardiovascular complications [4-6] and health costs.[7] Therefore, an early diagnosis and treatment of PA are key for increasing the chance of improvement and even cure of hypertension, and for preventing cardiovascular complications [8-10]. In primary care centres, reported prevalences vary from 6% to 13%; in secondary care centres, prevalences of 23% to almost 30% have been reported.[11-13]

In this article, we provide a systematic review and meta-analysis on the prevalence of PA in both primary care and referral centres, conducted according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.[14] In our attempt to obtain a reliable estimate of the prevalence of PA, we encountered substantial methodological heterogeneity. Therefore, we also set out to identify those factors that contribute to the wide variability in estimates of PA prevalence, using meta-regression analysis.

MATERIALS AND METHODS

DATA SOURCES AND SEARCHES

The objectives and methods of this meta-analysis, including databases that were to be searched, search terms, inclusion criteria, and method of analysis were defined before the start of the review and not modified thereafter. Reporting of this systematic review is in accordance with the Meta-analysis of Observational Studies in Epidemiology statement, a structured checklist for reporting meta-analyses.[14]

We conducted a systematic search on four electronic databases: PubMed, EMBASE, Web of Science, and the Cochrane Library; these were searched for English, German, French, Spanish, and Dutch articles on the prevalence of PA published between January 1, 1990, and January 31, 2015. We used the following search terms:

((“Hyperaldosteronism” [Mesh]) OR (hyperaldosteronism [title/abstract]) OR (aldosteronism [title/abstract]) OR (Conn syndrome [title/abstract]) OR (Conns syndrome [title/abstract]) OR (Conn’s syndrome [title/abstract]) OR (hyperaldosteronism [other term]) OR (aldosteronism [other term]) OR (Conn syndrome [other term]) OR (Conn’s syndrome [other term]))

AND

((“Prevalence” [Mesh]) OR (prevalence [title/abstract]) OR (prevalences [title/abstract]) OR (occurrence [title/abstract]) OR (occurrences [title/abstract]) OR (“Incidence” [Mesh]) OR (incidence [title/abstract]) OR (incidences [title/abstract]) OR (“Epidemiology” [Mesh]) OR (“epidemiology” [subheading]) OR (epidemiology [title/abstract]) OR (epidemiologic [title/abstract]) OR (epidemiological [title/abstract]) OR (prevalence [other term]) OR (prevalences [other term]) OR (incidence [other term]) OR (incidences [other term]) OR (occurrence [other term]) OR (occurrences [other term]) OR (epidemiology [other term]) OR (epidemiologic [other term]) OR (epidemiological [other term])) (the Supplemental Data).

We checked reference lists of all provisionally included studies (i.e., studies that were eligible for further assessment) and reviews for additional, relevant studies published in or after 1990. When articles could not be retrieved from electronic databases or national university archives, we contacted the corresponding authors.

We merged search results from the four databases and checked automatically and manually for duplicates (S.C.K. and T.D.). We used no restrictions other than language and year of publication. Studies published before 1990 were excluded to reduce excessive diversity in used assays, cut-off values, and confirmation tests. The final literature search was performed on February 17, 2015.

STUDY SELECTION

Two researchers (S.C.K. and T.D.) independently assessed eligibility of retrieved articles on title and abstract. Full-text articles were retrieved if necessary. Studies were considered eligible for inclusion if they met the following criteria:

1. Data presented as an original study, short report, or letter on the prevalence of PA;
2. Prospective, retrospective, or cross-sectional study design;

3. Study population of adult patients (≥18 years of age) with hypertension;
4. Use of a confirmation test (IV salt-loading test (IV SLT), oral SLT, captopril suppression test, or fludrocortisone suppression test) to verify the diagnosis of PA (performed in at least 50% of the patients with positive screening test).[13]

Studies were excluded if:

1. The prevalence of PA was investigated in patient groups with a specific morbidity (e.g., diabetes mellitus);
2. The article was a case report;
3. The reported prevalences were solely based on aldosterone-renin ratio (ARR) or on another screening test, computed tomography scan results, adrenal venous sampling, blood pressure response to spironolactone or on postoperative histopathology reports.

Disagreements on eligibility were resolved by consensus among the two reviewers or, when necessary, by a third researcher (J.D.).

DATA EXTRACTION AND QUALITY ASSESSMENT

Two researchers (S.C.K. and T.D.) independently scored all included studies on a data extraction form for author(s), year of publication, country, study design, health care setting (primary care or referral centre), number of included patients, patient characteristics (gender, age, severity of hypertension), number of patients with hypokalemia, antihypertensive medication, screening method(s) with cut-off value(s), position during screening method (supine vs. not supine), number of patients in whom screening was positive, confirmation method(s) with cut-off value(s), number of patients with a positive screening who underwent confirmation, the prevalence of PA, and if the study was included or excluded for analysis. Differences in extraction were resolved by consensus or, if necessary, by a third researcher (J.D.).

We contacted corresponding authors (by e-mail or telephone) in case of missing or ambiguous information. If there was an indication that the same group of patients was used in multiple papers on PA prevalence, we contacted corresponding authors to check. In case of multiple reports, we included the study in which the methods were reported in most detail.

After the final inclusion, S.C.K. and T.D. rated the methodological quality and risk of bias in individual studies using the Methodological Evaluation of Observational Research (MORE) – Observational Studies of Incidence or Prevalence of Chronic Diseases protocol.[15] This

protocol comprises the following items:

1. Funding, ethical approval, conflict of interest;
2. Aim of the study and study design;
3. External validity: population, patient selection, inclusion criteria, sampling bias;
4. Internal validity: source of measurements, validation and reliability of estimates, type of outcome.

The MORE protocol provides a descriptive quality assessment of individual studies without an overall quality score.

DATA SYNTHESIS AND ANALYSIS

To estimate the prevalence of PA, we computed random effect pooled proportions for primary care and referral centres separately.[16] Logit transformation was used to get quantities from prevalence. To explore sources of heterogeneity, we performed random effects logistic regression analysis with prevalence of PA as dependent variable.[17,18] We based the choice of variables on controversies discussed in the Endocrine Society Guideline[13] and on our expectations of explanatory factors for bias in prevalence studies. We distinguished three categories of potential predictors of prevalence estimates:

1. Time: studies published in different periods (two categories: 1990 till 2000, and after 2000);
2. Geographic region where studies were performed: Asia, Australia, Europe, Latin America, and United States of America;
3. Factors concerning study design:
    a. Data collection (prospective or retrospective);
    b. Study objective (to assess the prevalence of PA, to assess the prevalence of secondary hypertension, other);
    c. Method of patient selection (consecutive, convenience, self-selection). We defined convenience as arbitrarily selected individuals from the target population other than general such that each individual had uncontrolled probability of selection[19];
    d. Limited to therapy resistant hypertension or not;
    e. Plasma potassium level at inclusion (normokalemia or hypokalemia [serum potassium $\leq$3.5 mmol/L]);
    f. Medication regimen (medication adjusted according to the Endocrine Society guideline, medication adjusted otherwise, only mineralocorticoid receptor antagonists discontinued or medication unchanged)[13];

g. Potassium level at confirmation testing (corrected hypokalemia or normokalemia);
h. Type of screening test (ARR-based test, no screening test, other screening test);
i. Number of screening tests (one test or multiple tests);
j. Patient position during screening tests (supine or not supine);
k. Cut-off levels used for screening tests (unrestrictive or restrictive). We included only studies using ARR-based tests. Unrestrictive was arbitrarily defined as an ARR cut-off value of 20–60 (aldosterone in ng/dl and renin in ng/ml/h); restrictive was defined as an ARR cut-off level of more than 60 or an ARR cut-off level of 20–60 with a plasma aldosterone level of more than 15 ng/dl and/or a suppressed renin level.
l. Percentage of patients with positive screening who underwent a confirmation test (100% or ≥80% or 50–80%);
m. Type of confirmation test (IV SLT, oral SLT, captopril suppression test, fludrocortisone suppression test)[13];
n. Cut-off levels used for the IV SLT confirmation test (unrestrictive or restrictive). Unrestrictive was defined if the used cut-off level of plasma aldosterone after saline was at least 8 ng/dl, and restrictive if that cut-off level was lower than 8 ng/dl. The number of studies concerning other confirmation tests was too low for analysis of the effects of different cut-off levels.

We explored the association of each of these factors with the estimate of the prevalence of PA individually in a univariate analysis. To correct for correlations between factors among studies, we built a model with the set of explanatory factors that remained significant in a multivariable model. We set the entry level of potentially valid predictors for the model at P = .10.

Because of the relatively low number of studies in primary care, we could only develop a model for referral centres. Because sex is not considered a factor in the diagnosis of PA and studies were unselective with respect to gender, we did not take sex into account in the statistical analysis.

Association between predictive factors and the prevalence estimates of PA was reported as odds ratios and their 95% confidence intervals. Prevalence of PA as predicted by the model was compared with the observed prevalence in the articles.

STATISTICAL ANALYSIS

We used the statistical package Meta 4.1–0 in the program R version 3.1.3 (R Foundation for Statistical Computing) to build forest plots and to compute the random effect pooled proportions. Package Meta 4.1–0 is specialized to perform meta-analyses. We also used the program SAS, version 9.2 (SAS Institute Incorporated), to perform a random effect logistic regression analysis using Procedure Glimmix (Proc Glimmix). In this model, the prevalence of PA is predicted by six explanatory variables. We used study as subject in the analysis, which means that the linear predictor contains an intercept term that randomly varies the level of the study.

RESULTS

SEARCH RESULTS AND STUDY SELECTION

The literature search in PubMed, EMBASE, Web of Science, and the Cochrane Library provided 2614 articles, of which 1679 remained after removal of duplicate entries. After review of title and abstract, we excluded 1586 papers (Figure 1), with 93 potentially relevant articles remaining. By reference checking, four more articles were found, of which one was also included. After full-text reading of all provisionally included articles, we excluded 60 articles (Supplemental Table 1). The main reason for exclusion was the lack of a confirmation test to verify the diagnosis of PA (31 studies). Two articles reported on more than one study, resulting in 39 studies (patient cohorts) derived from 36 articles. Overall concordance on (de)selection of studies between the two raters was high: interrater agreement was 95%, Cohen's kappa was 0.89 (0.79–0.99).

PREVALENCE OF PA IN PRIMARY CARE.

Of the 39 studies included, nine were performed within a primary care setting (Table 1 and Supplemental Table 2). The number of patients included ranged from 52 to 3000 (median, 347), with a total of 5896. PA prevalences ranged from 3.2% to 12.7%.

PREVALENCE OF PA IN REFERRAL CENTRES

Thirty studies were conducted in hypertension referral centres, providing data for 36 614 patients (Table 1 and Supplemental Table 2). The number of included patients varied from 50 to 7343 (median, 322.5). PA prevalence ranged from 1.0% to 29.8%.

DIFFERENCES ACROSS STUDIES IN THE REPORTED PREVALENCE OF PA

Forest plots show the weighted mean and the confidence intervals for the prevalence of PA (Figure 2 and Figure 3; Supplemental Figure 1). Heterogeneity (I^2) was large: in primary care, I^2 = 57.6% (0–78%); in referral centres, I^2 = 97.1% (96.7–97.5%). Therefore, we used meta-regression analysis to explore possible sources of heterogeneity (see the following section).

PREVALENCE OF HYPOKALEMIA IN PATIENTS WITH PA

Twenty-eight of the 39 studies reported the number of PA patients with hypokalemia. In primary care studies, hypokalemia was present in 0–37.5% of the patients with confirmed PA (n = 6). In referral centres, hypokalemia ranged from 0% to 67% among patients with confirmed PA (n = 22). Five studies (two primary care studies [20,21] and three studies from referral centres [22-24]) restricted inclusion to normokalemic patients (Supplemental Table 3).

PREVALENCE OF PA IN PATIENTS WITH VARYING SEVERITY OF HYPERTENSION

Seven studies provided data on patients with resistant hypertension and five studies reported on the relation between prevalence of PA and severity of hypertension. The weighted mean PA prevalence was 5.5%, 4.2%, 10.2%, and 16.4% for high-normal blood pressure, stage 1, stage 2, and stage 3 hypertension, respectively.[25-29]

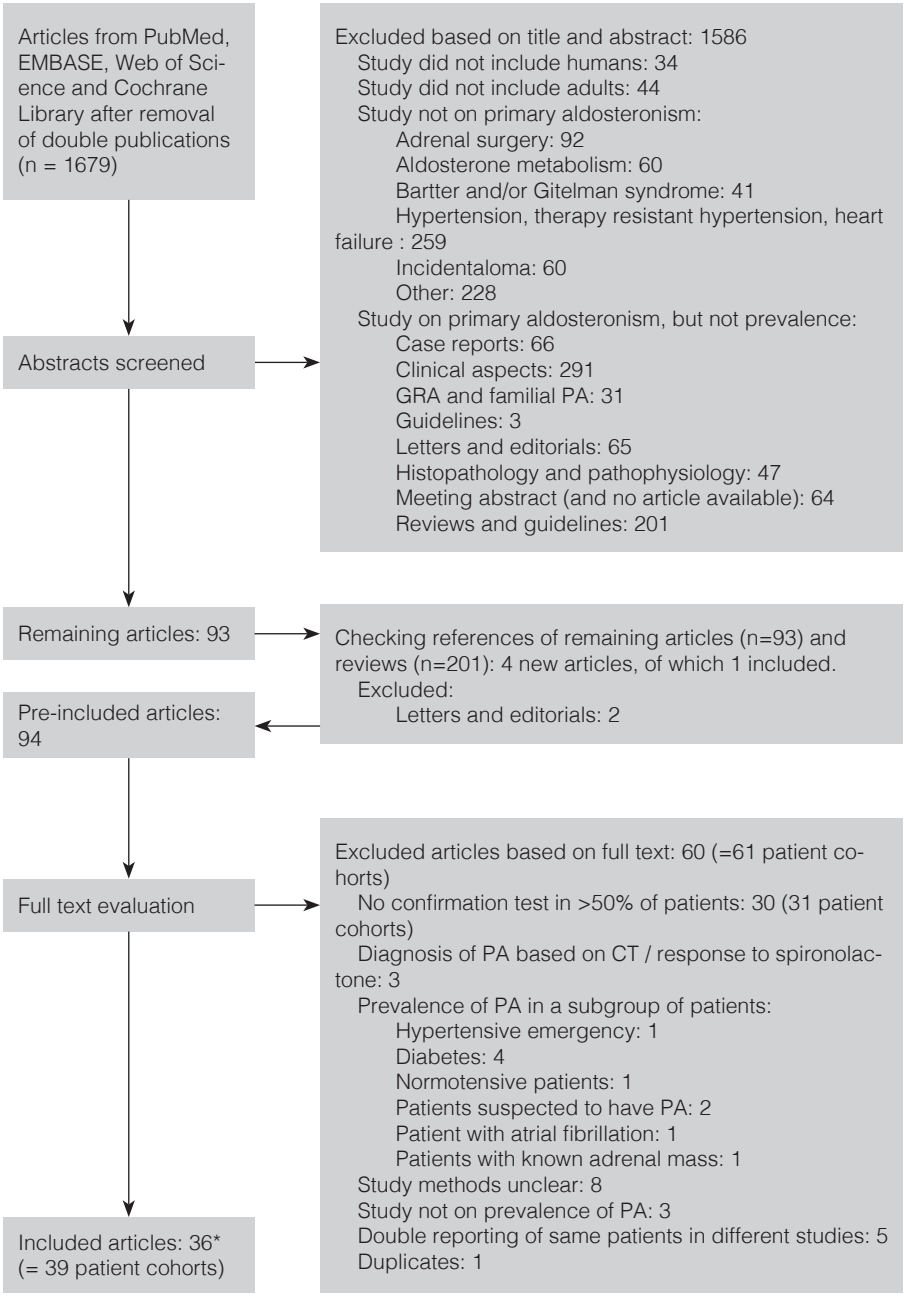


Figure 1. Flow diagram of studies considered for systematic review.

Mulatero[25] and Rossi[30] report five and three cohorts, respectively, of which four and one, respectively, were included. The reason for exclusion of the cohorts are explained in Supplemental Table 1. As a result 36 (included articles) + 60 (excluded articles) = 94. *The 36 articles contain 39 studies (patient cohorts).

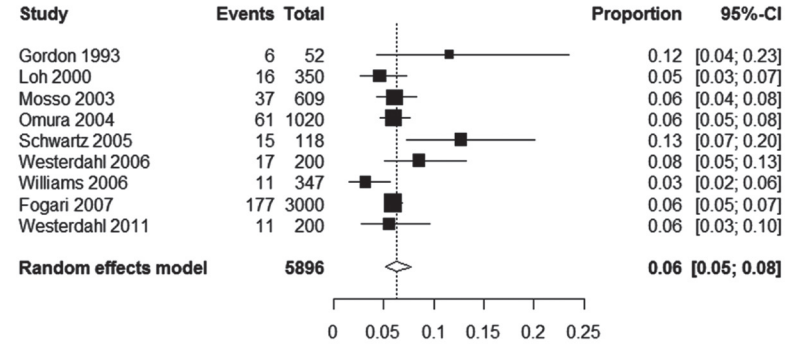


Figure 2. Forest plot for the prevalence of primary aldosteronism in primary care.

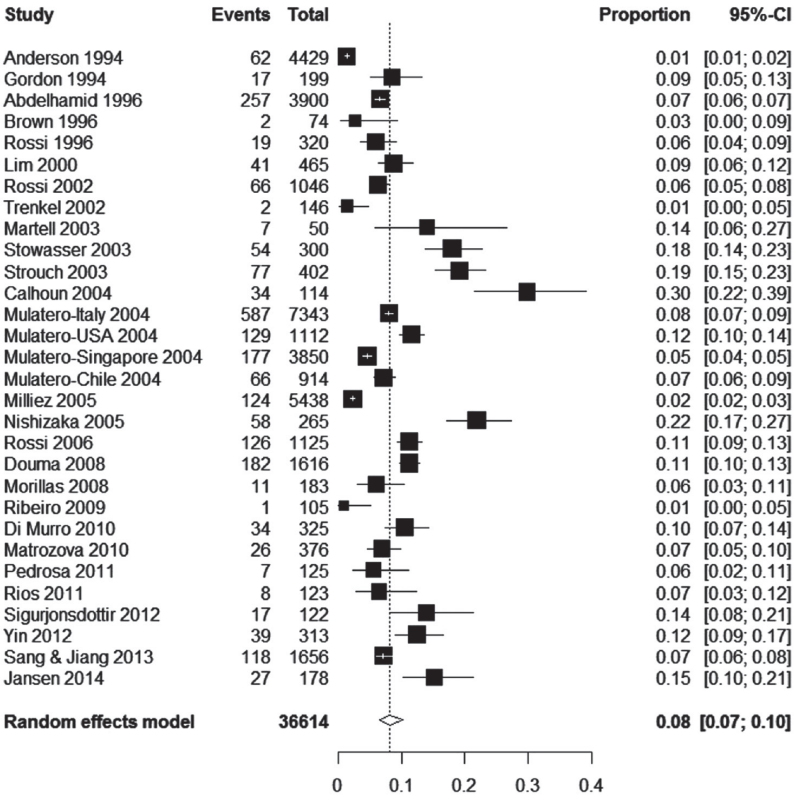


Figure 3. Forest plot for the prevalence of primary aldosteronism in referral centres.

GRA, glucocorticoid remediable aldosteronism; PA, primary aldosteronism.

Table 1. Summary of Studies on Prevalence of Primary Aldosteronism in Primary Care and Referral Centres

Author, Year (ref)	Country	Setting	Design	Period	n	Population	Screening Test	Confirmation Test	Prevalence (%)
Gordon, 1993 [31] [a]	Australia	PC	Prosp	NR	52	HT	ARR	FST	11.5
Loh, 2000 [32] [a]	Singapore	PC	Prosp	1998	350	HT	ARR and PAC	IV SLT	4.6
Mosso, 2003 [26] [a]	Chile	PC	Retro, prosp[b]	1998–2002	609	HT	ARR	FST	6.1
Omura, 2004 [33] [a]	Japan	PC	Prosp	1995–1999	1020	New HT	PAC and PRA	Captopril test	6.0
Schwartz, 2005 [20] [a]	United States	PC	Prosp	2000–2002	118	HT	No screening	Oral SLT	12.7
Westerdahl, 2006 [34]	Sweden	PC	Cross	NR	200	HT	ARR	FST	8.5
Williams, 2006 [21] [a]	United States	PC	Cross	1996–2005	347	HT	ARR and PAC	Oral SLT	3.2
Fogari, 2007 [35]	Italy	PC	Prosp	1999–2002	3000	HT	ARR	IV SLT	5.9
Westerdahl, 2011 [36]	Sweden	PC	Cross	NR	200	New HT	ARR	FST	5.5
Anderson, 1994 [37]	United States	RC	Prosp	1976–1991	4429	HT	IV SLT	Oral SLT	1.4
Gordon, 1994 [22] [a]	Australia	RC	Retro	1992–1993	199	HT	ARR	FST	8.5
Abdelhamid, 1996 [38]	Germany	RC	Prosp	NR	3900	HT	Urinary aldo	IV SLT	6.6
Brown,1996 [23]	Australia	RC	Prosp	1988–1992	74	HT	ARR	IV SLT and FST	2.7
Rossi, 1998 [30]	Italy	RC	Prosp	NR	320	HT	ARR	IV SLT	5.9
Lim, 2000 [39] [c]	UK	RC	Prosp	1995–1997	465	HT	ARR	FST	8.8
Rossi, 2002 [40]	Italy	RC	Prosp	1997–1999	1046	HT	ARR post-captopril	IV SLT	6.3
Trenkel, 2002 [41]	Germany	RC	Prosp	1997–1999	146	HT	ARR	IV SLT	1.4
Martell, 2003 [24] [a]	Spain	RC	Prosp	2000–2002	50	RHT	No screening	IV SLT	15.9
Stowasser, 2003 [42a]	Australia	RC	Prosp	2000–2002	300	HT	ARR	FST	18
Strauch, 2003 [43] [a]	Czech Republic	RC	Retro	1997–2001	402	HT	ARR	IV SLT	19.2
Calhoun, 2004 [44]	United States	RC	Prosp	2000–2002	114	RHT	Urinary aldo and PRA	Oral SLT	29.8

Mulatero, 2004 [25d]	Italy	RC	Retro	1994–2002	7343	HT	ARR and PAC	IV SLT	8.0
	United States	RC	Retro	1999	1112	HT	ARR and PAC	Oral SLT	10.8
	Singapore	RC	Retro	1995–2001	3850	HT	ARR and PAC	IV SLT	4.6
	Chile	RC	Retro	2000–2002	914	HT	ARR	FST	7.2
Milliez, 2005 [1a]	France	RC	Prosp	1997–1999	5438	HT	ARR and PAC	Captopril test	2.3
Nishizaka, 2005 [45]	United States	RC	Prosp	2000–2004	265	RHT	Urinary aldo	Oral SLT	21.9
Rossi, 2006 [27]	Italy	RC	Prosp	2001–2004	1125	New HT	ARR[e]	Captopril testf	11.2
Douma, 2008 [46a]	Greece	RC	Retro	1988–2008	1616	RHT	ARR and SAC	IV SLT and FST	11.3
Morillas, 2008 [47]	Spain	RC	Prosp	2005–2006	183	HT	ARR and PAC	IV SLT	6.0
Ribeiro, 2009 [48]	Brazil	RC	Prosp	2007	105	HT	ARR	IV SLT	1.0
Di Murro, 2010 [49 a]	Italy	RC	Retro	2007–2008	325	New HT	ARR and PAC	IV SLT	13.2
Matrozova, 2010 [28 a,g]	Bulgaria	RC	Prosp	2005–2008	376	HT	ARR and PAC	Captopril test	6.9
Pedrosa, 2011 [50]	Brazil	RC	Cross	2008–2010	125	RHT	ARR	IV SLT	5.6
Rios, 2011 [51]	Argentina	RC	Prosp	2006–2009	123	HT	ARR	IV SLT	6.5
Sigurjonsdottir, 2012 [52 a,h]	Sweden	RC	Prosp	2000–2003	122	HT	ARR and SA	Oral SLT	13.9
Yin, 2012 [53 a]	China	RC	Prosp	2007–2010	313	HT	ARR	Captopril and IV SLT	12.5
Sang & Jiang, 2013 [54 a]	China	RC	Cross	2010–2011	1656	RHT	ARR	IV SLT	7.1
Jansen, 2014 [55 a]	Netherlands	RC	Prosp	2006–2011	178	RHT	No screening	IV SLT	15.2

Abbreviations: aldo, aldosterone; ARR, aldosterone to renin ratio; cross, cross-sectional; FST, fludrocortisone suppression test; HT, hypertension; IV SLT, IV sodium-loading test; n, number of patients; NR, not reported; oral SLT, oral sodium-loading test; PAC, plasma aldosterone concentration; PC, primary care; PRA, plasma renin activity; prosp, prospective; RC, referral centre; ref, reference; retro, retrospective; RHT, resistant hypertension; SAC, serum aldosterone concentration.

[a] Additional data received from author. [b] Study design: partly retrospective. [c] In this review, only patients who were assessed by our predefined inclusion criteria were included in the analysis (prevalence is 41/464 = 8.8%); however, usually when cited, a prevalence of 9.2% is reported (56). [d] Because of missing number of included patients, the study from Australia (Brisbane) is excluded. [e] ARR ≥40 and/or post-captopril ARR ≥30 and/or LDF (logistic discriminant function) score ≥0.50. [f] ARR ≥40 plus post-captopril ARR ≥30 and/or LDF score ≥ 0.50. [g] Patients who were analyzed because of an incidentaloma were excluded. [h] Patients studied in primary care were excluded because of a <50% confirmation test.

DIFFERENCES IN DIAGNOSTIC METHODS

The methods and cut-offs used for screening and confirmation tests varied widely between the included studies. The ARR with or without the use of an absolute level of plasma aldosterone, with varying cut-off values and restrictions, was used for screening in 29 of 39 studies. In four studies, no screening test was performed and in six, other screening tests were used. For confirmation of PA were used: IV SLT (n = 20), oral SLT (n = 7), captopril suppression test (n = 5), fludrocortisone suppression test (n = 4), or a combination of two confirmation tests (n = 3).

Medication regimens during the diagnostic process were reported in most studies and varied from unaltered regimen to complete cessation of all hypertensive medication. In 15 studies, medication regimen was based on the Endocrine Society Clinical Practice Guideline.[13]

QUALITY ASSESSMENT

The results of the quality assessment using the MORE protocol showed that all studies had minor flaws including assessment of sampling bias and type of outcome. More importantly, five studies were classified as having a major flaw because of a patient exclusion rate of more than 10%. For individual quality assessments, see Supplemental Table 4 and Supplemental Figure 2. Some descriptive items or items concerning internal and external validity were neither reported nor addressed in many studies such as role of funding, precision and reliability of estimates, and consideration of sampling bias.

META-REGRESSION ANALYSIS

In primary care, univariate analysis showed a significant association between PA prevalence and five factors: year of publication ($P < .001$), region ($P < .001$), study objective ($P < .001$), medication regimen ($P = .04$), and type of screening test ($P < .001$) (Supplemental Table 5). The highest prevalence estimates were found when the publication year was before 2000, when the study was performed in Australia, when the primary study objective was other than to assess the prevalence of PA, when medication regimen was unchanged, and when no screening test was performed.

Univariate analysis in referral centres showed a significant association between PA prevalence and five variables: year of publication ($P = .04$), study objective ($P = .02$), method of patient selection ($P < .0001$), type of hypertension ($P = .01$), and type of screening test ($P < .001$). The highest prevalence estimates were found when the year of publication was after 2000, when the primary study objective was other than to assess the prevalence of PA,

when patient inclusion was consecutive, when the study population comprised patients with therapy resistant hypertension, and when no screening test was performed.

MULTIVARIATE ANALYSIS

By combining the possible explanatory variables in a single model (only possible for referral centres), we found a set of six variables to independently affect the prevalence of PA: year of publication ($P < .001$), region ($P = .002$), study design ($P = .004$), study objective ($P = .044$), method of patient selection ($P < .001$), and type of screening test ($P = .02$) (Table 2). This model for referral centres showed the highest prevalence in studies that were performed after 2000, when the study was performed in Australia, when the study was retrospective, when the study objective was to assess the prevalence of secondary hypertension, when patient inclusion was consecutive, and in studies in which no screening test was performed. To clarify the prediction of the random effect logistic regression model, we provide a table with examples how variation of the six explanatory variables affects the predicted prevalence (Supplemental Table 6).

Table 2. Solutions for the Fixed Effects of the Random Effect Logistic Regression Model in Referral Centres

Variable	Description OR	OR (95% CI)	Overall P Value
Publication year	2000–current vs. 1990–2000	9.29 (3.17–27.16)	<.001
Region	United States vs. Europe	4.88 (2.07–11.57)	.002
	Latin America vs. Europe	0.53 (0.28–1.01)	
	Asia vs. Europe	1.50 (0.71–3.17)	
	Australia vs. Europe	5.57 (1.94–15.99)	
Study design	Retrospective vs. Prospective	2.31 (1.39–3.84)	.004
Study objective	Prevalence PA vs. other	1.71 (0.81–3.62)	.044
	Prevalence secondary HT vs. other	2.83 (1.12–7.17)	
	Prevalence PA vs. prevalence secondary HT	0.60 (0.40–0.91)	
Patient selection	Consecutive vs. convenience	4.95 (1.82–13.48)	<.001
method	Self-selection vs. convenience	3.40 (0.90–12.89)	
	Consecutive vs. self-selection	1.46 (0.88–2.42)	
Screening test	No screening vs. other	3.25 (1.51–7.01)	.02
	ARR vs. other	0.75 (0.39–1.43)	
	No screening vs. ARR	4.36 (1.52–12.54)	

Abbreviations: ARR, aldosterone to renin ratio; CI, confidence interval; HT, hypertension; OR, odds ratio; PA, primary aldosteronism. The model estimates the prevalence of PA as a function of the six variables. The resulting ORs (according to the model) represent the ratios of the odds for PA of two groups.

DISCUSSION

In this systematically performed review and meta-regression analysis, we confirm the previously reported wide variations in prevalences, both in studies performed in the primary care setting (3.2–12.7%) and in those performed in referral centres (1.0–29.8%). Although previous reviews and meta-analysis studies [56-58] reported mean prevalences, our study shows that it is pointless to provide point estimates in the absence of reporting contextual key factors. We established several factors that, at least partially, are responsible for the gross heterogeneity among studies on prevalence of primary aldosteronism.

In our analysis studies in referral centres published after 2000 showed nearly 9-fold higher odds for the prevalence than studies before 2000, and this was independent from other factors. This might be explained by increasing awareness of the presence of primary aldosteronism over time.

The very first studies that investigated the prevalence of PA were performed in centres in Australia in self-selected patients or on the basis of retrospective data.[22,31] This might partially explain why studies from Australia have a more than 5.5-fold higher odds than those that were carried out in Europe. An alternative explanation is that the prevalence of PA is indeed higher in Australia. Studies performed in the United States also showed nearly 5-fold higher odds. Whether this is due to the same reasons as may apply to Australian studies cannot be ascertained.

It is plausible that prospective studies are more appropriate to estimate prevalences. Our finding that retrospective studies report higher prevalences than prospective ones suggest that the current "epidemic" of PA is partly explained by reliance on retrospective studies.[59] It is difficult to explain why studies that had the objective to assess the prevalence of secondary hypertension showed a nearly 3-fold higher prevalence of PA than studies that had other objectives, including studies that had the objective to assess specifically the prevalence of PA. However, the latter category was small and this may be a fortuitous finding. The higher yield in the diagnosis of PA when testing consecutive patients than using other methods of patient selection is to be expected since fewer patients will be missed.

As a screening test, most studies (n = 20) used the ARR. The reliability of the ARR is disputed because of its susceptibility to disturbances by external factors, variable cut-off levels and its mediocre sensitivity and specificity.[55,60-62] This might explain why studies that did not use any screening test showed the highest prevalences. One can speculate that when using the ARR,

some patients may be missed and this would argue for performing directly a confirmation test when attempting to detect PA.

VARIATION IN DIAGNOSTIC STRATEGIES

The test conditions, medication regimens, and cut-offs used for screening and confirmation tests varied largely among the included studies. It is generally accepted that patients with an elevated ARR should undergo further confirmatory testing to establish the diagnosis of PA.[13] For this reason, we chose to include only those studies that used some kind of confirmatory testing.

Because use of medication can affect the laboratory results of plasma aldosterone, renin, and ARR, the Endocrine Society guideline advocates adjustment of medication so that plasma aldosterone and renin are minimally affected. In contrast, several studies have suggested that screening and confirmation testing is still reliable when patients continue their antihypertensive medication during testing.[63,64] Our meta-regression model confirms that adjustment of medication regimen has no effect on the prevalence of PA. This challenges the Endocrine Society Guideline's recommendation.[13]

Hypokalemia is often viewed as a clue to screen for PA although only about one-third of the patients with PA presented with hypokalemia. The wide range of hypokalemia in the studies underlines that hypokalemia is not a prerequisite for further testing for PA. Moreover, (mild) hypokalemia may also reflect diuretic treatment of essential hypertension.

IMPORTANCE OF PROPER PREVALENCE ESTIMATES FOR CASE IDENTIFICATION

As recently noted by Funder, considerably less than 1% of the hypertensive patients are screened for PA each year, not to mention diagnosed and properly treated. While the prevalence of PA remains under debate, undiagnosed and untreated PA has important medical implications, such as the detrimental effect on the cardiovascular and renal systems due to aldosterone.[1-4,65-73] Proper treatment of PA, both surgically and with medication, appears to reduce the risk of both cardiovascular and renal complications.[70,74] It is therefore self-evident that identifying PA in hypertensive patients has important benefits. To design a strategy for identification of PA or to allocate health care resources to PA, it is important to know the prevalence of PA among hypertensive patients. Although our study shows that this knowledge is currently insufficient, it also provides us with clues as to what factors cause under or overestimation of the prevalence of PA. Based on that, we would urge to perform a

multicontinental prospective study in which consecutive hypertensive patients are screened for PA by a standardized confirmation test.

LIMITATIONS

We performed separate analyses for primary care and referral centres because the variables that determine the prevalence evidently differ between primary care and referral centres. Unfortunately, the model built with the set of explanatory factors derived from the univariate analysis, could only be used for the studies performed in the referral centres because of the relatively low number of studies in the primary care setting. A final limitation is that we did not exclude any articles by quality assessment because the validated protocol (MORE) we used for our quality assessment is not developed to "weight" or to exclude studies. However, studies with a "major flaw" according to the MORE protocol did not show higher or lower prevalences than studies without "major flaws" (not shown).

CONCLUSIONS

This study of 5896 patients in primary care and 36 614 patients in referral centres demonstrates that the wide range in reported prevalences of primary aldosteronism is associated with year of publication, study region, study objective, modes of data collection, patient selection, and use of screening test. The heterogeneity of studies precludes a reliable estimate of the prevalence of PA. Because of the significant impact of a diagnosis of primary aldosteronism on health care resources and the necessary facilities, our findings urge for better designed prospective prevalence studies. Prerequisites for such a study are international or even intercontinental agreement on a uniform screening and a confirmation test. Next, a survey by screening and, if screening is positive, a confirmation test for PA in all hypertensive patients should be performed, in both primary care and referral centres, with all untested patients being accounted for.

ACKNOWLEDGMENTS

Disclosure Summary: J.D. received a grant from the Dutch Organization for Health Research and Development (ZonMw, Grant 171002102) and participates in EU Horizon 2020 Grant 633983, ENSAT-HT. J.W.L. received a grant from the Dutch Organization for Health Research and Development (ZonMw, Grant 171002102) and from the Deutsche Forschungsgemeinschaft (LE 3660/1-1 KFO 252). S.C.K., T.D., H.J.G., G.J.v.d.W., M.C.v.d.W., and A.R.H. have nothing to declare. S.C.K. and T.D. contributed equally to the study. Dr. C. Bakx died during the study.

SUPPLEMENTAL DATA

SUPPLEMENTAL SEARCH

PUBMED

Search ((((((“Hyperaldosteronism” OR hyperaldosteronism[Title/Abstract]) OR aldosteronism[Title/Abstract]) OR Conn syndrome[Title/Abstract]) OR Conns syndrome[Title/Abstract]) OR Conn’s syndrome[Title/Abstract]) OR (((hyperaldosteronism[Other Term] OR aldosteronism[Other Term]) OR Conn syndrome[Other Term]) OR Conn’s syndrome[Other Term])) AND (((((((“Prevalence” OR prevalence[Title/Abstract]) OR prevalences[Title/Abstract]) OR occurrence[Title/Abstract]) OR occurrences[Title/Abstract]) OR ((“Incidence” OR incidence[Title/Abstract]) OR incidences[Title/Abstract])) OR ((((“Epidemiology” OR “epidemiology”[Subheading]) OR epidemiology[Title/Abstract]) OR epidemiologic[Title/Abstract]) OR epidemiological[Title/Abstract])) OR (((((((prevalence[Other Term] OR prevalences[Other Term]) OR incidence[Other Term]) OR incidences[Other Term]) OR occurrence[Other Term]) OR occurrences[Other Term]) OR epidemiology[Other Term]) OR epidemiologic[Other Term]) OR epidemiological[Other Term])) AND (“1990/01/01”[PDAT] : “2015/01/31”[PDAT]) Filters: Dutch; English; French; German; Spanish

Items found: 624

EMBASE

1. exp hyperaldosteronism/
2. (hyperaldosteronism or aldosteronism).ti,ab.
3. (Conn syndrome or Conns syndrome or Conn’s syndrome).ti,ab.
4. 1 or 2 or 3
5. exp prevalence/
6. exp incidence/
7. (incidence or incidences).ti,ab.
8. 6 or 7
9. exp epidemiology/
10. (epidemiology or epidemiologic or epidemiological).ti,ab.
11. 9 or 10

12. (prevalence or prevalences or occurrence or occurrences).ti,ab.
13. 5 or 12
14. 8 or 11 or 13
15. 4 and 14
16. limit 15 to ((Dutch or English or French or German or Spanish) and yr="1990 - Current")
17. limit 16 to ((Dutch or English or French or German or Spanish) and yr="1990 - Current")

Items found: 1239

WEB OF SCIENCE

#1	**TOPIC:** (hyperaldosteronism) *OR* **TOPIC:** (aldosteronism) *OR* **TOPIC:** (Conn syndrome) *OR* **TOPIC:** (Conns syndrome) *OR* **TOPIC:** (Conn's syndrome) *Indexes=SCI-EXPANDED, SSCI, A&HCI Timespan=1990-2015*	4086
#2	**TOPIC:** (prevalence) *OR* **TOPIC:** (prevalences) *OR* **TOPIC:** (incidence) *OR* **TOPIC:** (incidences) *OR* **TOPIC:** (occurrence) *OR* **TOPIC:** (occurrences) *OR* **TOPIC:** (epidemiology) *OR* **TOPIC:** (epidemiologic)*OR* **TOPIC:** (epidemiological) *Indexes=SCI-EXPANDED, SSCI, A&HCI Timespan=1990-2015*	1355259
#3	#2 AND #1 *Indexes=SCI-EXPANDED, SSCI, A&HCI Timespan=1990-2015*	743
#4	(#2 AND #1) AND **LANGUAGE:** (English OR Dutch OR French OR German OR Spanish) *Indexes=SCI-EXPANDED, SSCI, A&HCI Timespan=1990-2015*	743

Items found: 743

COCHRANE LIBRARY

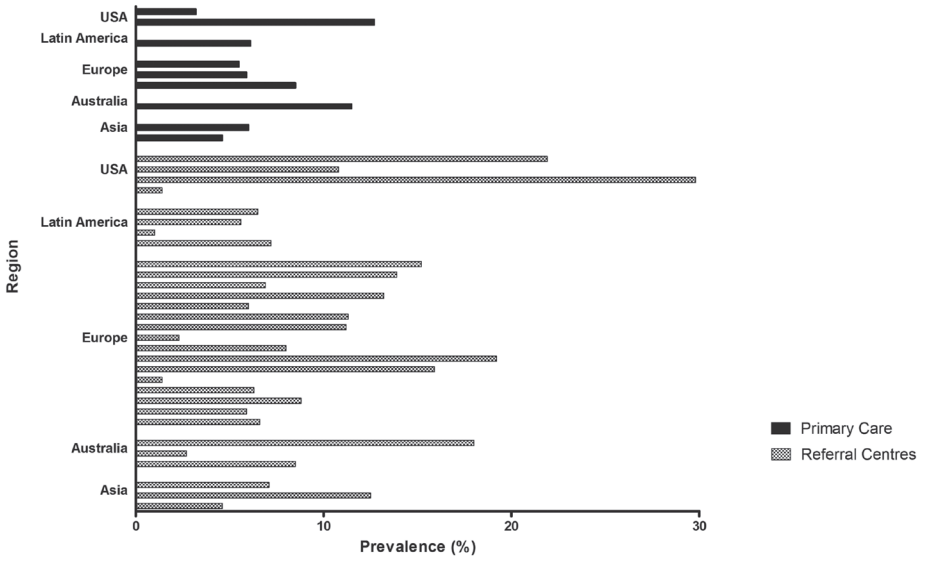
ID	Search Hits	
#1	MeSH descriptor: [Hyperaldosteronism] explode all trees	50
#2	MeSH descriptor: [Prevalence] explode all trees	3937
#3	MeSH descriptor: [Incidence] explode all trees	7910
#4	MeSH descriptor: [Epidemiology] explode all trees	43
#5	hyperaldosteronism:ti,ab,kw or aldosteronism:ti,ab,kw or Conn syndrome:ti,ab,kw or Conns syndrome:ti,ab,kw or Conn's syndrome:ti,ab,kw (Word variations have been searched)	100
#6	prevalence:ti,ab,kw or prevalences:ti,ab,kw or occurence:ti,ab,kw or occurences:ti,ab,kw (Word variations have been searched)	14443
#7	incidence:ti,ab,kw or incidences:ti,ab,kw (Word variations have been searched)	54614
#8	epidemiology:ti,ab,kw or epidemiologic:ti,ab,kw or epidemiological:ti,ab,kw (Word variations have been searched)	10554
#9	#2 or #3 or #4	11464
#10	#6 or #7 or #8	75212
#11	#9 or #10	75221
#12	#1 or #5	102
#13	#12 and #11 Publication Year from 1990 to 2015	8

Items found: 8

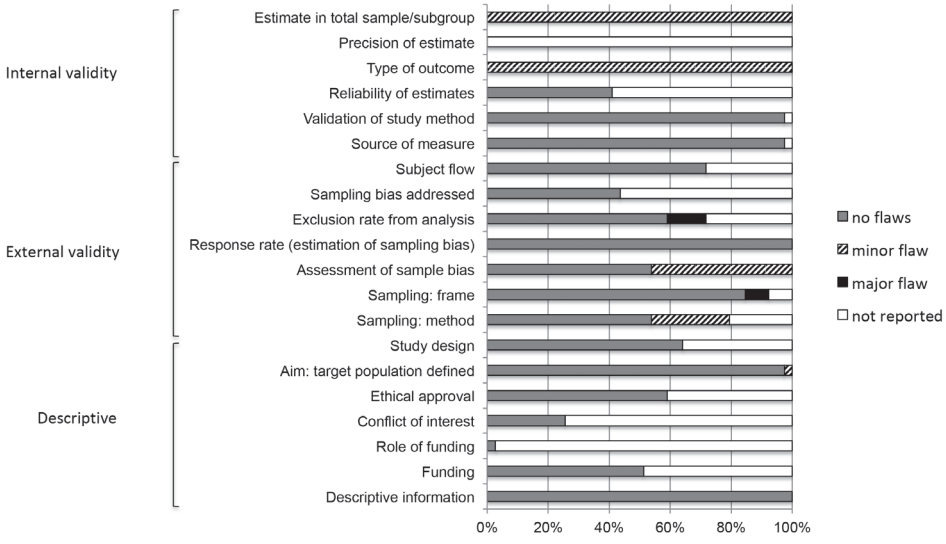
The final literature search was performed on 17th February 2015 (all databases, by SK).

Total items found: 2614

SUPPLEMENTARY FIGURES



Supplemental Figure 1. PA prevalence per geographical region



Supplemental Figure 2. Quality Assessment of the 39 Included Studies Using the MORE Criteria

SUPPLEMENTARY TABLES

Supplemental Table 1. Excluded Studies Based on Full Text Reading

First Author	Year	Country	Setting	Remark
No Confirmation Test in >50% of Patients				
Cortes [75]	2000	Chile	RC	
Daimon [76]	2014	Japan	PC	
Denolle [77]	2000	France	RC	
Ducher [78]	2012	France	RC	
Eide [11]	2004	Norway	RC	
Gallay [79]	2001	USA	RC	
Gallego [80]	2007	Spain	RC	
Garcia [81]	2011	USA	NR	
Gombet [82]	2007	France	RC	
Gonzaga [83]	2010	USA	RC	
Gregori[84]	2014	Italy	RC	
Hannemann [85]	2012	Germany	NR	
Ito [86]	2011	Japan	PC	
Jefic [87]	2006	USA	RC	
Lim [88]	1999	UK	PC	
Mosso [89]	1999	Chile	PC	
Mysliwiec [90]	2010	Poland	RC	
Olivieri [91]	2004	Italy	PC	
Pardes [92]	2010	Argentina	RC	
Rayner [93]	2000	S. Africa	RC	
Rayner [94]	2001	S. Africa	PC	
Rosenbaum [95]	2012	France	PC	
Rossi [30]	1998	Italy	RC	Article comprising three studies: 2 studies without >50% confirmation test were excluded from analysis, 1 study was
Sabio [96]	2005	Spain	RC	
Schmiemann [97]	2012	Germany	PC	
Schwartz [60]	2002	USA	PC	
Sharma [98]	1994	India	RC	
Takayanagi [99]	2000	Japan	RC	
Volpe [100]	2012	Sweden	PC	
Williams [101]	2006	UK	RC	
Diagnosis of Primary Aldosteronism Based on CT or Reaction to Spironolactone				
Hood [102]	2005	UK	PC	
Niizuma [103]	2008	Japan	RC	
Nogueira [104]	2008	Brazil	RC	

Prevalence of Primary Aldosteronism in a Subgroup of Patients				
Hypertensive Emergency				
Börgel [105]	2010	Germany	RC	
Diabetes				
Li [106]	2013	China	NR	
Mukherjee [107]	2010	Singapore	RC	
Murase[108]	2013	Japan	RC	
Umpierrez[109]	2007	USA	NR	
Normotensive Patients				
Markou [110]	2013	Greece	RC	
Patients Suspected to Have PA				
Solar [111]	2012	Czech rep.	RC	
Ye [112]	2012	China	RC	
Patients with Atrial Fibrillation				
Rossi [113]	2013	Italy	RC	
Patients with Known Adrenal Mass				
Godula[114]	2013	Portugal	RC	
Study Methods Unclear				
Benchetrit [115]	2002	Israel	NR	
Gouli [116]	2011	Greece	RC	
Mulatero[25]	2004	Italy	RC	Article comprising five studies: 1 study was excluded (Australia)
Mysliwiec [117]	2012	Poland	RC	
Papanastasiou [118]	2014	Greece	RC	Is the same as Gouli (2011)
Sy [119]	2012	China	PC	
Trifanescu [120]	2013	Romania	RC	
Wu [121]	2014	Taiwan	RC	
Study not on Prevalence of Primary Aldosteronism				
Adlin [122]	2013	USA	NR	Study on aldosterone
Kao [123]	2013	Taiwan	NR	Clinical aspects
Sakthiswary [124]	2012	Malaysia	NR	Study on aldosterone
Double Reporting of Same Patients in Different Studies				
Calhoun [125]	2002	USA	NR	= Nishizaka 2005
Fardella [126]	2000	Chile	NR	= Mosso 2003
Nishikawa [127]	2000	Japan	NR	= Omura 2004
Rossi [128]	2007	Italy	NR	= Rossi 2006

Abbreviations: NR; Not Reported; PC, Primary Care; R, Referral Center; S, South.

Supplemental Table 2. Studies on Prevalence of Primary Aldosteronism in Primary Care and Referral Centres

PRIMARY CARE										
Author Year (Ref)	**Country**	**Design**	**Period**	**n**	**Population % male Age: mean (SD)**	**Medication[a]**	**Screening test[b]**	**Screening positive**	**Confirmation test Cut-off**	**Prevalence**
Gordon 1993 [31]	Australia	Prosp[c]	NR	52	HT 65% male Age: 56y (7y)[d]	MRA withdrawn 3 weeks, rest unchanged	ARR >30	6 (11.5%)	FST (in 6/6) Cut-off NR	6 (11.5%)
Loh 2000 [32]	Singapore	Prosp[c]	1998	350	HT 39% male Age: 55y (9y)	Unchanged (patients using MRA were excluded)	ARR >20 and PAC >15 ng/dL	63 (18%)	IV SLT (in 56/63) PAC >10 ng/dL	16 (4.6%)
Mosso 2003 [26]	Chile	Retro and prosp[c,e]	1998-1999[c] 2001-2002[c]	609	HT 36% male Age: 54y (11y)	Withdrawn ≥15 days, CCB allowed	ARR >25	63 (10.3%)	FST (in 62/63) PAC ≥5 ng/dL	37 (6.1%)
Omura 2004 [33]	Japan	Prosp	1995-1999	1020	HT newly diagnosed[c] 55% male Age: 52y[c]	No medication (unless budralazin)	PAC >12 ng/dL and PRA <1 ng/mL/h	134 (13.1%)	Captopril test (in 83/134[c]) ARR >20	61 (6.0%)
Schwartz 2005 [20]	USA	Prosp[c]	2000-2002	118	HT normoK+ 62% male Age: 29-63y[f]	All withdrawn 2 weeks	Screening not used for prevalence analysis	None	Oral SLT Urinary aldo ≥12 µg/24h and PRA ≤1 ng/mL/h	15 (12.7%)
Westerdahl 2006 [34]	Sweden	Cross	NR	200	HT % male NR Age: ≤75y[f]	Withdrawn 2 weeks, CCB allowed	ARR >100 pmol/L per ng/L	50 (25%)	FST (in 26/50) PAC >160 pmol/L	17 (8.5%)
Williams 2006 [21]	USA	Cross[c]	1996-2005[c]	347	HT normoK+ 54% male Age: 49y (7y)[d]	Standard	ARR >25 and PAC >8 ng/dL	26 (7.5%)	Oral SLT (in 26/26[c]) Urinary aldo >17 µg/24h	11 (3.2%)
Fogari 2007 [35]	Italy	Prosp	1999-2002	3000	HT 48% male Age: 51y (6y)	Standard + all medication withdrawn 1 week	ARR >25	684 (22.8%)	IV SLT (in 650/684) PAC >7.5 ng/dL	177 (5.9%)
Westerdahl 2011 [36]	Sweden	Cross	NR	200	HT newly diagnosed 43% male Age: 24-75y[f]	Standard	ARR >65 pmol/L per mU/L	36 (18%)	FST (in 27/36) PAC >225 pmol/L (day 4) or PAC >305 nmol/L (day 3)	11 (5.5%)
Total number				**5896**						**351**

REFERRAL CENTERS										
Author Year (Ref)	**Country**	**Design**	**Period**	**n**	**Population % male Age: mean (SD)**	**Medication[a]**	**Screening test[b]**	**Screening positive**	**Confirmation test Cut-off**	**Prevalence**
Anderson 1994 [37]	USA	Prosp	1976-1991	4429	HT[g] % male NR Age: NR	Withdrawn 1 week (when possible)	IV SLT (afternoon) Aldosterone >8.5 ng/dL	NR	Oral SLT 3 days (saline + fludrocortisone or deoxycorticosterone) (in NR/NR) Urinary aldo <8 µg/24h	62 (1.4%)
Gordon 1994 [22]	Australia	Retro[c]	1992-1993[c]	199	HT normoK$^+$ 50% male[c] Age: 54y (16y)	Unchanged	ARR >30	22 (11.1%)	FST (in 17/22) Cut-off NA[c]	17 (8.5%)
Abdelhamid 1996[38]	Germany	Prosp	NR	3900	HT % male NA[c] Age: NA[c]	Standard	Urinary aldosterone >50 nmol/24h or 18-OH-B >20 nmol/24h	NA[c]	IV SLT (in 257/257) Cut-off NR	257 (6.6%)
Brown[h] 1996 [23]	Australia	Prosp	1988-1992	74	HT normoK$^+$ % male NA[c] Age: NA[c]	Withdrawn 3 days	ARR >2000 pmol/L per pmol A1/mL/h (PRC)[c] ARR >525 pmol/L per pmol A1/mL/h (PRA)[c]	6 (8.1%) 4 (5.4%)	IV SLT and FST (in 6/6[i]) PAC >140 pmol/L	2 (2.7%)
Rossi 1998 [30]	Italy	Prosp	NR	320	HT	Standard	ARR >30	NR	IV SLT (in all) PAC >208 pmol/L	19 (5.9%)
Lim 2000 [39]	UK	Prosp	1995-1997	465	HT % male NR Age: NR	Withdrawn 7-10days (60%), 'no MRA or α-blocker allowed'	ARR ≥750 pmol/L per ng/mL/h	77 (16.6%)	FST (in 45/77) PAC ≥7.5 ng/dL	41[j] (8.8%)
Rossi 2002 [40]	Italy	Prosp	1997-1999	1046	HT 51% male Age: 50y (12y)	Standard	ARR post-captopril ≥35	134 (12.8%)	IV SLT (in 134/134) PAC ≥7.5 ng/dL	66 (6.3%)
Trenkel[h] 2002 [41]	Germany	Prosp	1997-1999	146	HT % male NA[c] Age: NA[c]	Unchanged	ARR ≥25 (pg/mL)/(pg/mL)	27 (18.4%)	IV SLT (in 14/27) PAC >100 pg/mL	2 (1.4%)

>>

REFERRAL CENTERS (CONTINUED)										
Author Year (Ref)	**Country**	**Design**	**Period**	**n**	**Population % male Age: mean (SD)**	**Medication[a]**	**Screening test[b]**	**Screening positive**	**Confirmation test Cut-off**	**Prevalence**
Martell 2003 [24]	Spain	Prosp[c]	2000-2002[c]	50	RHT normoK+ 52% male Age: 52y (9y)	Withdrawn 7-10 days	None	None	IV SLT (in 44/50; 6 excluded due to white coat HT) <50% suppression of aldosterone compared to baseline value	7 (15.9%)
Stowasser 2003 [42]	Australia	Prosp[c]	2000-2002	300	HT % male NA[c] Age: NA[c]	BB withdrawn 2 weeks, MRA 4 weeks, ARB or ACE-I allowed	ARR >30 or ARR >20 when RHT	59 (19.7%)	FST (in 59/59) PAC ≥6 ng/dL	54 (18%)
Strauch 2003 [43]	Czech Republic	Retro[c]	1997-2001	402	HT 43% male Age: 51y (12y)	Withdrawn 2 weeks, α-blocker allowed	ARR ≥50	87 (21.6%)	IV SLT (in 87/87[c]) PAC >85 ng/L	77 (19.2%)
Calhoun 2004 [44]	USA	Prosp	2000-2002	114	RHT 37% male Age: 57y (11y)	MRA withdrawn ≥6 weeks, rest unchanged	Urinary aldo >12 µg/24h and PRA <1.0 ng/mL/h	NR	Oral SLT (NA[c]) Urinary aldo >12 µg/24h and PRA <1.0 ng/mL/h with urinary sodium >200 mEq/24h	34 (29.8%)
Mulatero[k] 2004 [25]	Italy	Retro	1994-2002	7343	HT >160/100mm Hg % male NA[c] Age: NA[c]	Standard[c]	ARR >40 and PAC >15 ng/dL	905[c]	IV SLT (in 905/905[c]) PAC >5 ng/dL	587 (8.0%)
	USA	Retro	1999	1112	HT % male NA[c] Age: NA[c]	Standard, although ACE-I and ARB have not been withdrawn when the ratio was positive under treatment[c]	ARR>20 and PAC >15 ng/dL	NA[c]	Oral SLT (in all[c]) Urine aldosterone >12 µg/d	120 (10.8%)
	Singapore	Retro	1995-2001	3850	HT % male NA[c] Age: NA[c]	NA[c]	ARR >20 and PAC >15 ng/dL	NA[c]	IV SLT (in all[c]) PAC >10 ng/dL	177 (4.6%)

	Chile	Retro	2000-2002	914	HT % male NA[c] Age: NA[c]	Interfering drugs such as diuretics, ACE-I, ARB and BB were stopped for ≥15 days[c]	ARR >25	NA[c]	FST (in all[c]) PAC >5 ng/dL and PRA <1 ng/mL/h	66 (7.2%)
Milliez 2005 [1]	France	Prosp[c]	1997-1999	5438[‡]	HT % male NA[c] Age: NA[c]	Standard	ARR >23 and PAC >178 pg/mL and PRA ≤5 pg/mL and urinary aldosterone >23 µg/24h[c]	NA[c]	Captopril test (in all[c]) <70% suppression of aldosterone compared to baseline PAC[c]	124 (2.3%)
Nishizaka 2005 [45]	USA	Prosp	2000-2004	265	RHT 44% male Age: 56y (12y)	MRA withdrawn ≥6 weeks, rest unchanged	Urinary aldo >12 µg/24h	58	Oral SLT (in 58/58[i]) Urinary aldo >12µg/24h and PRA <1.0 ng/mL/h with urinary sodium >200 mEq/24h	58 (21.9%)
Rossi 2006 [27]	Italy	Prosp	2001-2004	1125	HT newly diagnosed 56% male Age: 46y (12y)	Standard	ARR ≥40 and/or post-captopril ARR ≥30 and/or LDH-score ≥0.50	230 (20.4%)	ARR ≥40 plus post-captopril ARR ≥30 and/or LDH-score ≥ 0.50 (in 230/230)	126 (11.2%)
Douma 2008 [46]	Greece	Retro	1988-2008[c]	1616	RHT 51% male Age: 56y (13y)	Standard	ARR >65.16 pmol/L per pmol/L/min and SAC >416 pmol/L	338 (20.9%)	IV SLT and FST (in 338/338[c,j]) IV SLT: SAC ≥222 pmol/L FST: SAC >139 pmol/L	182 (11.3%)
Morillas 2008 [47]	Spain	Prosp	2005-2006	183	HT 61% male Age: 58y (13y)	Unchanged	ARR >30 and PAC >20 ng/dL	NR	IV SLT (in NR/NR) PAC >10 ng/dL	11 (6.0%)
Ribeiro 2009[48]	Brazil	Prosp	2007	105	HT (90%borderline or stage 1) 25% male Age: 55y (11y)	Use of MRA and BB were excluded: unchanged	ARR >25	9 (8.6%)	IV SLT (in 8/9) PAC >5 ng/dL	1 (1.0%)
Di Murro 2010 [49]	Italy	Retro[c]	2007-2008	325	HT newly diagnosed 61% male[c] Age: 51y (10y[‡])	Standard	ARR >40 and PAC >15 ng/dL with suppressed PRA	almost 17%[c]	IV SLT (in all[c]) PAC ≥5 ng/dL	43 (13.2%)

REFERRAL CENTERS (CONTINUED)										
Author Year (Ref)	**Country**	**Design**	**Period**	**n**	**Population % male Age: mean (SD)**	**Medication[a]**	**Screening test[b]**	**Screening positive**	**Confirmation test Cut-off**	**Prevalence**
Matrozova[m] 2010 [28]	Bulgaria	Prosp[c]	2005-2008[c]	376[k]	HT 34% male[c] Age:48y (14y)[c]	MRA with-drawn 45 days, rest stopped 7-10days. CCB or α-blocker allowed	ARR >750 pmol/L per ng/mL/h and PAC >416 pmol/L	94[c,n]	Captopril test (in 87/94[c]): ARR >970 (pmol/L)/(ng/mL/h)	26[n] (6.9%)
Pedrosa 2011 [50]	Brazil	Cross	2008-2010	125	RHT 43% male Age:52y (10y)	MRA withdrawn 3 weeks, rest unchanged	ARR >20	14 (11.2%)	IV SLT (in 14/14) PAC >10 ng/dL	7 (5.6%)
Rios 2011 [51]	Argentina	Prosp[c]	2006-2009	123	HT 39% male Age:43y (11y)	Standard	ARR >25	20 (16.3%)	IV SLT (18/20) PAC >5 ng/dL	8 (6.5%)
Sigurjons-dottir[o] 2012 [52]	Sweden	Prosp	2000-2003[c]	122[p]	HT 61% male[c] Age: 56y (12y)[c]	Standard	ARR >1.28 and SA >0.43 nmol/l	28 (22.8%)	Oral SLT (in 25/28[c]) Urinary aldo >28 nmol/24h	17[q] (13.9%)
Yin 2012 [53]	China	Prosp[c]	2007-2010	313	HT 46% male Age: 46y (13y[c])	Standard	ARR >25	72 (23%)	IV SLT (in 2/72[r]) SAC >6.75 ng/dL Captopril test (in 72/72) ARR >13 ng/dL	39 (12.5%)
Sang & Jiang 2013 [54]	China	Cross[c]	2010-2011	1656	RHT 57% male Age: 18-65y[f]	MRA withdrawn 4 weeks, rest unchanged	ARR >20	494 (29.8%)	IV SLT (in 494/494) PAC >8 ng/dL	118 (7.1%)
Jansen 2014 [55]	The Nethelands	Prosp	2006-2011[c]	178	RHT 53 % male Age: 49y (9y)	MRA and BB withdrawn 4 weeks: rest unchanged	Screening not used for prev-alence analysis	-	IV SLT (in 178/178) PAC >235 pmol/L	27 (15.2%)
Total number				**36614**						**2375**

SI conversion factors: to convert aldosterone (ng/dL) to pmol/L, multiply values by 27.74; to convert renin (pg/mL) to pmol/L, multiply values by 0.0237. To preserve authenticity of the original article, we did not convert the cut-off values to conventional units.

Abbreviations: A1, Angiotensin 1; ACE-I, Angiotensin Converting Enzyme Inhibitor(s); aldo, aldosterone; ARB, Angiotensin Receptor Blocker; ARR, Aldosterone to Renin Ratio; BB, Beta-Blocker; CCB, Calcium Channel Blocker; Cross, Cross-sectional; FST, Fludrocortisone Suppression Test; HT, Hypertension: blood pressure >140/90 with or without medication; IV SLT, Intravenous Sodium Loading Test; LDH, Logistic Discriminant Function; MRA, Mineralocorticoid Receptor antagonist; n, number of patients; NA, Non Available (data untraceable due to elapsed time); NAp, Not Applicable; normoK+ , normokalemia; NR, Not Reported; Oral SLT, Oral Sodium Loading Test; PAC, Plasma

Aldosterone Concentration; PC, Primary Care; PRA, Plasma Renin Activity; Prosp, Prospective; RC, Referral Center; ref, reference; Retro, Retrospective; RHT, Resistant Hypertension; SAC, Serum Aldosterone Concentration; y, year.[a] Standard (=according to the Endocrine Society guideline13: MRAs stopped for at least 4 weeks, all other anti-hypertensive drugs stopped for at least 2 weeks, except for calcium antagonists and α-blockers.[b] ARR calculated with PAC in ng/dL and PRA in ng/mL/h, unless stated otherwise. [c] Data received from author. [d] Standard error of the mean converted to standard deviation. [e] Study design: partly retrospective. 305 patients from a previous study were included126, the other patients were prospectively included. [f] Mean age and standard deviation not reported. [g] The study population consisted of poorly controlled hypertensive patients. [h] In this analysis only the hypertensive study population is included. [i] Hypertensive patients with elevated ARR performed a saline infusion test as well as a fludrocortisone suppression test. [j] In the original article a prevalence of 43/465 is reported. However, 2/465 have not been identified by screening and confirmation testing: one patient with a negative FST had a right adrenal adenoma, which was detected on CT scanning (histological examination after adrenalectomy confirmed a Conn's adenoma), and one patient had already been diagnosed with PA. In this review, only patients who were assessed by our pre-defined inclusion criteria were included in the analysis (prevalence is 41/464 = 8.8%). However, usually when cited, a prevalence of 9.2% is reported.129 [k] Due to missing number of included patients, the study from Australia (Brisbane) is excluded [l] If urinary aldosterone was elevated (>12 µg/24h), but urinary sodium was low (<200 mEq/24h), the 24h urinary assessments were repeated after 3 days of dietary salt supplementation. However, if urinary aldosterone and urinary sodium exceeds cut-off values during normal diet (routine high sodium diet), additional sodium loading was omitted (because of risks and little additional value). So, the confirmatory test is the 24h urine under high sodium diet. [m] Patients who were analyzed because of an incidentaloma were excluded. [n] In this number incidentalomas are excluded (n=376+96=472). [o] Patients studied in primary care were excluded due to <50% confirmation test (6/18 = 33%, data confirmed by author). [p] Information from author by email: the original paper states that the number of patients is 123. [q] Including dropouts in analysis. [r] All of the patients with elevated ARR underwent the captopril test, and two of the patients underwent the saline infusion because of the confused results of the captopril test (data received from author).

Supplemental Table 3. Studies in Patients with Primary Aldosteronism Reporting the Number of Patients with Hypokalemia

PRIMARY CARE		
Author (Year)	**Number of PA Patients Assessed**	**Number of Patients with Hypokalemia (%)**
Gordon, 1993 [31]	6	0 (0%)
Loh, 2000 [32]	16	6 (37.5%)
Mosso, 2003 [26]	37	1 (2.7%)
Omura, 2004 [33]	61	15 (24.6%)
Fogari, 2007 [35]	177	44 (24.8%)
Westerdahl, 2011 [36]	11	3 (27.3%)†
Total	**308**	**69**
REFERRAL CENTERS		
Anderson, 1994[37]	62	19 (30%)
Lim, 2000 [39]	41	2 (4.4%)
Rossi, 2002 [40]	66	26 (39.4%)
Stowasser, 2003 [42]	54	7 (13%)
Strauch, 2003 [43]	77	15 (19%)*
Mulatero, 2004 [25]		
Italy	587	146 (24.9%)
USA	120	44 (36.7%)
Singapore	177	66 (37.3%)
Chile	66	6 (9.1%)
Milliez, 2005 [1]	124	121 (98%)
Nishizaka, 2005[45]	58	23 (39.7%)
Rossi, 2006 [27]	126	12 (9.6%)
Douma, 2008 [46]	182	83 (45.6%)
Ribeiro, 2009 [48]	1	0 (0%)
Di Murro, 2010 [49]	43	18 (42%)*
Matrozova, 2010 [28]	38	21 (55.3%)‡
Pedrosa, 2011 [50]	7	0 (0%)*
Rios, 2011 [51]	8	4 (50%)
Sigurjonsdottir, 2012 [52]	17	5 (29%)*
Yin, 2012 [53]	39	26*(67%)
Sang & Jiang, 2013 [54]	118	62 (52.5%)
Jansen, 2014 [55]	27	13 (48.1)
Total	**2038**	**719**

* Data obtained from the authors. † Estimated from box plot. ‡ Including 12 patients who were diagnosed with PA after analysis for incidentaloma. Five studies included only patients with normokalemia.[20-24] Six studies did not report the number of patients who had hypokalemia.[12,30,34,38,41,47]
Abbreviations: PA, Primary Aldosteronism

Supplemental Table 4A. Quality Assessment According to the MORE Protocol, part A

Author, Year (Ref)	DESCRIPTIVE							EXTERNAL VALIDITY							INTERNAL VALIDITY					
								Sampling of Subjects				Subject flow				Measurements of Outcome		Reporting of Outcome		
	Descriptive Information	Funding	Role of Funding	Conflict of Interest	Ethical Approval	Aim	Study Design	Sampling Method	Sampling Frame	Sampling Bias	Response Rate (%)	Eligibility Fract (%)	Enroll Fract (%)	Recruit Fract (%)	Source of Measure	Validation Study Method	Reliability Estimates	Type of Outcome	Precision of Estimate	Estimate in Total Sample
Abdelhamid, 1996 [38]	C	-	-	-	Y	PA/O	Pro	-	HC	-	> 60	100	100	100	OM	C-ES	-	PP	-	-
Anderson, 1994 [37]	C	-	-	-	-	O	Pro	-	HC	Disc	> 60	100	100	100	OM	C-ES	-	PP	-	-
Brown, 1996 [23]	C	G	-	-	Y	PA	Pro	Self	DB	-	40-60	-	32	-	OM	C-ES	-	PP	-	-
Calhoun, 2004 [12]	C	G	-	-	Y	PA	Pro	Cs	HC	-	> 60	100	100	100	OM	C-ES	-	PP	-	-
Di Murro, 2010 [49]	C	-	-	-	-	PA	-	Cs	HC	-	> 60	100	100	100	OM	C-ES	-	PP	-	-
Douma, 2008 [46]	C	NF	NA	No	Y	PA	Ret	Cs	HC	Disc	> 60	70	100	70	MR	C-ES	-	PP	-	-
Fogari, 2007 [35]	C	G	-	-	Y	PA	Pro	Cv	HC	-	> 60	100	100	100	OM	C-ES	Pub	PP	-	-
Gordon, 1993 [31]	C	-	-	-	-	-	-	Self	-	Asse	> 60	-	100	-	OM	C-ES	-	PP	-	-
Gordon, 1994 [22]	C	-	-	-	-	PA	-	Cs	HC	Disc	> 60	100	100	100	OM	C-ES	-	PP	-	-
Jansen, 2014 [55]	C	G/I	No	No	Y	O	Pro	Cs	HC	-	> 60	97	99	96	OM	C-ES	Pub	PP	-	-
Lim, 2000 [39]	C	NF	NA	No	-	PA	-	Cs	HC	-	> 60	94	100	94	OM	C-ES	Discus	PP	-	-
Loh, 2000 [32]	C	G	-	-	Y	PA	-	Cs	HC	-	> 60	96	92	89	OM	C-ES	Discus	PP	-	-
Martell, 2003 [24]	C	-	-	-	Y	O	-	Cs	HC	Disc	> 60	88	100	88	OM	C-ES	-	PP	-	-
Matrozova, 2010[28]	C	G	-	-	Y	PA	-	Cs	HC	Disc	> 60	81	91	74	OM	C-ES	Pub	PP	-	-
Milliez, 2005 [1]	C	-	-	-	-	O	Ret	Cs	MR	Disc	> 60	100	100	100	MR	C-ES	-	PP	-	-
Morillas, 2008 [47]	C	-	-	-	-	PA	Pro	-	HC	-	> 60	100	100	100	OM	C-ES	-	PP	-	-
Mosso, 2003 [26]	C	G	-	-	Y	PA	Cro	Cs	MR	Disc	> 60	100	100	100	MR	C-ES	Pub	PP	-	-
Mulatero, 2004 [25]																				
Italy	C	-	-	-	-	PA	Ret	Cv	HC	Disc	> 60	-	100	-	OM	C-ES	Discus	PP	-	-
USA	C	-	-	-	-	PA	Ret	Cv	HC	Disc	> 60	-	100	-	OM	C-ES	Discus	PP	-	-
Singapore	C	-	-	-	-	PA	Ret	Cv	HC	Disc	> 60	-	100	-	OM	C-ES	Discus	PP	-	-
Chile	C	-	-	-	-	PA	Ret	Cs	HC	Disc	> 60	100	100	100	OM	C-ES	Discus	PP	-	-

Supplemental data Table 4A. Continued

Author, Year (Ref)	DESCRIPTIVE							EXTERNAL VALIDITY							INTERNAL VALIDITY					
								Sampling of Subjects				Subject flow				Measurements of Outcome		Reporting of Outcome		
	Descriptive Information	Funding	Role of Funding	Conflict of Interest	Ethical Approval	Aim	Study Design	Sampling Method	Sampling Frame	Sampling Bias	Response Rate (%)	Eligibility Fract (%)	Enroll Fract (%)	Recruit Fract (%)	Source of Measure	Validation Study Method	Reliability Estimates	Type of Outcome	Precision of Estimate	Estimate in Total Sample
Nishizaka, 2005 [45]	C	G	-	-	Y	O	Pro	Cs	HC	-	> 60	100	100	100	OM	C-ES	Pub	PP	-	-
Omura, 2004 [33]	C	-	-	-	Y	O	Pro	-	HC	-	> 60	-	100	-	OM	C-ES	-	PP	-	-
Pedrosa, 2011 [50]	C	G/I	-	No	Y	O	Cro	Cs	HC	Disc	> 60	82	82	67	OM	C-ES	-	PP	-	-
Ribeiro, 2009 [48]	C	NF	NA	No	Y	PA	-	Cv	HC	Disc	> 60	100	98	98	OM	C-ES	-	PP	-	-
Rios, 2011 [51]	C	-	-	No	-	PA	Cro	-	-	-	> 60	97	83	90	OM	C-ES	Pub	PP	-	-
Rossi, 1998 [30]	C	-	-	-	-	O	-	-	HC	Disc	> 60	100	100	100	OM	C-ES	-	PP	-	-
Rossi, 2002 [40]	C	-	-	-	Y	PA/O	Pro	Cs	HC	Disc	> 60	98	100	98	OM	C-ES /Val	Pub	PP	-	-
Rossi, 2006 [27]	C	G	-	-	Y	PA	Pro	Cs	HC	Disc	> 60	99	95	94	OM	C-ES	Pub	PP	-	-
Sang & Jiang, 2013 [54]	C	G	-	No	Y	PA	Cro	Cs	HC	-	> 60	84	100	84	-	-	Pub	PP	-	-
Schwartz, 2005 [20]	C	G	-	-	Y	O	-	Self	O	Disc	> 60	50	84	42	OM	C-ES	-	PP	-	-
Sigurjonsdottir, 2012[52]	C	-	-	No	Y	O	Pro	Cs	HC	-	> 60	-	90	-	OM	C-ES	-	PP	-	-
Stowasser, 2003 [42]	C	G	-	-	-	O	-	-	HC	Disc	> 60	-	100	-	OM	C-ES	-	PP	-	-
Strauch, 2003 [43]	C	O	-	-	-	PA	-	Cs	HC	Disc	> 60	100	100	100	OM	C-ES	-	PP	-	-
Trenkel, 2002 [41]	C	-	-	-	-	O	Pro	Cs	HC	-	> 60	-	100	-	OM	C-ES	-	PP	-	-
Westerdahl, 2006 [34]	C	-	-	-	Y	PA/O	Cro	Self	DB	Disc	40-60	84	49	41	OM	C-ES	-	PP	-	-
Westerdahl, 2011 [36]	C	O	-	No	Y	PA/O	Cro	Cs	-	-	> 60	-	100	-	OM	C-ES	-	PP	-	-
Williams, 2006 [21]	C	G	-	-	Y	PA	-	Self	O	-	> 60	-	100	-	OM	Pub	Pub	PP	-	-
Yin, 2012 [53]	C	O	-	No	Y	O	-	-	HC	-	> 60	88	100	88	OM	C-ES	-	PP	-	-

Data that were not reported are indicated by '-'. **Abbreviations:** Asse, Assessed; C, Complete; C-ES, Conformation test according to the Endocrine Society Guideline[13]; Cs, Consecutive; Cv, Convenience; Cro, Cross Sectional; DB, Database; Disc, Discussed; Discus, Discussion; Eligibility Fract, Eligibility Fraction (Eligible/Screened); Enroll Fract, Enrollment Fraction (Enrolled/Eligible); G, Grant; HC, Health Care; I, Industry; MR, Medical Records, NA, Not Available; NF, No Funding; O, Other; OM, Objectively Measured; PA, Aim to Assess Prevalence of Primary Aldosteronism; PP, point prevalence; Pro, Prospective; Pub, Published; Recruit Fract, Recruitment Fraction (Enrolled/screened); Ref, Reference; Ret, Retrospective; Self, Self Selection; Val, Validated; Y, Yes

Supplemental Table 4B. Quality Assessment According to the MORE Protocol, part B

Author (Year)	DESCRIPTIVE	EXTERNAL VALIDITY		INTERNAL VALIDITY
	Minor Flaw	Major Flaw	Minor Flaw	Minor Flaw
Abdelhamid, 1996 [38]	-	-	-	PP-OCE
Anderson, 1994 [37]	-	-	-	PP-OCE
Brown, 1996 [23]	-	Exclusion rate from analysis >10%	Sampling method: self selection; Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Calhoun, 2004 [12]	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Di Murro, 2010 [49]	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Douma, 2008 [46]	-	Sampling frame: medical records	-	PP-OCE
Fogari, 2007 [35]	-	-	Sampling method: convenience; Sampling bias not addressed in analysis/discussed	PP-OCE
Gordon, 1993 [31]	-	-	Subject flow not reported	PP-OCE
Gordon, 1994 [22]	Target population not defined	-	-	PP-OCE
Jansen, 2014 [55]	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Lim, 2000 [39]	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Loh, 2000 [32]	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Martell, 2003 [24]	-	-	-	PP-OCE
Matrozova, 2010 [28]	-	-	-	PP-OCE
Milliez, 2005 [1]	-	Sampling frame: medical records	-	PP-OCE
Morillas, 2008 [47]	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Mosso, 2003 [26]	-	Sampling frame: medical records	-	PP-OCE
Mulatero, 2004 [25]				
Italy	-	-	Sampling method: convenience; Subject flow not reported	PP-OCE
USA	-	-	Sampling method: convenience; Subject flow not reported	PP-OCE
Singapore	-	-	Sampling method: convenience; Subject flow not reported	PP-OCE
Chile	-	-	-	PP-OCE

Supplemental data Table 4B. Continued

Author (Year)	DESCRIPTIVE	EXTERNAL VALIDITY		INTERNAL VALIDITY
	Minor Flaw	Major Flaw	Minor Flaw	Minor Flaw
Nishizaka, 2005 [45]	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Omura, 2004 [33]	-	-	Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Pedrosa, 2011 [50]	-	Exclusion rate from analysis >10%	-	PP-OCE
Ribeiro, 2009 [48]	-	-	Sampling method: convenience	PP-OCE
Rios, 2011 [51]	-	Exclusion rate from analysis >10%	Sampling bias not addressed in analysis/discussed; Sampling method not reported	PP-OCE
Rossi, 1998 [30]	-	-	-	PP-OCE
Rossi, 2002 [40]	-	-	-	PP-OCE
Rossi, 2006 [27]	-	-	-	PP-OCE
Sang & Jiang, 2013 [54]	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Schwartz, 2005 [20]	-	Exclusion rate from analysis >10%	Sampling method: self selection	PP-OCE
Sigurjonsdottir, 2012 [52]	-	-	Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Stowasser, 2003 [42]	-	-	Subject flow not reported	PP-OCE
Strauch, 2003 [43]	-	-	-	PP-OCE
Trenkel, 2002 [41]	-	-	Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Westerdahl, 2006 [34]	-	Exclusion rate from analysis >10%	Sampling method: self selection; Sampling frame: database	PP-OCE
Westerdahl, 2011 [36]	-	-	Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Williams, 2006 [21]	-	-	Sampling method: self selection; Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Yin, 2012 [53]	-	-	Sampling bias not addressed in analysis/discussed; Sampling method not reported.	PP-OCE

Data that were not reported are indicated by '-'. Abbreviations: PP-OCE, Point Prevalence, only Crude Estimates.

Supplemental Table 5. Univariate Analysis

Variable	Setting	Comparison	OR [95% CI]	Overall P-value
Publication Year	PC	2000-current vs. 1990-2000	0.49 [0.38 ; 0.64]	<.001
	RC	2000-current vs. 1990-2000	2.18 [1.04 ; 4.58]	0.04
Region	PC	USA vs. Europe	0.99 [0.22 ; 4.44]	<.001
	PC	Latin America vs. Europe	0.93 [0.68 ; 1.27]	
	PC	Asia vs. Europe	0.81 [0.53 ; 1.22]	
	PC	Australia vs. Europe	1.87 [1.38 ; 2.56]	
	RC	USA vs. Europe	1.32 [0.33 ; 5.29]	0.52
	RC	Latin America vs. Europe	0.56 [0.28 ; 1.15]	
	RC	Asia vs. Europe	0.89 [0.48 ; 1.67]	
	RC	Australia vs. Europe	1.08 [0.41 ; 2.80]	
Study Design	PC	Retrospective vs. Prospective	NA	NA
	RC	Retrospective vs. Prospective	1.33 [0.80 ; 2.22]	0.26
Study Objective	PC	Prevalence PA vs. Other	0.42 [0.34 ; 0.52]	<.001
	PC	Prevalence Secondary HT vs. Other	NA	NA
	PC	Prevalence PA vs. Prevalence Secondary HT	0.96 [0.77 ; 1.18]	
	RC	Prevalence PA vs. Other	0.88 [1.63 ; 1.95]	0.02
	RC	Prevalence Secondary HT vs. Other	0.63 [0.33 ; 1.18]	
	RC	Prevalence PA vs. Prevalence Secondary HT	1.40 [1.07 ; 1.82]	
Patient Selection Method	PC	Consecutive vs. Convenience	0.73 [0.35 ; 1.53]	0.35
	PC	Self Selection vs. Convenience	NA	
	PC	Consecutive vs. Self Selection	NA	
	RC	Consecutive vs. Convenience	1.82 [0.86 ; 3.85]	<.001
	RC	Self Selection vs. Convenience	0.46 [0.23 ; 0.91]	
	RC	Consecutive vs. Self Selection	3.95 [2.87 ; 5.45]	
Type of HT	PC	Therapy resistant HT vs. HT	NA	NA
	RC	Therapy resistant HT vs. HT	2.13 [1.19 ; 3.83]	0.01
Patient Selection on Potassium	PC	No Selection vs. Only Normokalemic Patients	0.98 [0.28 ; 3.46]	0.97
	RC	No Selection vs. Only Normokalemic Patients	1.06 [0.47 ; 2.39]	0.88
Patient Selection on Medication	PC	Endocrine Society Guideline vs. Unchanged	0.43 [0.33 ; 0.56]	0.04
	PC	Changed vs. Unchanged	0.68 [0.42 ; 1.12]	
	PC	MRA Stop vs. Unchanged	NA	
	PC	Endocrine Society Guideline vs. Changed	0.63 [0.36 ; 1.10]	
	PC	Endocrine Society Guideline vs. MRA Stop	1.17 [0.89 ; 1.53]	
	PC	Changed vs. MRA Stop	1.86 [1.13 ; 3.05]	
	RC	Endocrine Society Guideline vs. Unchanged	1.40 [0.58 ; 3.38]	0.58

>>

Supplemental data Table 5. Continued

Variable	Setting	Comparison	OR [95% CI]	Overall P-value
Patient Selection on Medication	RC	Changed vs. Unchanged	1.51 [0.57 ; 4.04]	
	RC	MRA Stop vs. Unchanged	2.33 [0.68 ; 8.08]	
	RC	Endocrine Society Guideline vs. Changed	0.93 [0.48 ; 1.78]	
	RC	Endocrine Society Guideline vs. MRA Stop	0.60 [0.22 ; 1.64]	
	RC	Changed vs. MRA Stop	0.65 [0.22 ; 1.93]	
Potassium Levels Corrected	PC	Hypokalemia Corrected vs. Normokalemia	0.98 [0.28 ; 3.46]	0.97
	RC	Hypokalemia Corrected vs. Normokalemia	1.06 [0.47 ; 2.39]	0.88
Screening Test	PC	No Screening vs. Other	2.81 [1.97 ; 4.02]	<.001
	PC	ARR vs. Other	1.32 [0.89; 1.95]	
	PC	No Screening vs. ARR	2.14 [1.81 ; 2.52]	
	RC	No Screening vs. Other	1.88 [1.23 ; 2.88]	<.001
	RC	ARR vs. Other	0.79 [0.43 ; 1.46]	
	RC	No Screening vs. ARR	2.38 [1.51 ; 3.77]	
Number of Screening Measurements	PC	One Measurement vs. Multiple measurements	0.85 [0.49 ; 1.47]	0.49
	RC	One Measurement vs. Multiple measurements	0.75 [0.39 ; 1.46]	0.38
Patient Position during Screening	PC	Supine vs. Not Supine	0.81 [0.50 ; 1.31]	0.32
	RC	Supine vs. Not Supine	0.53 [0.22 ; 1.24]	0.13
Cut-off Screening Test with ARR	PC	All Unrestrictive	NA	NA
	RC	All Unrestrictive	NA	NA
Percentage of Patients with Positive Screening Test who Underwent Confirmation Test	PC	100% vs. <80%	1.15 [0.39 ; 3.40]	0.40
	PC	>80% vs. <80%	0.84 [0.61 ; 1.16]	
	PC	100% vs. >80%	1.37 [0.47 ; 3.96]	
	RC	100% vs. <80%	1.88 [0.73 ; 4.81]	0.24
	RC	>80% vs. <80%	1.12 [0.34 ; 3.62]	
	RC	100% vs. >80%	1.68 [0.71 ; 3.98]	
Type of Confirmation Test	PC	IV SLT vs. Fludrocortisone	0.88 [0.69 ; 1.11]	0.33
	PC	Oral SLT vs. Fludrocortisone	1.09 [0.28 ; 4.21]	
	PC	Captopril vs. Fludrocortisone	1.24 [0.86; 1.76]	
	PC	IV SLT vs. oral SLT	0.81 [0.20 ; 3.18]	
	PC	IV SLT vs. Captopril	0.71 [0.46 ; 1.09]	
	PC	Oral SLT vs. Captopril	0.88 [0.22 ; 3.57]	
	RC	IV SLT vs. Fludrocortisone	1.30 [0.53 ; 3.23]	0.38
	RC	Oral SLT vs. Fludrocortisone	2.08 [0.52 ; 8.36]	
	RC	Captopril vs. Fludrocortisone	1.86 [0.72 ; 4.79]	

	RC	IV SLT vs. Oral SLT	0.63 [0.20 ; 1.96]	
	RC	IV SLT vs. Captopril	0.70 [0.41 ; 1.18]	
	RC	Oral SLT vs. Captopril	1.12 [0.35 ; 3.63]	
Cut-off IV SLT	PC	Restrictive vs. Unrestrictive	NA	NA
	RC	Restrictive vs. Unrestrictive	0.85 [0.43 ; 1.73]	0.64

Abbreviations: ARR, Aldosterone to Renin Ratio; IV SLT, Intravenous Salt Loading Test; HT, Hypertension; NA, Not Applicable; OR, Odds ratio; Oral SLT, Oral Salt Loading Test; PA, Primary Aldosteronism; PC, Primary Care; RC, Referral Center

Supplemental Table 6. Predicted Prevalences according to the Model

Changing variable	Publication Year	Region	Study Design	Study Purpose	Patient Selection	Screening Test	Prevalence [CI]
Publication Year	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 [0.06345 - 0.17958]**
	1999-2000	Europe	Retrospective	Other	Consecutive	Other	0.01295 [0.00344 - 0.04749]
Region	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 [0.06345 - 0.17958]**
	2000-curren	USA	Retrospective	Other	Consecutive	Other	0.37317 [0.24056 - 0.52804]
	2000-current	Latin America	Retrospective	Other	Consecutive	Other	0.06088 [0.03073 - 0.11704]
	2000-current	Asia	Retrospective	Other	Consecutive	Other	0.15451 [0.07984 - 0.27792]
	2000-current	Australia	Retrospective	Other	Consecutive	Other	0.40424 [0.23498 - 0.59983]
Study Design	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 [0.06345 - 0.17958]**
	2000-current	Europe	Prospective	Other	Consecutive	Other	0.05010 [0.02570 - 0.09542]
Study Purpose	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 [0.06345 - 0.17958]**
	2000-current	Europe	Retrospective	Prevalence PA	Consecutive	Other	0.17246 [0.11326 - 0.25376]
	2000-current	Europe	Retrospective	Prevalence Secondary Hypertension	Consecutive	Other	0.25620 [0.13712 - 0.42748]
Patient Selection Method	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 [0.06345 - 0.17958]**
	2000-current	Europe	Retrospective	Other	Convenience	Other	0.02400 [0.00746 - 0.07443]
	2000-current	Europe	Retrospective	Other	Self Selection	Other	0.07716 [0.03636 - 0.15631]
Screening Test	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 [0.06345 - 0.17958]**
	2000-current	Europe	Retrospective	Other	Consecutive	No Screening	0.28402 [0.19936 - 0.38724]
	2000-current	Europe	Retrospective	Other	Consecutive	ARR	0.08337 [0.03770 - 0.17436]

Combination for Lowest Prevalence	1999-2000	Latin America	Prospective	Other	Convenience	ARR	0.00046 [0.00004 - 0.00527]
Combination for Highest Prevalence	2000-Current	Australia	Retrospective	Prevalence Secondary Hypertension	Consecutive	No screening	0.40238 [0.18153 - 0.67148]

Predicted prevalences according to the model as a function of the six variables. The study of Di Murro, 2010[49] is chosen as reference study (bold).

REFERENCES

1. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. Journal of the American College of Cardiology 2005;45:1243-8.
2. Abad-Cardiel M, Alvarez-Alvarez B, Luque-Fernandez L, Fernandez C, Fernandez-Cruz A, Martell-Claros N. Hypertension caused by primary hyperaldosteronism: increased heart damage and cardiovascular risk. Revista espanola de cardiologia (English ed) 2013;66:47-52.
3. Mulatero P, Monticone S, Bertello C, et al. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. The Journal of clinical endocrinology and metabolism 2013;98:4826-33.
4. Catena C, Colussi G, Nadalini E, et al. Cardiovascular outcomes in patients with primary aldosteronism after treatment. Archives of internal medicine 2008;168:80-5.
5. Rossi GP, Cesari M, Cuspidi C, et al. Long-term control of arterial hypertension and regression of left ventricular hypertrophy with treatment of primary aldosteronism. Hypertension 2013;62:62-9.
6. Turchi F, Ronconi V, di Tizio V, Ceccoli L, Boscaro M, Giacchetti G. Primary aldosteronism and essential hypertension: assessment of cardiovascular risk at diagnosis and after treatment. Nutrition, metabolism, and cardiovascular diseases : NMCD 2014;24:476-82.
7. Sywak M, Pasieka JL. Long-term follow-up and cost benefit of adrenalectomy in patients with primary hyperaldosteronism. The British journal of surgery 2002;89:1587-93.
8. Streeten DH, Anderson GH, Jr., Wagner S. Effect of age on response of secondary hypertension to specific treatment. American journal of hypertension 1990;3:360-5.
9. Celen O, O'Brien MJ, Melby JC, Beazley RM. Factors influencing outcome of surgery for primary aldosteronism. Archives of surgery (Chicago, Ill : 1960) 1996;131:646-50.
10. Sawka AM, Young WF, Thompson GB, et al. Primary aldosteronism: factors associated with normalization of blood pressure after surgery. Annals of internal medicine 2001;135:258-61.
11. Eide IK, Torjesen PA, Drolsum A, Babovic A, Lilledahl NP. Low-renin status in therapy-resistant hypertension: a clue to efficient treatment. Journal of hypertension 2004;22:2217-26.
12. Calhoun DA, Nishizaka MK, Zaman MA, Harding SM. Aldosterone excretion among subjects with resistant hypertension and symptoms of sleep apnea. Chest 2004;125:112-7.
13. Funder JW, Carey RM, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. The Journal of clinical endocrinology and metabolism 2016;101:1889-916.

14. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. Jama 2000;283:2008-12.
15. Shamliyan T, Ansari MT, Raman G, et al. Development and Implementation of the Standards for Evaluating and Reporting Epidemiologic Studies on Chronic Disease Incidence or Prevalence. American Journal of Public Health Research 2013;1:183-90.
16. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled clinical trials 1986;7:177-88.
17. Sutton AJ, Higgins JP. Recent developments in meta-analysis. Statistics in medicine 2008;27:625-50.
18. Thompson SG, Higgins JP. How should meta-regression analyses be undertaken and interpreted? Statistics in medicine 2002;21:1559-73.
19. Shamliyan TA, Kane RL, Ansari MT, et al. AHRQ Methods for Effective Health Care. Development of Quality Criteria To Evaluate Nontherapeutic Studies of Incidence, Prevalence, or Risk Factors of Chronic Diseases: Pilot Study of New Checklists. Rockville (MD): Agency for Healthcare Research and Quality (US); 2011.
20. Schwartz GL, Turner ST. Screening for primary aldosteronism in essential hypertension: diagnostic accuracy of the ratio of plasma aldosterone concentration to plasma renin activity. Clinical chemistry 2005;51:386-94.
21. Williams JS, Williams GH, Raji A, et al. Prevalence of primary hyperaldosteronism in mild to moderate hypertension without hypokalaemia. Journal of human hypertension 2006;20:129-36.
22. Gordon RD, Stowasser M, Tunny TJ, Klemm SA, Rutherford JC. High incidence of primary aldosteronism in 199 patients referred with hypertension. Clinical and experimental pharmacology & physiology 1994;21:315-8.
23. Brown MA, Cramp HA, Zammit VC, Whitworth JA. Primary hyperaldosteronism: a missed diagnosis in 'essential hypertensives'? Australian and New Zealand journal of medicine 1996;26:533-8.
24. Martell N, Rodriguez-Cerrillo M, Grobbee DE, et al. High prevalence of secondary hypertension and insulin resistance in patients with refractory hypertension. Blood pressure 2003;12:149-54.
25. Mulatero P, Stowasser M, Loh KC, et al. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. The Journal of clinical endocrinology and metabolism 2004;89:1045-50.
26. Mosso L, Carvajal C, Gonzalez A, et al. Primary aldosteronism and hypertensive disease. Hypertension 2003;42:161-5.

27. Rossi GP, Bernini G, Caliumi C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. Journal of the American College of Cardiology 2006;48:2293-300.
28. Matrozova JA, Zacharieva SZ, Kirilov GG, Boyanov MA. Prevalence of primary aldosteronism among bulgarian hypertensive patients. Central European Journal of Medicine 2010;5:399-405.
29. Rios MC, Izquierdo A, Sotelo M, et al. Aldosterone/renin ratio in the diagnosis of primary aldosteronism Utilidad de la relacion aldosterona y actividad de renina plasmatica en el diagnostico de hiperaldosteronismo primario. Medicina (B Aires) 2011;71:525-30.
30. Rossi GP, Rossi E, Pavan E, et al. Screening for primary aldosteronism with a logistic multivariate discriminant analysis. Clinical endocrinology 1998;49:713-23.
31. Gordon RD, Ziesak MD, Tunny TJ, Stowasser M, Klemm SA. Evidence that primary aldosteronism may not be uncommon: 12% incidence among antihypertensive drug trial volunteers. Clinical and experimental pharmacology & physiology 1993;20:296-8.
32. Loh KC, Koay ES, Khaw MC, Emmanuel SC, Young WF, Jr. Prevalence of primary aldosteronism among Asian hypertensive patients in Singapore. The Journal of clinical endocrinology and metabolism 2000;85:2854-9.
33. Omura M, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T. Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan. Hypertension research : official journal of the Japanese Society of Hypertension 2004;27:193-202.
34. Westerdahl C, Bergenfelz A, Isaksson A, Wihl A, Nerbrand C, Valdemarsson S. High frequency of primary hyperaldosteronism among hypertensive patients from a primary care area in Sweden. Scandinavian journal of primary health care 2006;24:154-9.
35. Fogari R, Preti P, Zoppi A, Rinaldi A, Fogari E, Mugellini A. Prevalence of primary aldosteronism among unselected hypertensive patients: a prospective study based on the use of an aldosterone/renin ratio above 25 as a screening test. Hypertension research : official journal of the Japanese Society of Hypertension 2007;30:111-7.
36. Westerdahl C, Bergenfelz A, Isaksson A, Nerbrand C, Valdemarsson S. Primary aldosteronism among newly diagnosed and untreated hypertensive patients in a Swedish primary care area. Scandinavian journal of primary health care 2011;29:57-62.
37. Anderson GH, Jr., Blakeman N, Streeten DH. The effect of age on prevalence of secondary forms of hypertension in 4429 consecutively referred patients. Journal of hypertension 1994;12:609-15.
38. Abdelhamid S, Muller-Lobeck H, Pahl S, et al. Prevalence of adrenal and extra-adrenal Conn syndrome in hypertensive patients. Archives of internal medicine 1996;156:1190-5.
39. Lim PO, Dow E, Brennan G, Jung RT, MacDonald TM. High prevalence of primary aldosteronism in the Tayside hypertension clinic population. Journal of human hypertension 2000;14:311-5.

40. Rossi E, Regolisti G, Negro A, Sani C, Davoli S, Perazzoli F. High prevalence of primary aldosteronism using postcaptopril plasma aldosterone to renin ratio as a screening test among Italian hypertensives. American journal of hypertension 2002;15:896-902.
41. Trenkel S, Seifarth C, Schobel H, Hahn EG, Hensen J. Ratio of serum aldosterone to plasma renin concentration in essential hypertension and primary aldosteronism. Experimental and Clinical Endocrinology & Diabetes 2002;110:80-5.
42. Stowasser M, Gordon RD, Gunasekera TG, et al. High rate of detection of primary aldosteronism, including surgically treatable forms, after 'non-selective' screening of hypertensive patients. Journal of hypertension 2003;21:2149-57.
43. Strauch B, Zelinka T, Hampf M, Bernhardt R, Widimsky J, Jr. Prevalence of primary hyperaldosteronism in moderate to severe hypertension in the Central Europe region. Journal of human hypertension 2003;17:349-52.
44. Calhoun D, Nishizaka M, Zaman A. Low prevalence of white-coat hypertension in subjects with resistant hypertension and hyperaldosteronism. Journal of hypertension 2004;22:S206-S.
45. Nishizaka MK, Pratt-Ubunama M, Zaman MA, Cofield S, Calhoun DA. Validity of plasma aldosterone-to-renin activity ratio in African American and white subjects with resistant hypertension. American journal of hypertension 2005;18:805-12.
46. Douma S, Petidis K, Doumas M, et al. Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. Lancet (London, England) 2008;371:1921-6.
47. Morillas P, Castillo J, Quiles J, et al. [Prevalence of primary aldosteronism in hypertensive patients and its effect on the heart]. Rev Esp Cardiol 2008;61:418-21.
48. Ribeiro MJ, Figueiredo Neto JA, Memoria EV, et al. Prevalence of primary hyperaldosteronism in a systemic arterial hypertension league. Arq Bras Cardiol 2009;92:39-45.
49. Di Murro A, Petramala L, Cotesta D, et al. Renin-angiotensin-aldosterone system in patients with sleep apnoea: prevalence of primary aldosteronism. Journal of the renin-angiotensin-aldosterone system : JRAAS 2010;11:165-72.
50. Pedrosa RP, Drager LF, Gonzaga CC, et al. Obstructive sleep apnea: the most common secondary cause of hypertension associated with resistant hypertension. Hypertension 2011;58:811-7.
51. Rios MC, Izquierdo A, Sotelo M, et al. [Aldosterone/renin ratio in the diagnosis of primary aldosteronism]. Medicina (B Aires) 2011;71:525-30.
52. Sigurjonsdottir HA, Gronowitz M, Andersson O, et al. Unilateral adrenal hyperplasia is a usual cause of primary hyperaldosteronism. Results from a Swedish screening study. BMC endocrine disorders 2012;12:17.

53. Yin GS, Zhang SL, Yan L, et al. Effect of age on aldosterone/renin ratio (ARR) and comparison of screening accuracy of ARR plus elevated serum aldosterone concentration for primary aldosteronism screening in different age groups. Endocrine 2012;42:182-9.
54. Sang X, Jiang Y, Wang W, et al. Prevalence of and risk factors for primary aldosteronism among patients with resistant hypertension in China. Journal of hypertension 2013;31:1465-71; discussion 71-2.
55. Jansen PM, van den Born BJ, Frenkel WJ, et al. Test characteristics of the aldosterone-to-renin ratio as a screening test for primary aldosteronism. Journal of hypertension 2014;32:115-26.
56. Jansen PM, Boomsma F, van den Meiracker AH. Aldosterone-to-renin ratio as a screening test for primary aldosteronism--the Dutch ARRAT Study. The Netherlands journal of medicine 2008;66:220-8.
57. Hannemann A, Wallaschofski H. Prevalence of primary aldosteronism in patient's cohorts and in population-based studies--a review of the current literature. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2012;44:157-62.
58. Plouin PF, Amar L, Chatellier G. Trends in the prevalence of primary aldosteronism, aldosterone-producing adenomas, and surgically correctable aldosterone-dependent hypertension. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2004;19:774-7.
59. Kaplan NM. Is there an unrecognized epidemic of primary aldosteronism? Con. Hypertension 2007;50:454-8; discussion -8.
60. Schwartz GL, Chapman AB, Boerwinkle E, Kisabeth RM, Turner ST. Screening for primary aldosteronism: implications of an increased plasma aldosterone/renin ratio. Clinical chemistry 2002;48:1919-23.
61. Gordon RD. The challenge of more robust and reproducible methodology in screening for primary aldosteronism. Journal of hypertension 2004;22:251-5.
62. Tomaschitz A, Pilz S. Aldosterone to renin ratio--a reliable screening tool for primary aldosteronism? Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2010;42:382-91.
63. Seifarth C, Trenkel S, Schobel H, Hahn EG, Hensen J. Influence of antihypertensive medication on aldosterone and renin concentration in the differential diagnosis of essential hypertension and primary aldosteronism. Clinical endocrinology 2002;57:457-65.
64. Mulatero P, Rabbia F, Milan A, et al. Drug effects on aldosterone/plasma renin activity ratio in primary aldosteronism. Hypertension 2002;40:897-902.

65. Funder JW. Primary aldosteronism: clinical lateralization and costs. The Journal of clinical endocrinology and metabolism 2012;97:3450-2.
66. Reincke M, Rump LC, Quinkler M, et al. Risk factors associated with a low glomerular filtration rate in primary aldosteronism. The Journal of clinical endocrinology and metabolism 2009;94:869-75.
67. Born-Frontsberg E, Reincke M, Rump LC, et al. Cardiovascular and cerebrovascular comorbidities of hypokalemic and normokalemic primary aldosteronism: results of the German Conn's Registry. The Journal of clinical endocrinology and metabolism 2009;94:1125-30.
68. Nishimura M, Uzu T, Fujii T, et al. Cardiovascular complications in patients with primary aldosteronism. American journal of kidney diseases : the official journal of the National Kidney Foundation 1999;33:261-6.
69. Rossi GP, Cesari M, Cuspidi C, et al. Changes of left ventricular filling indexes during long-term follow-up after adrenalectomy or medical treatment for primary aldosteronism. Hypertension 2010;56 (5):e78-e9.
70. Catena C, Colussi G, Lapenna R, et al. Long-term cardiac effects of adrenalectomy or mineralocorticoid antagonists in patients with primary aldosteronism. Hypertension 2007;50:911-8.
71. Muiesan ML, Salvetti M, Paini A, et al. Inappropriate left ventricular mass in patients with primary aldosteronism. Hypertension 2008;52:529-34.
72. Rizzoni D, Muiesan ML, Porteri E, et al. Relations between cardiac and vascular structure in patients with primary and secondary hypertension. Journal of the American College of Cardiology 1998;32:985-92.
73. Holaj R, Zelinka T, Wichterle D, Petrak O, Strauch B, Widimsky J. Increased intima-media thickness of the common carotid artery in primary aldosteronism in comparison with essential hypertension. Journal of hypertension 2007;25:1451-7.
74. Catena C, Colussi G, Sechi LA. Treatment of Primary Aldosteronism and Organ Protection. International journal of endocrinology 2015;2015:597247.
75. Cortes P, Fardella C, Oestreicher E, et al. [Excess of mineralocorticoids in essential hypertension: clinical-diagnostic approach]. Rev Med Chil 2000;128:955-61.
76. Daimon M, Konta T, Oizumi T, et al. Lower aldosterone-renin ratio is a risk factor for total and cancer death in Japanese individuals: the Takahata study. Clinical endocrinology 2015;82:489-96.
77. Denolle T, Hanon O, Mounier-Vehier C, et al. [What tests should be conducted for secondary arterial hypertension in hypertensive patients resistant to treatment?]. Arch Mal Coeur Vaiss 2000;93:1037-9.

78. Ducher M, Mounier-Vehier C, Baguet JP, et al. Aldosterone-to-renin ratio for diagnosing aldosterone-producing adenoma: A multicentre study. Archives of Cardiovascular Diseases 2012;105:623-30.
79. Gallay BJ, Ahmad S, Xu L, Toivola B, Davidson RC. Screening for primary aldosteronism without discontinuing hypertensive medications: plasma aldosterone-renin ratio. American journal of kidney diseases : the official journal of the National Kidney Foundation 2001;37:699-705.
80. Gallego S, Covarsi A, Luengo J, Gonzalez P, Suarez MA, Novillo R. [Our experience in primary hyperaldosteronism]. Nefrologia 2007;27:704-9.
81. Garcia EA, Lopez JR, Meier JL, Swislocki ALM, Siegel D. Resistant Hypertension and Undiagnosed Primary Hyperaldosteronism Detected by Use of a Computerized Database. Journal of Clinical Hypertension 2011;13:487-91.
82. Gombet T, Steichen O, Plouin PF. [Hypertensive disease in subjects born in sub-Saharan Africa or in Europe referred to a hypertension unit: a cross-sectional study]. Bull Acad Natl Med 2007;191:1745-54; discussion 54-5.
83. Gonzaga CC, Gaddam KK, Ahmed MI, et al. Severity of obstructive sleep apnea is related to aldosterone status in subjects with resistant hypertension. Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine 2010;6:363-8.
84. Gregori M, Giammarioli B, Tocci G, et al. Synergic effects of renin and aldosterone on right ventricular function in hypertension: a tissue Doppler study. Journal of cardiovascular medicine (Hagerstown, Md) 2015;16:831-8.
85. Hannemann A, Bidlingmaier M, Friedrich N, et al. Screening for Primary Aldosteronism in Hypertensive Subjects - Results From Two German Epidemiological Studies. European journal of endocrinology / European Federation of Endocrine Societies 2012.
86. Ito Y, Takeda R, Karashima S, Yamamoto Y, Yoneda T, Takeda Y. Prevalence of primary aldosteronism among prehypertensive and stage 1 hypertensive subjects. Hypertension research : official journal of the Japanese Society of Hypertension 2011;34:98-102.
87. Jefic D, Mohiuddin N, Alsabbagh R, Fadanelli M, Steigerwalt S. The prevalence of primary aldosteronism in diabetic patients. Journal of clinical hypertension (Greenwich, Conn) 2006;8:253-6.
88. Lim PO, Rodgers P, Cardale K, Watson AD, MacDonald TM. Potentially high prevalence of primary aldosteronism in a primary-care population. Lancet (London, England) 1999;353:40.
89. Mosso L, Fardella C, Montero J, et al. [High prevalence of undiagnosed primary hyperaldosteronism among patients with essential hypertension]. Rev Med Chil 1999;127:800-6.
90. Mysliwiec J, Zukowski L, Grodzka A, et al. Problems in diagnostics of primary aldosteronism - analysis of the own data. Endokrynol Pol 2010;61:2-5.

91. Olivieri O, Ciacciarelli A, Signorelli D, et al. Aldosterone to renin ratio in a primary care setting: The Bussolengo study. Journal of Clinical Endocrinology & Metabolism 2004;89:4221-6.
92. Pardes E, Belli S, Cornalo D, et al. Determination of the aldosterone/plasma renin activity ratio for the screening of primary hyperaldosteronism in essential hypertension: A multicentric study. [Spanish] Determinacion del valor de corte de la relacion aldosterona/actividad de renina plasmatica para la deteccion de hiperaldosteronismo primario en hipertension arterial esencial: Estudio multicentrico. Revista Argentina de Endocrinologia y Metabolismo 2010;47:27-39.
93. Rayner BL, Opie LH, Davidson JS. The aldosterone/renin ratio as a screening test for primary aldosteronism. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde 2000;90:394-400.
94. Rayner BL, Myers JE, Opie LH, Trinder YA, Davidson JS. Screening for primary aldosteronism--normal ranges for aldosterone and renin in three South African population groups. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde 2001;91:594-9.
95. Rosenbaum D, Rigabert J, Villeneuve F, Girerd X. An abdominal CT scan in first-line is an efficient investigation of uncontrolled hypertensives suspected to have an adrenal cause. [French] evaluation de l'efficience diagnostique de la tomodensitometrie abdominale de premiere intention chez l'hypertendu non controle. Ann Cardiol Angeiol (Paris) 2012;61:209-12.
96. Sabio JM, Mediavilla-Garcia JD, Jaen F, Fernandez-Torres C, Aliaga L, Jimenez-Alonso J. [Primary aldosteronism: analysis of a series of 54 patients]. Med Clin (Barc) 2005;124:765-8.
97. Schmiemann G, Gebhardt K, Hummers-Pradier E, Egidi G. Prevalence of hyperaldosteronism in primary care patients with resistant hypertension. Journal of the American Board of Family Medicine : JABFM 2012;25:98-103.
98. Sharma BK, Singh G, Sagar S. Malignant hypertension in north west India. A hospital based study. Japanese heart journal 1994;35:601-9.
99. Takayanagi R, Miura K, Nakagawa H, Nawata H. Epidemiologic study of adrenal gland disorders in Japan. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2000;54 Suppl 1:164s-8s.
100. Volpe C, Wahrenberg H, Hamberger B, Thoren M. Screening for primary aldosteronism in a primary care unit. Journal of the renin-angiotensin-aldosterone system : JRAAS 2012.
101. Williams D, Croal B, Furnace J, et al. The prevalence of a raised aldosterone-renin ratio (ARR) among new referrals to a hypertension clinic. Blood pressure 2006;15:164-8.
102. Hood S, Cannon J, Foo R, Brown M. Prevalence of primary hyperaldosteronism assessed by aldosterone/renin ratio and spironolactone testing. Clinical medicine (London, England) 2005;5:55-60.

103. Niizuma S, Nakahama H, Kamide K, et al. The cutoff value of aldosterone-to-renin ratio for the diagnosis of primary aldosteronism in patients taking antihypertensive medicine. Clinical and Experimental Hypertension 2008;30:640-7.

104. Nogueira AR, Bloch KV. Screening for primary aldosteronism in a cohort of Brazilian patients with resistant hypertension. Journal of clinical hypertension (Greenwich, Conn) 2008;10:619-23.

105. Borgel J, Springer S, Ghafoor J, et al. Unrecognized secondary causes of hypertension in patients with hypertensive urgency/emergency: prevalence and co-prevalence. Clinical research in cardiology : official journal of the German Cardiac Society 2010;99:499-506.

106. Li N, Wang M, Wang H, et al. Prevalence of Primary Aldosteronism in Hypertensive Subjects with Hyperglycemia. Clinical and experimental hypertension (New York, NY : 1993) 2012.

107. Mukherjee JJ, Khoo CM, Thai AC, Chionh SB, Pin L, Lee KO. Type 2 diabetic patients with resistant hypertension should be screened for primary aldosteronism. Diabetes & vascular disease research : official journal of the International Society of Diabetes and Vascular Disease 2010;7:6-13.

108. Murase K, Nagaishi R, Takenoshita H, Nomiyama T, Akehi Y, Yanase T. Prevalence and clinical characteristics of primary aldosteronism in Japanese patients with type 2 diabetes mellitus and hypertension. Endocrine journal 2013;60:967-76.

109. Umpierrez GE, Cantey P, Smiley D, et al. Primary aldosteronism in diabetic subjects with resistant hypertension. Diabetes Care 2007;30:1699-703.

110. Markou A, Pappa T, Kaltsas G, et al. Evidence of primary aldosteronism in a predominantly female cohort of normotensive individuals: a very high odds ratio for progression into arterial hypertension. The Journal of clinical endocrinology and metabolism 2013;98:1409-16.

111. Solar M, Malirova E, Ballon M, Pelouch R, Ceral J. Confirmatory testing in primary aldosteronism: extensive medication switching is not needed in all patients. European journal of endocrinology / European Federation of Endocrine Societies 2012;166:679-86.

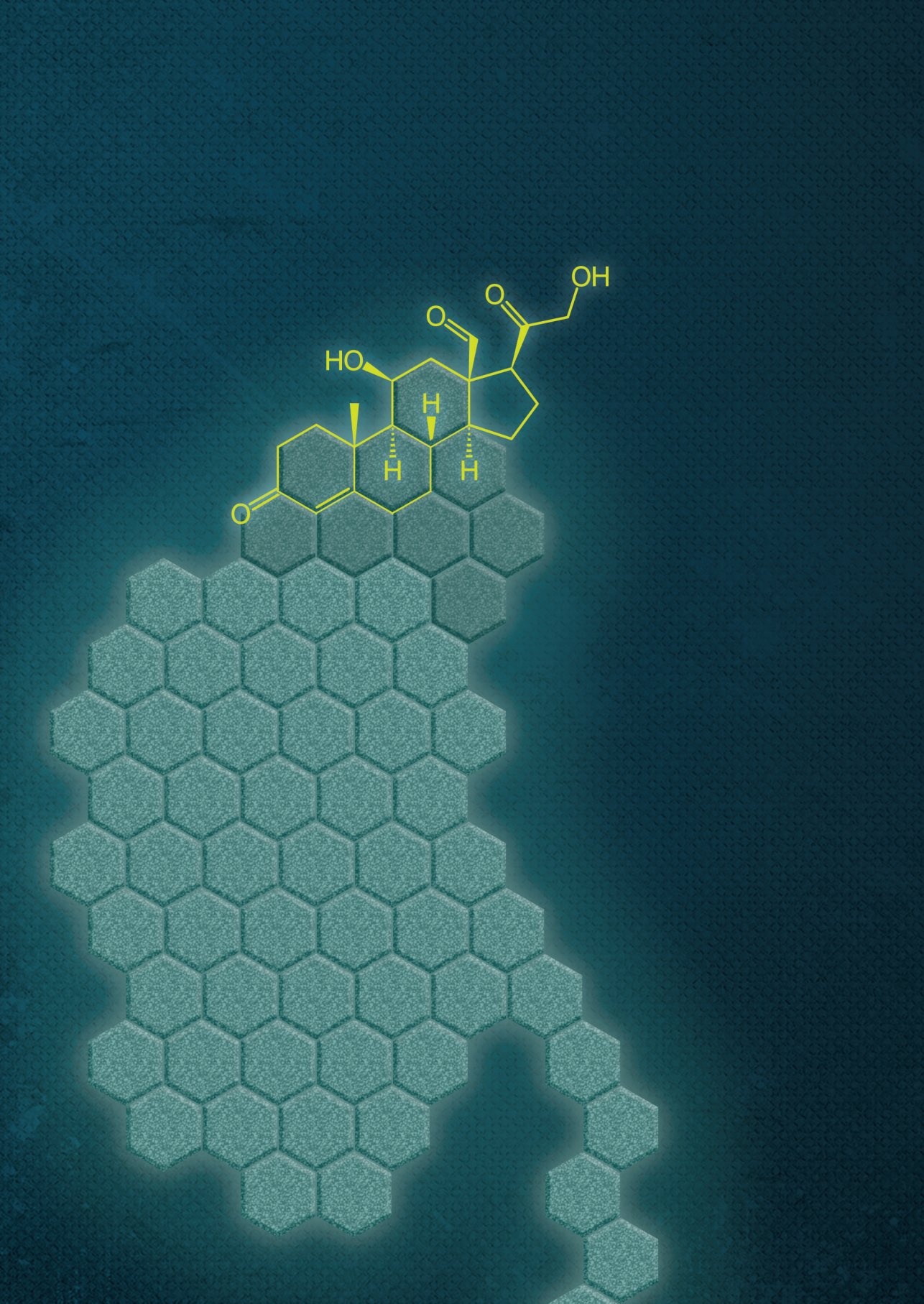
112. Ye D, Dong FQ, Lu XL, Zhang Z, Feng YF, Li CJ. Analysis of various etiologies of hypertension in patients hospitalized in the endocrinology division. Endocrine 2012;42:174-81.

113. Rossi GP, Seccia TM, Gallina V, et al. Prospective appraisal of the prevalence of primary aldosteronism in hypertensive patients presenting with atrial flutter or fibrillation (PAPPHY Study): rationale and study design. Journal of human hypertension 2013;27:158-63.

114. Godula K, Cunha J, Simões PS, Lauro F, Oubina C. The 10 year observational study of adrenal mass pathology in Prof. Dr. Fernando Fonseca Hospital. European Journal of Internal Medicine 2013;24:e110.

115. Benchetrit S, Bernheim J, Podjarny E. Normokalemic hyperaldosteronism in patients with resistant hypertension. Isr Med Assoc J 2002;4:17-20.

116. Gouli A, Kaltsas G, Tzonou A, et al. High prevalence of autonomous aldosterone secretion among patients with essential hypertension. Eur J Clin Invest 2011;41:1227-36.
117. Mysliwiec J, Zukowski L, Grodzka A, Pilaszewicz A, Dragowski S, Gorska M. Diagnostics of primary aldosteronism: is obligatory use of confirmatory tests justified? Journal of the Renin-Angiotensin-Aldosterone System 2012;13:367-71.
118. Papanastasiou L, Markou A, Pappa T, et al. Primary aldosteronism in hypertensive patients: clinical implications and target therapy. Eur J Clin Invest 2014;44:697-706.
119. Sy WM, Fu SN, Luk W, Wong CK, Fung LM. Primary hyperaldosteronism among Chinese hypertensive patients: how are we doing in a local district in Hong Kong. Hong Kong medical journal = Xianggang yi xue za zhi / Hong Kong Academy of Medicine 2012;18:193-200.
120. Trifanescu R, Carsote M, Caragheorgheopol A, et al. Screening for secondary endocrine hypertension in young patients. Maedica 2013;8:108-15.
121. Wu VC, Hu YH, Wu CH, et al. Administrative data on diagnosis and mineralocorticoid receptor antagonist prescription identified patients with primary aldosteronism in Taiwan. Journal of clinical epidemiology 2014;67:1139-49.
122. Adlin EV, Braitman LE, Vasan RS. Bimodal aldosterone distribution in low-renin hypertension. American journal of hypertension 2013;26:1076-85.
123. Kao CC, Wu VC, Kuo CC, et al. Delayed diagnosis of primary aldosteronism in patients with autosomal dominant polycystic kidney diseases. Journal of the renin-angiotensin-aldosterone system : JRAAS 2012.
124. Sakthiswary R, Wong M, Isa ZM, Azmi KN. Spot urine potassium as a potential screening test for aldosterone breakthrough. Clinica Terapeutica 2012;163:195-8.
125. Calhoun DA, Nishizaka MK, Zaman MA, Thakkar RB, Weissmann P. Hyperaldosteronism among black and white subjects with resistant hypertension. Hypertension 2002;40:892-6.
126. Fardella CE, Mosso L, Gomez-Sanchez C, et al. Primary hyperaldosteronism in essential hypertensives: prevalence, biochemical profile, and molecular biology. The Journal of clinical endocrinology and metabolism 2000;85:1863-7.
127. Nishikawa T, Omura M. Clinical characteristics of primary aldosteronism: its prevalence and comparative studies on various causes of primary aldosteronism in Yokohama Rosai Hospital. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2000;54 Suppl 1:83s-5s.
128. Rossi GP. Primary aldosteronism in hypertensive patients. Cardiology Review 2007;24:27-33.
129. Fardella CE, Mosso L. Authors' response: Prevalence of primary aldosteronism in unselected hypertensive populations - Screening and definitive diagnosis. Journal of Clinical Endocrinology & Metabolism 2001;86:4003-4.


OH
O
O
HO
H
H
H
O

Adrenal vein sampling versus CT-scan to determine treatment in primary aldosteronism: an outcome-based randomised diagnostic trial

Tanja Dekkers[1] | Aleksander Prejbisz[7] | Leo J. Schultze Kool[3]
Hans J.M.M. Groenewoud[6] | Marieke S. Velema[2] | Wilko Spiering[9]
Sylwia Kołodziejczyk-Kruk[7] | Mark Arntz[3] | Jacek Kądziela[8]
Johannes F. Langenhuijsen[4] | Michiel N. Kerstens[10], | Anton H. van den Meiracker[11]
Bert-Jan van den Born[12] | Fred C.G.J. Sweep[5] | Ad R.M.M. Hermus[2]
Andrzej Januszewicz[7] | Alike F. Ligthart-Naber[1] | Peter Makai[6]
Gert-Jan van der Wilt[6] | Jacques W.M. Lenders[1,13] | Jaap Deinum[1]
for the SPARTACUS Investigators*

[1] Department of Internal Medicine, Division of Vascular Medicine, Radboud University Medical Center, Nijmegen, Netherlands
[2] Department of Internal Medicine, Division of Endocrinology, Radboud University Medical Center, Nijmegen, Netherlands
[3] Department of Radiology, Radboud University Medical Center, Nijmegen, Netherlands
[4] Department of Urology, Radboud University Medical Center, Nijmegen, Netherlands
[5] Department of Laboratory Medicine, Radboud University Medical Center, Nijmegen, Netherlands
[6] Department of Health Evidence, Radboud University Medical Center, Nijmegen, Netherlands
[7] Department of Hypertension, Institute of Cardiology, Warsaw, Poland
[8] Department of Interventional Cardiology and Angiology, Institute of Cardiology, Warsaw, Poland
[9] Department of Vascular Medicine, University Medical Center Utrecht, Utrecht, Netherlands
[10] Department of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands
[11] Division of Pharmacology and Vascular Medicine, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands
[12] Department of Internal and Vascular Medicine, Academic Medical Center, Amsterdam, Netherlands
[13] Department of Internal Medicine III, University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany
* Complete list of investigators provided in the supplemental data

Lancet Diabetes Endocrinol. 2016 Sep;4(9):739-746.

ABSTRACT

Background. The distinction between unilateral aldosterone-producing adenoma and bilateral adrenal hyperplasia as causes of primary aldosteronism is usually made by adrenal CT or by adrenal vein sampling (AVS). Whether CT or AVS represents the best test for diagnosis remains unknown. We aimed to compare the outcome of CT-based management with AVS-based management for patients with primary aldosteronism.

Methods. In a randomised controlled trial, we randomly assigned patients with aldosteronism to undergo either adrenal CT or AVS to determine the presence of aldosterone-producing adenoma (with subsequent treatment consisting of adrenalectomy) or bilateral adrenal hyperplasia (subsequent treatment with mineralocorticoid receptor antagonists). The primary endpoint was the intensity of drug treatment for obtaining target blood pressure after 1 year of follow-up, in the intention-to-diagnose population. Intensity of drug treatment was expressed as daily defined doses. Key secondary endpoints included biochemical outcome in patients who received adrenalectomy, health-related quality of life, cost-effectiveness, and adverse events. This trial is registered with ClinicalTrials.gov, number NCT01096654.

Findings. We recruited 200 patients between July 6, 2010, and May 30, 2013. Of the 184 patients that completed follow-up, 92 received CT-based treatment (46 adrenalectomy and 46 mineralocorticoid receptor antagonist) and 92 received AVS-based treatment (46 adrenalectomy and 46 mineralocorticoid receptor antagonist). We found no differences in the intensity of antihypertensive medication required to control blood pressure between patients with CT-based treatment and those with AVS-based treatment (median daily defined doses 3.0 [IQR 1.0–5.0] vs. 3.0 [1.1–5.9], p=0.52; median number of drugs 2 [IQR 1–3] vs. 2 [1–3], p=0.87). Target blood pressure was reached in 39 (42%) patients and 41 (45%) patients, respectively (p=0.82). On secondary endpoints we found no differences in health-related quality of life (median RAND-36 physical scores 52.7 [IQR 43.9–56.8] vs. 53.2 [44.0–56.8], p=0.83; RAND-36 mental scores 49.8 [43.1–54.6] vs. 52.7 [44.9–55.5], p=0.17)

for CT-based and AVS-based treatment. Biochemically, 37 (80%) of patients with CT-based adrenalectomy and 41 (89%) of those with AVS-based adrenalectomy had resolved hyperaldosteronism (p=0.25). A non-significant mean difference of 0.05 (95% CI –0.04 to 0.13) in quality-adjusted life-years (QALYs) was found to the advantage of the AVS group, associated with a significant increase in mean health-care costs of €2285 per patient (95% CI 1323–3248). At a willingness-to-pay value of €30 000 per QALY, the probability that AVS compared with CT constitutes an efficient use of health-care resources in the diagnostic work-up of patients with primary aldosteronism is less than 0.2. There was no difference in adverse events between groups (159 events of which nine were serious vs. 187 events of which 12 were serious) for CT-based and AVS-based treatment.

Interpretation. 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. This finding challenges the current recommendation to perform AVS in all patients with primary aldosteronism.

Funding. Netherlands Organisation for Health Research and Development–Medical Sciences, Institute of Cardiology, Warsaw.

INTRODUCTION

Primary aldosteronism is an important cause of secondary hypertension, affecting 5–15% of the hypertensive population.[1] Early diagnosis and treatment are important because patients have higher cardiovascular morbidity and mortality than blood-pressure-matched controls with primary hypertension.[2] In most cases, primary aldosteronism is caused by either a unilateral aldosterone-producing adenoma or by bilateral hyperplasia.[1] Proper distinction between the two is crucial, because the former is treated by adrenalectomy, and the latter by mineralocorticoid receptor antagonists.[1] For the diagnosis of these two subtypes, adrenal CT scanning or bilateral adrenal vein sampling (AVS) is used.[1] Adrenal CT is readily available and cheap, but the accuracy for diagnosing aldosterone-producing adenomas is limited.

AVS is expensive and requires considerable technical skill.[3] It is therefore less widely available than CT. AVS can have the advantage of obtaining a functional diagnosis in CT-identified nodules. Additionally, it can uncover aldosterone-producing adenomas below the detection limit of CT. Therefore, AVS has emerged as the reference standard for primary aldosteronism subtyping.[1,4-6] In a systematic review of predominantly retrospective studies, we found a diagnostic discordance between CT and AVS in 38% of cases.[7] However, evidence for superiority of AVS is limited when it comes to treatment outcome.[8] Therefore, we set out to perform a diagnostic, randomised trial to compare CT-based and AVS-based management of patients with primary aldosteronism. Crucial to the design of our study, in the absence of a reference test for subtyping of primary aldosteronism, is the concept that the better diagnostic strategy is expected to translate to a better clinical outcome. To circumvent bias by more vigorous drug treatment, we chose as primary endpoint the intensity of drug treatment needed to achieve target blood pressure.

METHODS

STUDY DESIGN AND PARTICIPANTS

We performed a diagnostic, randomised clinical trial. The study was done in 12 Dutch medical centres and one Polish centre. The study was approved by the institutional review boards of the centres. We planned no interim analyses and did not install a data monitoring

committee. We planned to enroll 200 patients. Criteria for inclusion were age 18 years or older, and hypertension needing three or more antihypertensive drugs in adequate doses, or hypertension accompanied by spontaneous or diuretic-induced hypokalemia (serum potassium <3.5 mmol/L). Before inclusion, primary aldosteronism was confirmed by an oral or intravenous salt-loading test.[1] Exclusion criteria were refusal by the patient to undergo AVS, CT, or adrenalectomy; pregnancy; suspicion of glucocorticoid remediable aldosteronism; suspicion of adrenocortical carcinoma; severe comorbidity potentially interfering with treatment or health-related quality of life; or requirement of medication interfering with the study protocol. All patients gave written informed consent.

RANDOMISATION AND MASKING

We randomly assigned patients to either adrenal CT or AVS using a web-based algorithm stratified by study centre and minimised for sex, age, blood pressure, and intensity of antihypertensive medication (in terms of defined daily dose); no block randomisation was applied. The daily defined dose is the assumed average maintenance dose per day for a drug used for its main indication in adults. For instance, 5 mg of amlodipine has a daily defined dose of 1, as does 10 mg of lisinopril. If both drugs are taken together, the daily defined dose is 2. Daily defined doses in this way provide an estimation of intensity of drug use for the same indication and can be used to compare different patient populations. Because we did not use any sham procedures, patients, investigators, and statisticians were not masked to treatment allocation.

PROCEDURES

At baseline all patients underwent 24 h ambulatory blood pressure measurement with registration of medication use. To assess the effect on quality of life, patients filled out the RAND-36 health-related quality of life question naire.[9,10] We treated patients randomised to CT by adrenalectomy in case of a unilaterally enlarged adrenal with a normal contra-lateral gland. In case of bilaterally enlarged or normal adrenal glands patients were treated with mineralocorticoid receptor antagonist. Adrenal glands with a thickness of 7 mm or more of body or limb on CT were considered enlarged.[11] Adrenal CT was done in all medical centres, assessed by a local radiologist and centrally revised in Nijmegen. The conclusions were communicated to the local centres. In case of discrepancy, the local centre determined the treatment strategy.

We performed AVS procedures at Radboud University, Medical Center and University Medical Center Groningen (Netherlands), and at the Institute of Cardiology in Warsaw (Poland). AVS was preceded by CT to determine adrenal vein anatomy. We performed AVS under continuous cosyntropin stimulation with sequential catheterisation of adrenal veins (supplemental data). Successful cannulation was defined as a selectivity index (defined as the ratio between adrenal and peripheral cortisol) of 3.0 or higher. Unilateral disease was diagnosed when the lateralisation index (defined as the ratio of aldosterone normalised to cortisol between dominant and the non-dominant adrenal gland) was 4.0 or higher, and the suppression index (defined as the ratio of aldosterone normalised to cortisol between the non-dominant adrenal gland and peripheral blood) was less than or equal to 1.0. We treated patients with unilateral disease by adrenalectomy. Patients without unilateral disease were treated with a mineralocorticoid receptor antagonist. In successful AVS procedures, CT outcome had no effect on treatment decisions. In case of technical AVS failure, patients were treated according to the CT findings.

During follow-up after adrenalectomy, antihypertensive medication was initiated and adjusted by the treating physician according to a recommended treatment algorithm to achieve a target blood pressure of less than 135/85 mmHg using a semiautomatic device, or of less than 140/90 mmHg using office measurement of blood pressure.[12] In case of treatment with mineralocorticoid receptor antagonist, patients started on 25 mg of spironolactone, if necessary to be increased to a maximum dose of two times 100 mg daily. In case of side-effects to spironolactone, we prescribed eplerenone to a maximum of two times 200 mg daily. Patients with a history of spironolactone intolerance were treated with eplerenone from the beginning. At the maximally tolerable dose of the mineralocorticoid receptor antagonist, we added conventional antihypertensive agents to reach target blood pressure if needed. At final evaluation after 1 year, we assessed 24 h ambulatory blood pressure and medication use and did a salt-loading test in patients who underwent adrenalectomy. Patients completed the RAND-36 health-related quality of life questionnaire at final assessment.[9,10]

OUTCOMES

The primary endpoint was the intensity of antihypertensive medication needed, expressed in daily defined doses. Because we aimed to achieve target blood pressure in both study groups, blood pressure per se was not the primary outcome of interest. The proportion of patients reaching target blood pressure (<135/85 mmHg according to daytime ambulatory blood

pressure monitoring) was included as a secondary endpoint. Other secondary endpoints included serum potassium and aldosterone after salt-loading post adrenalectomy. We classified patients with suppressible aldosterone as having resolved primary aldosteronism (cut-offs provided in the supplemental data). In case of indeterminate aldosterone values, classification as resolved or persistent primary aldosteronism was reached by consensus. All other patients had persistent aldosteronism. Further secondary endpoints were health-related quality of life expressed as the RAND-36 physical (PCS) and mental (MCS) component summary scores,[8-10] adverse events, and cost-effectiveness of the treatment. The cost-effectiveness analysis assessed whether an improvement in quality of life, if present, would outweigh the anticipated increase in costs associated with AVS. The analysis was conducted from a health-care perspective and all health effects and costs that were incurred from the time of randomization to the end of follow-up were taken into account. Quality-adjusted life-years (QALYs) were based on the SF-6D, representing health state utilities derived from the RAND-36 that was measured at baseline, at 6 months, and at 12 months of follow-up. We constructed a cost-effectiveness acceptability curve, using a range of willingness-to-pay values for the gain of an extra QALY from €0 to €80 000. Details on data acquisition and analysis are provided in the supplemental data.

STATISTICAL ANALYSIS

The trial was designed to have a power of 80% to detect a difference between the two groups in daily defined dose of 0.8 (supplemental data). Assuming an SD of 1.8, 81 patients needed to be enrolled in each group (two-sided α of 0.05). Taking into account a potential dropout rate of 20%, we aimed to include 100 patients in each group. We analysed only patients who were eligible for the study, received the diagnostic intervention, and completed follow-up. Analysis was on an intention-to-diagnose basis, meaning that patients were analysed in the diagnostic group to which they had been randomly assigned; those assigned to AVS in whom AVS failed remained in the AVS group for analysis, even though their treatment was determined by CT scanning. Data are expressed as means and SDs or, in case of skewed distributions, as medians and IQRs. To assess significance of differences between CT-based and AVS-based treatments, we decided to use χ^2 test or Fisher's exact test for categorical data and unpaired t test and Mann-Whitney U test for continuous data with and without a normal distribution, respectively, when at baseline the two randomisation groups were comparable. For the assessment of health-related quality of life, missing data were imputed non-statistically according to the manual,[9] provided that at least 50% of subscale questions

had been answered. We made comparisons between baseline and final evaluation with a Wilcoxon signed rank test. A p value less than 0.05 was considered significant. We used IBM SPSS statistics 20 for Windows for statistical analysis and the R statistical package version 3.1.0 for cost-effectiveness analysis.

ROLE OF THE FUNDING SOURCE

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

From July 6, 2010, to May 30, 2013, 275 patients met our inclusion criteria after screening, of whom 41 declined to participate and 34 met exclusion criteria. Four patients were found to be ineligible after randomisation and 12 patients did not complete the study (figure). 184 patients, of whom 92 were randomised to CT and 92 to AVS, completed the follow-up period and were included in the intention-to-diagnose analysis. Baseline characteristics were similar between the patients allocated to CT and those allocated to AVS (table 1). Last follow-up was completed on Aug 25, 2015.

In the CT group, CT indicated unilateral right-sided enlargement in 12 (12%) of 98 patients, unilateral left-sided enlargement in 39 (40%), bilateral enlargement in 22 (22%), and bilaterally normal glands in 25 patients (26%). Central and local CT conclusions were discordant in 14 patients. In three of these cases, consensus was reached between the assessing radiologists, in one case the patient underwent adrenalectomy based on the local CT diagnosis, and in ten cases the treating physician opted for treatment with mineralocorticoid receptor antagonist, on the basis of local radiology report or because of uncertain diagnosis.

In the AVS group, AVS was successful in 92 (96%) of the 96 procedures (supplemental data). The four unsuccessful procedures were due to failure of cannulation of the right adrenal vein. 22 successful procedures showed lateralisation of aldosterone production to the right

and 26 to the left (supplemental data). In the four unsuccessful procedures, CT indicated unilateral disease in two patients and bilateral disease in the other two. 45 (50%) of 90 patients with both conclusive CT and AVS had discordant results (supplemental data). We did adrenalectomy in 50 patients in the AVS group and 49 patients in the CT group using a transperitoneal (n=64) or retroperitoneal (n=35) endoscopic approach.

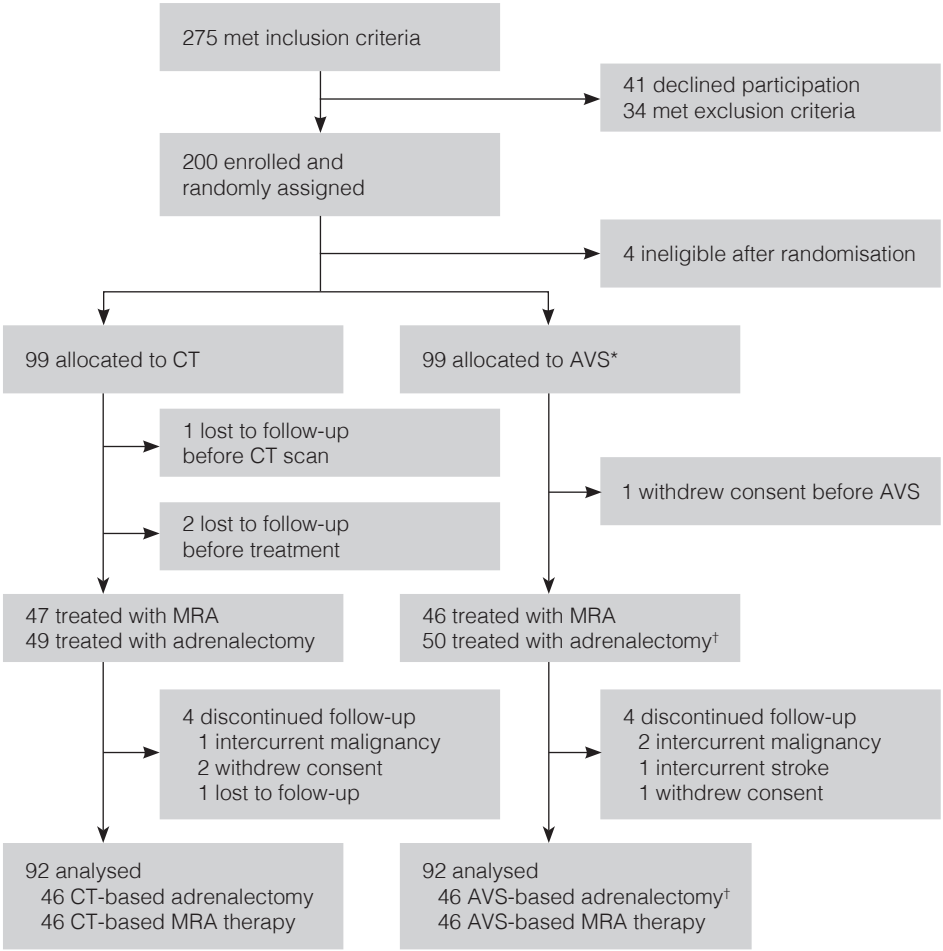


Figure. Trial profile

Reasons for declining participation are shown in the supplemental data. Reasons for ineligibility after randomisation were: suspicion of adrenal carcinoma (n=1) and suspicion of secondary hyperaldosteronism (n=3, violation of protocol). AVS=adrenal vein sampling. MRA=mineralocorticoid receptor antagonist. *AVS was successful in 92 of 96 sampled patients. Four patients with unsuccessful procedures were treated on the basis of outcomes from pre-AVS CT and were included in the AVS group in the intention-to-diagnose analysis. Of these four patients, one discontinued follow-up because of melanoma. †In one patient, partial adrenalectomy was performed at the patient's request.

Table 1. Patient Baseline Characteristics

	CT (n=92)	AVS (n=92)
Male – no. (%)	69 (75%)	75 (82%)
Age – year*	53.1±9.4	53.1±9.7
BMI – kg/m2*	28.4±4.1	29.5±4.7
Residence: Netherlands/Poland	76/16	74/18
Hypokalemia (< 3.5 mmol/l) or K+ suppletion	63 (69%)	63 (69%)
History of uncontrolled HT with ≥ 2 antihypertensives	66 (72%)	74 (80%)
Systolic /diastolic ABP 24-hrs – mmHg	143 (129-155)/89 (82-98)	148 (133-161)/89 (84-98)
Systolic /diastolic ABP day – mmHg	147 (134-158)/92 (85-100)	153 (135-165)/92 (86-99)
Systolic /diastolic ABP night – mmHg	135 (121-149)/82 (74-91)	137 (123-149)/84 (75-91)
DDD	3.0 (1.1-4.0)	3.0 (2.0-4.0)
No. of antihypertensive drugs	2.0 (1.0-3.0)	2.0 (1.0-2.0)
Serum Sodium – mmol/l	142 (140-143)	142 (140-143)
Serum Potassium – mmol/l	3.5 (3.2-4.0)	3.5 (3.2-3.8)
Serum Creatinine – µmol/l	83.0 (70.2-101.8)	84.0 (71.8-94)
Plasma Aldosterone – pmol/l	645 (442-943)	685 (500-1178)
Plasma Direct Renin Concentration – mU/l (n= 55/n=53)	3.5 (3.0-6.2)	4.2 (3.0-7.9)
Plasma Renin Activity – µg/l/hr (n=37/n=39)	0.3 (0.2-0.6)	0.3 (0.1-0.4)
Post SLT Plasma Aldosterone – pmol/l (n=80/n=80)	418 (282-665)	406 (304-775)
Post SLT Urinary Aldosterone – nmol/24hr (n=12/n=12)	119 (83-158)	99 (63-147)
RAND-36 PCS	48.1 (38.0-53.8)	48.2 (37.2-54.7)
RAND-36 MCS	49.2 (39.8-54.4)	47.3 (34.4-53.1)

Data presented as median and interquartile range (unmarked) or mean±SD (*). Conversion to conventional units: Sodium mmol/l to mEq/l conversion factor 1.0; Potassium mmol/l to mEq/l conversion factor 1.0; Creatinine µmol/l to mg/dl conversion factor 0.0113; Aldosterone pmol/l to ng/dl conversion factor 0.03605. Aldosterone urine nmol/24hr to µg/24hr conversion factor 0.3605. Plasma Renin Activity µg/l/hr to ng/ml/hr conversion factor 1.0. Plasma Direct Renin concentration mU/l to ng/l conversion factor 0.635. AVS = Adrenal Vein Sampling; BMI = Body Mass Index. ABP = Ambulatory Blood Pressure. DDD = Defined Daily Dosage; SLT = Salt Loading Test; PCS = physical component summary score; MCS = mental component summary score.

46 patients with AVS-based adrenalectomy and 46 patients with CT-based adrenalectomy completed 1-year follow-up and were included in the analysis (figure).

93 patients (47 CT and 46 AVS) diagnosed with bilateral disease started on mineralocorticoid receptor antagonist therapy, 75 on spironolactone and 18 on eplerenone. During follow-up, 27 patients switched from spironolactone to eplerenone. Complete follow-up was obtained from 46 patients with AVS-based therapy and 46 patients with CT-based mineralocorticoid receptor antagonist therapy (figure).

For the primary endpoint, we found no difference in medication use (neither in daily defined doses nor in number of medications) between patients managed on the basis of either CT results or AVS results (median daily defined dose, 3.0 [IQR 1.0–5.0] for CT vs. 3.0 [1.1–5.9] for AVS, p=0.52; median number of medications, 2 [IQR 1–3] for CT vs. 2 [1–3] for AVS, p=0.87; table 2). Mean blood pressure and the number of patients reaching target blood pressure at final assessment were also similar between patients managed on the basis of CT and those managed on the basis of AVS (table 2).

Serum potassium did not differ between the two diagnostic groups at final assessment (table 2). Three patients had persistent hypokalemia, two during treatment with mineralocorticoid receptor antagonist and one after CT-based adrenalectomy. Salt-loading tests in operated patients showed suppressed aldosterone in 56 (61%) patients, indeterminate test results in 25 (27%), and unsuppressed aldosterone in 10 (11%; table 3). One patient with persistent postoperative hypoaldosteronism did not undergo a salt-loading test. Of 25 cases with indeterminate test results, four patients were judged to have persistent primary aldosteronism as determined by consensus (supplemental data). This resulted in a total of 14 (15%) patients with persistent primary aldosteronism. Of these, five patients had been diagnosed by AVS and nine by CT (p=0.25).

Response rates for the RAND-36 questionnaires were 96% (88 patients) for CT and 92% (85 patients) for AVS at baseline, and 96% (87 patients) for CT and 95% (88 patients) for AVS at 1-year follow-up. We did not note any differences in RAND-36 PCS or MCS between the CT group and the AVS group at baseline or final assessment (tables 1, 2).

During the study, 346 adverse events were reported in 131 patients, of which 21 were

serious adverse events (supplemental data). The number of patients experiencing adverse events or serious adverse events did not differ between the CT and AVS group (nine serious adverse events and 150 adverse events in the CT group vs. 12 serious adverse events and 175 adverse events in the AVS group). The most commonly reported adverse events were medication side-effects such as gynaecomastia in mineralocorticoid receptor antagonist treatment.

Mean total costs per patient were €6746 for the AVS group and €4228 for the CT group, with a mean difference of €2285 (supplemental data). A non-significant difference of 0.05 QALY (95% CI –0.04 to 0.13) was found to the advantage of patients in the AVS group, resulting in an incremental cost-effectiveness ratio of €45 700 per QALY. At a willingness-to-pay value of €30 000 per QALY, the probability that AVS as compared with CT constitutes an efficient use of health-care resources in the diagnostic work-up of patients with primary aldosteronism was less than 0.2 (supplemental data).

We also did post-hoc analyses. Baseline data (not shown) and primary and secondary endpoints did not differ between the AVS group and the CT group when patients who received adrenalectomy and those who received mineralocorticoid receptor antagonist treatment were analysed separately (table 2). Per-protocol analyses excluding all patients with a failed AVS showed no clinically relevant differences in the primary or secondary endpoints (supplemental data). Increasing the AVS selectivity index from 3.0 to 5.0 in a per-protocol analysis also did not change the study outcomes (supplemental data).
In the adrenalectomy group, characteristics did not differ between the 14 patients with persistent aldosteronism and the 78 with resolved aldosteronism. All five patients with persistent aldosteronism in the AVS group had discordant results with the preceding CT (supplemental data). At final assessment, 49 of 92 patients of the mineralocorticoid receptor antagonist group used spironolactone and 43 patients used eplerenone. Eplerenone was as frequently used in the CT group as in the AVS group (20 [43%] patients vs. 23 [50%] patients, p=0.53). Intensity of therapy with mineralocorticoid receptor antagonist and non-mineralocorticoid receptor antagonist antihypertensives was similar in the CT and AVS group (mineralocorticoid receptor antagonists, median daily defined dose 1.3 [IQR 1.0–2.8] vs. 2.7 [1.3–4.0], p=0.07; non-mineralocorticoid receptor antagonists, 2.0 [0.3–4.0] vs. 2.3 [1.0–5.0], p=0.34). Primary or secondary endpoints between CT and AVS did not differ in patients younger than 40 years or those aged 40 years or older (supplemental data).

Table 2. Outcome at one year follow-up for the total cohort, and for the patients treated by adrenalectomy or MRA separately.

	Total cohort			Adrenalectomy			MRA		
	CT (n=92)	AVS (n=92)	p-value	CT (n=46)	AVS (n=46)	p-value	CT (n=46)	AVS (n=46)	p-value
DDD	3.0 (1.0-5.0)	3.0 (1.1-5.9)	0.52	1.2 (0-3.0)	1.2 (0-3.0)	0.42	4.0 (2.3 – 6.7)	5.7 (3.4-8.7)	0.05
No. of antihypertensive drugs	2 (1-3)	2 (1-3)	0.87	1 (0-2)	1 (0-2)	0.31	2 (2-3)	3 (2-4)	0.39
sABP / dABP 24-hrs - mmHg	127(120-138) /80(75-86)	128(121-135) /81(76-85)	0.93/0.76	129(121-141) /82(76-87)	128(121-137) /81(77-85)	0.53/0.98	125(120-135) /80(74-86)	128(122-133) /81(75-85)	0.57/0.71
sABP / dABP day - mmHg	131(124-141) /83(77-89)	131(124-138) /84(78-88)	0.89/0.91	133(123-143) /83(78-91)	132(124-139) /84(79-88)	0.48/0.84	128(124-142) /84(77-89)	130(125-138) /84(77-87)	0.60/0.97
sABP / dABP night - mmHg	117(109-131) /71(65-78)	120(112-127) /72(68-79)	0.54/0.29	116(111-131) /71(66-80)	120(112-127) /72(69-81)	0.92/0.41	117(109-128) /72(65-78)	119(112-128) /73(68-77)	0.53/0.53
No. at target day ABP (%)[#]	39 (42.9%)	41 (44.6%)	0.82	18 (40%)	20 (43.5%)	0.74	21 (46%)	21 (46%)	1.00
Potassium – mmol/l	4.3 (4.0-4.6)	4.2 (4.0-4.6)	0.72	4.3(3.9-4.6)	4.2 (4.0-4.4)	0.48	4.3 (4.0-4.6)	4.4 (4.1-4.6)	0.82
RAND-36 PCS [§]	52.7 (43.9-56.8)	53.2 (44.0-56.8)	0.83	54.3 (45.2-58.2)	53.9 (46.7-57.8)	0.87	50.9 (40.7-56.4)	51.7 (41.4-56.2)	1.00
RAND-36 MCS [§]	49.8 (43.1-54.6)	52.7 (44.9-55.5)	0.17	53.8 (48.8-55.8)	50.8 (45.1-56.1)	0.42	51.1 (41.7-55.0)	49.0 (42.1-53.3)	0.35

Data presented as median and interquartile range. There were no significant differences between the AVS-group and the CT-group in the total cohort, nor within the two treatment modalities.

[#] Target daytime ABP: <135/85 mmHg. Fourteen patients reached target daytime ABP without the use of any antihypertensive drugs: 10 after AVS-based adrenalectomy (22%) and 4 after CT-based adrenalectomy (9%, p=0.08). Conversion to conventional units: Potassium mmol/l to mEq conversion factor 1.0.

[§] Dutch and Polish patients combined. In a subgroup analysis of Dutch patients only (n=150) we found no statistically significant differences in RAND-36 physical and mental health summary scores between CT-group and AVS-group at baseline or final evaluation (data not shown).

MRA=mineralocorticoid receptor antagonist; AVS = Adrenal Vein Sampling; BMI = Body Mass Index; sABP = Systolic Ambulatory Blood Pressure; dABP = Diastolic Ambulatory Blood Pressure; DDD = Defined Daily Dosage. PCS = physical component summary score; MCS = mental component summary score .

Table 3. Biochemical outcome after adrenalectomy

	CT (n=46)	AVS (n=46)	p-value
Potassium (mmol/l)	4.3(3.9-4.6)	4.2 (4.0-4.4)	0.48
Plasma Aldosterone (pmol/l)	230 (150-360)	260 (170-360)	0.35
Plasma Direct Renin – mU/l (n=34/n=38)	14.0 (9.2-20.3)	14.1 (9.1-22.1)	0.85
Plasma Renin Activity – µg/l/hr (n=10/n=6)	0.63 (0.38-1.94)	2.74 (1.68-4.05)	0.06
Post SLT Plasma aldosterone – pmol/l (n=41/n=40)*	120 (71-175)	112 (73-158)	0.80
Post SLT Urine aldosterone – nmol/24hr (n=5/n=5)	22.0 (12.9-46.5)	26.0 (12.5-35.0)	1.00
Post SLT aldosterone*			
suppressed	24 (52%)	32 (71%)	
indeterminate	17 (37%)	8 (18%)	0.10
not suppressed	5 (11%)	5 (11%)	
Biochemical outcome			
persistent primary aldosteronism	9 (20%)	5 (11%)	0.25
resolved aldosteronism	37 (80%)	41* (89%)	

Data presented as median and interquartile range. There were no significant between-group differences.

*One patient in the AVS-group did not undergo SLT because of persistent hypoaldosteronism. He was considered as having resolved aldosteronism.

Post SLT aldosterone: suppressed: plasma aldosterone <140 pmol/l (<5.0 ng/dl) or 24-hr urine aldosterone <27.7 nmol/24hr (<10.0 µg/24hr); indeterminate: plasma aldosterone 140-280 pmol/l (5.0-10.0 ng/dl) or 24-hr urine aldosterone 27.7-38.8 nmol/24hr (10.0–14.0 µg/24hr); elevated: plasma aldosterone >280 pmol/l (>10 ng/dl) or 24-hr urine aldosterone >38.8 nmol/24hr (>14.0 µ g/24hr).

Conversion to conventional units: Aldosterone pmol/l to ng/dl conversion factor 0.03605. Aldosterone urine nmol/24hr to µg/24hr conversion factor 0.3605. Plasma Renin Activity µg/l/hr to ng/ml/hr conversion factor 1.0. Plasma Direct Renin concentration mU/l to ng/l conversion factor 0.635.

AVS = Adrenal Vein Sampling; SLT = Salt Loading Test.

In the combined group of CT and AVS patients who underwent adrenalectomy, health-related quality-of-life summary scores improved significantly between baseline and 1-year follow-up after adrenalectomy (median PCS from 47.2 [IQR 37.7–54.8] to 54.2 [46.2–58.0], p<0.0001; MCS from 47.1 [32.4–53.9] to 53.1 [46.1–55.9], p<0.0001; supplemental data). After treatment with mineralocorticoid receptor antagonist, both summary scores did not improve significantly (PCS from 48.4 [38.0–54.6] to 51.2 [41.3–56.3], p=0.08; MCS from 49.2 [40.8–53.8] to 49.8 [41.8–54.5], p=0.10; data shown graphically in supplemental data). Patients treated with adrenalectomy scored significantly higher on PCS (p=0.04) and MCS (p=0.02) at 12-month assessment compared with patients treated with mineralocorticoid receptor antagonist.

DISCUSSION

In this randomised diagnostic trial, we were unable to demonstrate any statistically significant or clinically meaningful difference in outcome between AVS-guided and CT-guided management of patients with primary aldosteronism. To our knowledge this is the first prospective, randomised diagnostic study in primary aldosteronism. Our study has several strong features, such as the selection of a primary endpoint that is highly relevant for patients with hypertension. Moreover, we used ambulatory blood pressure monitoring, the most objective measurement method for blood pressure, to assess treatment response.[12] Additionally, the study was done in patients with primary aldosteronism not selected for unilateral disease, by contrast with most previous retrospective studies. We also performed the AVS procedures according to accepted protocols[1,13,14] and achieved a high success rate of bilateral adrenal vein cannulation of 96%.

Our results challenge the current recommendation of the Endocrine Society guideline to perform AVS in all patients with primary aldosteronism to select those who may benefit from surgery.[1] The studies on which this recommendation was based were observational and retrospective, with ill-defined selection criteria for AVS and treatment based only on AVS.[6,15,16] Additionally, in these studies the clinical benefit of the AVS-based strategy was not rigorously assessed.[7] In our secondary endpoint, persistent versus resolved primary aldosteronism, we found a non-significant trend in favour of AVS. This trend was also present when the number of patients that reached normotension without medication was assessed. In a larger cohort, this difference might become statistically significant. However, the question is whether it would be clinically relevant, because the magnitude of the difference is very small. We cannot exclude that specific subgroups of patients such as those with bilaterally normal or enlarged adrenals could benefit from AVS, but we cannot ascertain this due to the design of our study.

Our findings suggest that both CT and AVS are imperfect tests to identify patients who might benefit from adrenalectomy, but each is imperfect for largely unknown reasons. CT may fail for obvious reasons such as restricted detection limit, resolution and specificity, and substantial interobserver variation. The asymmetric distribution of right-sided and left-sided adrenal enlargement (12 right side, 38 left side enlargement) might indicate false-negative

results in the right adrenal gland, or false-positive results in the left adrenal gland. This disparity might result from physiological size difference between both adrenal glands (in favour of the left gland), or the effect of patient sex and weight on adrenal size.[17] Our findings suggest that the criterion of 7 mm or larger might be too low for the left adrenal gland and that cut-off values should be balanced for sex and bodyweight.

Challenges in interpreting results from AVS include multiple vein drainage, selective cannulation of contributory veins not draining an aldosterone-producing adenoma, or asymmetrical cortisol secretion.[18,19] Additionally, several other AVS procedure-related factors, such as use of cosyntropin,[20,21] sequential or simultaneous sampling of adrenal veins,[22] or varying criteria for selectivity and lateralisation[23,24] can affect AVS conclusions.[8,25] In the AVS group we observed a nearly 50% discordance between the diagnostic conclusions derived from the CT and AVS, similar to the results of our systematic review.[7] This finding in the context of identical rates of adrenalectomy and similar outcomes in the CT and the AVS group suggests that both methods identify different patients amenable to adrenalectomy. However, the biological mechanisms that underlie these findings are not yet clear. Recent data, suggesting that adrenocortical aldosterone production could be multifocal and even bilateral, might provide a clue for such a mechanism.[26,27]

Our study also has some limitations. The results may not apply to AVS procedures without cosyntropin stimulation or with different cut-off values used for selectivity and lateralisation. Additionally, although we did not deviate from the current guideline, we did not do dexamethasone suppression tests in all patients to exclude subclinical hypercortisolism.[28] However, the prevalence of synchronous primary aldosteronism and unilateral adrenal hypercortisolism is low and it is therefore unlikely that this affected our results.[29] Finally, our study does not allow conclusions about long-term cardiovascular mortality and morbidity. Normalisation of blood pressure and aldosterone, however, are the most practical and best proxy outcomes to assess the clinical value of management decisions. Given the similar results for blood pressure and aldosterone levels in both arms of the study after 1-year follow-up, differences in long-term cardiovascular outcome are not to be expected.

In conclusion, treatment of primary aldosteronism on the basis of CT or AVS did not show significant differences in clinical benefits for patients after 1 year of follow-up. In the diagnostic work-up of patients with primary aldosteronism, AVS results in extra health-care costs that cannot be justified by proportional improvements in the quality of life of

these patients. These findings challenge the recommendation to do AVS in all patients with primary aldosteronism. Neither AVS nor CT should be considered as gold standard tests for identifying aldosterone-producing adenoma in all patients with primary aldosteronism.

CONTRIBUTORS

JD, JWML, G-JvdW, and ARMMH designed the study. TD, JD, JWML, AP, SK-K, WS, MNK, AHvdM, B-JvdB, and ARMMH contributed to patient enrolment. HJMMG generated the random allocation sequence. TD, JD, JWML, MA, LJSK, JFL, FCGJS, ARMMH, AP, SK-K, JK, AJ, WS, MNK, and AHvdM collected the data. TD, JD, MV, AFL-N, PM, AP, JWML, and HJMMG analysed the data. TD, JD, JWML, and G-JvdW wrote the first draft of this report. All authors made critical revisions of the manuscript. A complete list of contributors is provided in the supplemental data.

DECLARATION OF INTERESTS

We declare no competing interests.

ACKNOWLEDGMENTS

This study was supported by a grant from the Netherlands Organisation for Health Research and Development–Medical Sciences 2010–2012 E&K (171002102) to JD and by a grant from the Institute of Cardiology, Warsaw, Poland (2.13/VII/12) to AP and AJ. Grant providers had no influence on the content of the manuscript.

Research in context

Evidence before this study

The best available treatment for primary aldosteronism is adrenalectomy if a unilateral aldosterone-producing adenoma is diagnosed. Detection of an aldosterone-producing adenoma is usually by CT scan or adrenal vein sampling (AVS). In recent years AVS—a difficult, expensive, and not widely available technique—has emerged as the reference standard for primary aldosteronism subtyping. A systematic review showed discordance between the diagnosis based on CT scan and on AVS in almost 40% of cases. It also showed that the evidence supporting the preference of AVS over CT scan is limited.

Added value of this study

Our study is the first prospective, randomised diagnostic study in primary aldosteronism to compare CT-based and AVS-based management. We were unable to demonstrate any statistically significant or clinically and economically meaningful difference in outcome between AVS-guided and CT-guided management of patients with primary aldosteronism. Our findings also indicate that both CT and AVS are imperfect tests to identify patients that may benefit from adrenalectomy.

Implications of all the available evidence

This study challenges the recommendation to perform AVS in all patients with primary aldosteronism. Centres with only CT scan facilities may obtain treatment results in their primary aldosteronism patients that are similar to centres that have access to AVS. Because there is room for improvement of both diagnostic strategies, better ways of selecting patients for adrenalectomy are needed.

SUPPLEMENTAL DATA

LISTING OF INVESTIGATORS PARTICIPATING IN DE SPARTACUS TRIAL

RADBOUD UNIVERSITY MEDICAL CENTER, NIJMEGEN, THE NETHERLANDS

Department of Internal Medicine, Division of Vascular Medicine: T. Dekkers, J.W.M. Lenders, J. Deinum, J.M.M. Booij Liewes, A.J.C. Hendriks, B.D.C. Pouwels

Department of Internal Medicine, Division of Endocrinology: A.R.M.M Hermus, M.S. Velema, H.J.L.M. Timmers

Department of Radiology: M. Arntz, L. Schultze Kool

Department of Urology: J.F. Langenhuijsen

Department of Laboratory Medicine: F.C.G.J. Sweep

Department of Health Evidence: G.J. van der Wilt, H. J.M.M. Groenewoud

INSTITUTE OF CARDIOLOGY, WARSAW, POLAND

Department of Hypertension: A. Prejbisz, S. Kołodziejczyk-Kruk, A. Januszewicz

Department of Interventional Cardiology and Angiology: J. Kądziela

UNIVERSITY MEDICAL CENTER UTRECHT, UTRECHT, THE NETHERLANDS

Department of Vascular Medicine: W. Spiering C.A. Flint, C.A.M. Joosten

Department of Nephrology: M.B. Rookmaaker

UNIVERSITY MEDICAL CENTER GRONINGEN, GRONINGEN, THE NETHERLANDS

Department of Endocrinology: M.N. Kerstens

ERASMUS MEDICAL CENTER, ROTTERDAM, THE NETHERLANDS

Department of Internal Medicine, Division of Pharmacology and Vascular Medicine: A.H. van den Meiracker, E. Jäger.

ACADEMIC MEDICAL CENTER, AMSTERDAM, THE NETHERLANDS

Department of Internal and Vascular Medicine: B.J. van den Born, M. Cammenga

MAASTRICHT UNIVERSITY MEDICAL CENTER, MAASTRICHT, THE NETHERLANDS

Department of Internal Medicine: F. Vanmolkot

VU MEDICAL CENTER, AMSTERDAM, THE NETHERLANDS

Department of Internal Medicine: A.J. Kooter

ZIEKENHUISGROEP TWENTE, ALMELO AND HENGELO, THE NETHERLANDS

Department of Nephrology: A.J. Woittiez

AMPHIA HOSPITAL, BREDA, THE NETHERLANDS

Department of internal medicine: S. van Thiel, M.H.W. Kappers

ONZE LIEVE VROUWE GASTHUIS, AMSTERDAM, THE NETHERLANDS

Department of Internal Medicine: S. van Wissen, P.S. van Dam

FLEVOZIEKENHUIS, ALMERE, THE NETHERLANDS

Department of Internal Medicine: M. ten Wolde, N. Smit

MEDICAL CENTER LEEUWARDEN, LEEUWARDEN, THE NETHERLANDS

Department of Internal Medicine: P.H.N. Oomen

INVESTIGATOR CONTRIBUTIONS

Study design: J. Deinum, J.W.M Lenders, G.J. van der Wilt, A. Hermus.

Patient enrolment: T. Dekkers, J. Deinum, J.W.M. Lenders, A. Prejbisz, S. Kołodziejczyk-Kruk, W. Spiering, M.N. Kerstens, A.H. van den Meiracker, B.J. van den Born, A.R.M.M Hermus, A.J. Woittiez, M.B. Rookmaaker, S. van Thiel, M.H.W. Kappers, F. Vanmolkot, A. J. Kooter, M. ten Wolde, N. Smit, P.H.N. Oomen, S. van Wissen and P.S. van Dam.

Generation of random allocation sequence: H. Groenewoud

Data collection: T. Dekkers, J. Deinum, J.W.M. Lenders, J.M.M. Booij Liewes, A.J.C. Hendriks, B.D.C. Pouwels, M. Arntz, L.J. Schultze Kool, H. Langenhuijsen, F.C.G.J. Sweep, A.R.R.M. Hermus, H.J.L.M. Timmers, A. Prejbisz, S. Kołodziejczyk-Kruk, J. Kądziela, A. Januszewicz, W. Spiering, C. Flint, C.A.M. Joosten, M.N. Kerstens, A.H. van den Meiracker, E. Jäger, B.J. van den Born, M. Cammenga, A.R.M.M. Hermus, A.J. Woittiez, M.B. Rookmaaker, S. van Thiel, M.H.W. Kappers, F. Vanmolkot, A.J. Kooter, M. ten Wolde, N. Smit, P.H. N. Oomen, S. van Wissen and P.S. van Dam.

Data analyses: T. Dekkers, J. Deinum, M.S. Velema, A. Prejbisz, J.W.M. Lenders, H.J.M.M. Groenewoud

First draft of the manuscript: T. Dekkers, J. Deinum, J.W.M. Lenders, G.J. van der Wilt

All authors made critical revisions of the manuscript.

All sources of funding are acknowledged in the main manuscript.

EXTENDED METHODS

SAMPLE SIZE CALCULATION

The power of this study to detect a difference in antihypertensive medication use, expressed as defined daily dosage (DDD), is 80%, based on the following assumptions: aldosterone-producing adenoma (APA) is diagnosed by adrenal vein sampling (AVS) in 66% of the cases (based on previous research). APA is diagnosed by CT-scan in 56% of the cases. With AVS as the reference, the CT-scan result is incorrect in 33% of the cases (i.e. of the 56% APA diagnosis, 37.4% is correct and 18.6% is incorrect).[7,30] Antihypertensive medication use after one year of follow-up, expressed as DDD (mean +/-SD), is 4.4 +/- 1.8 in patients with BAH and 1.7 +/- 1.8 in patients that have been operated for APA.[30] We assumed that biochemical failure of operation results in the same use of medication as in cases with BAH. Mean number of DDD after one year follow-up in the CT group is therefore (37.4x 1.7 + 62.6x4.4)/100 = 3.4 and in the AVS group (66x1.7 + 34x4.4)/100 = 2.6. Calculation of the sample size (s=1.8; δ=0,8; α=0,05; β=0,2), with a two-sided significance level of 0.05, indicates a required total sample size of 162 patients, 81 in each group. To account for a ~20 % drop out rate we aimed at a sample size of 200 patients.

DIAGNOSTIC CONFIRMATION TEST

No diagnostic screening test was mandatory before performing a diagnostic confirmation test. The diagnosis of primary aldosteronism was confirmed by salt loading test (SLT) by means of an intravenous saline infusion test (n=160) with measurement of plasma aldosterone or oral salt loading test (n=12) with measurement of urine aldosterone according to the Endocrine society guidelines.[1] One centre used a 3-day intravenous salt loading test (n=12) infusing 2L of NaCl 0.9% per 24hr with measurement of 24 hr urine aldosterone excretion on day three.[31,32] Prior to the tests we stopped antihypertensive medication, with the exception of calcium channel blockers, doxazosin or hydralazine. We prescribed oral potassium chloride in case of hypokalemia to reach a potassium level of at least 3.5 mmol/L. The intravenous saline infusion test was considered positive in case of insufficient suppression (post-infusion aldosterone >280pmol/l (>10.0 ng/dl). In case aldosterone was between 140 - 280 pmol/l (5.0 – 10.0 ng/dl), the test was considered indeterminate and the test was repeated or the patient was discussed in a multidisciplinary board to decide on the presence or absence of aldosteronism (n=28). In case of an oral salt loading test or 3-day intravenous salt loading test with urinary aldosterone measurement tests were considered adequate when urinary sodium excretion exceeded 200mmol/24h (200 mmol/24h). Urinary aldosterone levels <27.7 nmol/24hr (<10.0 µg/24hr), 27.7-38.8 nmol/24hr (10.0 – 14.0 µg/24hr) and >38.8 nmol/24hr (>14.0 µg/24hr) were considered as a test with adequate, indeterminate and insufficient suppression of aldosterone, respectively.

ALLOCATION OF DIAGNOSTIC STRATEGY

We randomised patients for either adrenal CT-scanning or AVS using a web-based application with an algorithm stratified by study centre and minimised for gender, age, blood pressure and intensity of antihypertensive medication (Defined Daily Dosage (DDD)). The following variables were divided into classes: antihypertensive medication: ≤3.5 DDD or >3.5 DDD, age: ≤50 years or >50 years; blood pressure: ≤135/85 mmHg or > 135/85 mmHg.

CT-SCAN

We performed CT-scans with a 64-row multidetector CT-scanner, with reconstruction on 1 mm slices and with the following parameters: 32 x 0.6mm detector, 120kVp, 200-250mAs (effective), 370 msec rotation time. Reconstructions of 0.75 x 0.5 mm en 3 x 3 mm were performed.[33] In case of an attenuation of less than 10HU in a lesion smaller than 4cm on unenhanced images, we diagnosed an adenoma. For lesions with attenuation of more than

10HU we performed contrast series with 100ml of intravenous contrast (300mg/ml) with a flow of 4ml per second. We used bolus tracking with a 100HU threshold and a post-threshold delay of 40 seconds, resulting in a delay of 60 seconds after injection. CT-images were acquired 60 seconds and 15 minutes after contrast infusion. We used an absolute percentage washout > 60% or a relative percentage washout > 40% as a cut-off to diagnose an adenoma.[33]

ADRENAL VEIN SAMPLING

Interfering antihypertensive agents were stopped before the AVS procedure with an interval of 4 to 6 weeks for mineralocorticoid receptor antagonists and potassium sparing diuretics, and 2 weeks for ACE-inhibitors, angiotensin receptor-blockers, diuretics, and beta-blockers.[1] In case of uncontrolled hypertension, treatment with calcium-blockers, doxazosin or hydralazine was allowed during diagnostic work-up. We admitted patients the day prior to AVS for timely correction of hypokalemia if present. Potassium was corrected orally or intravenously to reach a serum potassium level ≥ 3.5 mmol/l. AVS was performed after at least three hours of recumbent position. We performed AVS under continuous cosyntropin stimulation of 50µg/hr started 30 minutes before the procedure. The adrenal veins were catheterised by a percutaneous femoral vein approach. Catheter tip position was confirmed by injection of a small amount of contrast. Blood samples were obtained sequentially by gravity or gentle negative pressure. During the procedure correct catheter position was verified by cortisol measurements. In case of incorrect catheter position new samples were obtained within the same sampling procedure. Formulas and cut-offs for selectivity and lateralisation are detailed below.

Formulas and cut-offs for selectivity, lateralisation and suppression index in AVS

	Formula	Cut-off
Selectivity index	$\text{Cortisol}_{\text{adrenal vein}} / \text{Cortisol}_{\text{iliac vein}}$	≥ 3.0
Lateralisation index	$\frac{[\text{Aldosterone}_{\text{dominant}}/\text{Cortisol}_{\text{dominant}}]}{[\text{Aldosterone}_{\text{non-dominant}}/\text{Cortisol}_{\text{non-dominant}}]}$	≥ 4.0
Suppression index	$\frac{[\text{Aldosterone}_{\text{non-dominant}}/\text{Cortisol}_{\text{non-dominant}}]}{[\text{Aldosterone}_{\text{iliac vein}}/\text{Cortisol}_{\text{iliac vein}}]}$	≤ 1.0

HEALTH-RELATED QUALITY OF LIFE

We measured HRQL using the Dutch or Polish version of the RAND-36. This questionnaire consists of 8 subscales: physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, and mental health. The Short Form-36 (SF-36) is a general HRQL instrument very similar to the RAND-36. The SF-36 provides summary scales for overall physical and mental health.[10] Considering the similarity of the two questionnaires, these can also be applied to the RAND-36. We calculated z-scores for each subscale using means and standard deviations from a Dutch reference population.[9] Subsequently the physical and mental summary scores were constructed with the use of utility weights for the Dutch population of these different subscales, according to *Ware et al*.[10,34] In this norm-based method, the scores are standardised to a mean of 50 and a SD of 10 (higher scores indicate better health status). As no Polish reference values or population utility weights were available, we performed an additional subgroup analysis for the Dutch patients only (see legend Table 2).

EXTENDED RESULTS

Table S1. Screened patients not included in the study.

	Number of patients
Patient declined participation	
Unwilling to participate in clinical studies in general	4
Declined randomisation (Insisted on AVS)	6
Unable to participate because of personal circumstances	6
No reason given*	25
Subtotal	**41**
Exclusion criteria met	
Declined to undergo AVS	13
Declined to undergo surgery	14
Severe comorbidity	2
Requirement of medication interfering with study protocol	1
Suspicion of adrenal carcinoma	1
Pregnancy	1
Regarded unsuitable for participation by clinician	2
Subtotal	**34**
Total	**75**

* Patients had the right to decline participation without specification.

Table S2 AVS procedures performed in the medical centres

Medical Centre	Number of AVS performing radiologists	Number of AVS	Success rate
Radboud University Medical Center	3	72	70 (97%)
Institute of Cardiology in Warsaw	1	18	16 (89%)
Medical Center Groningen	1	6	6 (100%)

Table S3. Selectivity index and lateralisation index of selective AVS procedures (N=92)

Selectivity index RAV (n=92)	26.4 (16.9-34.4)
Selectivity index LAV (n=92)	14.3 (10.8-19.5)
Selectivity index RAV 3.0 – 5.0 / ≥ 5.0	3 (3%) / 89 (97%)
Selectivity index LAV 3.0 – 5.0 / ≥5.0	2 (2%) / 90 (98%)
Lateralisation index dominant RAV (n=22)	16.6 (9.5-37.3)
Suppression index non-dominant LAV (n=22)	0.30 (0.14-0.52)
Lateralisation index dominant LAV (n=26)	15.3 (8.7-29.5)
Suppression index non-dominant RAV (n=26)	0.22 (0.11-0.38)

Data are presented as median and interquartile range. In one patient in whom the selectivity index was just below the cut-off AVS was regarded selective based on former hospital guidelines and the patient was treated according to the AVS result. RAV = Right Adrenal Vein; LAV = Left Adrenal Vein. See supplemental methods for the formulas for selectivity index, lateralisation index and suppression index.

Table S4. Consensus on persistent or resolved PA in adrenalectomised patients with indeterminate aldosterone values after saline infusion test at 12 months

nr	M/F	age (yrs)	ABP 24h mmHg	ABP Day mmHg	ABP Night mmHg	DDD	No. Drugs	Na+ mmol/l	K+ mmol/l	Creat. µmol/l	SLT Potassium mmol/l	SLT aldo T=0 pmol/l	SLT aldo T=240 pmol/l	SLT renin T=0 mU/l	SLT renin T=240 mU/l	consensus conclusion on PA
SLT after AVS-based adrenalectomy																
29	M	48	137/80	137/80	-	0.0	0	141	4.4	106	4.2	169	188	12.1	8.1	resolved
89	M	48	137/93	142/98	128/85	0.2	1	140	4.8	111	4.2	219	241	28.3	19.1	resolved
138	F	43	131/91	134/84	120/71	0.0	0	-	3.6	70	3.9	269	249	11.1	7.0	resolved
147	M	56	124/81	130/85	116/73	0.0	0	-	4.0	-	4.3	260	150	6.0	5.1	resolved
187	M	70	141/90	146/93	126/81	2.3	2	137	4.1	89	4.1	391	161	16.1	13.0	resolved
193	M	68	129/77	129/79	128/71	6.5	3	141	4.5	76	4.6	377	177	15.1	8.0	resolved
31	M	47	131/88	136/93	123/82	0.0	0	143	3.7	79	Oral SLT: Urine sodium: 232 mmol/24hr. Urine aldosterone: 34.1 nmol/24hr Plasma aldosterone (No SLT): 302 pmol/l, plasma renin 23.1 mU/l					resolved
38	M	58	144/78	148/81	137/71	4.0	2	143	4.4	105	Oral SLT: Urine sodium: 432 mmol/24hr. Urine aldosterone: 35.5 nmol/24hr. Plasma aldosterone (No SLT): 183 pmol/l, plasma renin: 22.1 mU/l					resolved
SLT after CT-based adrenalectomy																
2	M	46	117/82	123/86	105/74	7.7	5	140	3.1	104	3.3	180	180	3.0	3.0	persistent
73	M	28	141/94	139/96	146/90	2.0	2	140	3.8	92	3.6	230	219	3.0	3.0	persistent
111	M	61	147/93	147/91	148/98	4.2	3	138	4.4	172	4.2	418	230	8.1	7.0	persistent
54	M	46	149/85	155/88	137/79	3.0	2	141	3.9	89	Oral SLT: Urine sodium 226 mmol/24hr. Urine aldosterone 31.9 nmol/24hr Plasma aldosterone (No SLT): 350 pmol/l, plasma renin 4.1 mU/l					persistent

>>

16	F	45	114/76	121/83	100/63	2.3	2	141	4.6	46	4.6	202	150	-	-	resolved
52	M	44	136/84	125/80	140/86	5.0	4	140	3.9	185	3.9	379	230	42.3	20.2	resolved
76	F	61	127/85	131/89	113/71	0.0	0	142	4.6	109	4.0	111	161	7.0	5.1	resolved
88	M	42	147/90	154/97	131/77	0.0	0	138	4.0	89	4.6	89	161	18.1	15.1	resolved
118	M	43	126/83	129/89	116/68	0.0	0	-	3.8	95	3.8	188	150	8.1	7.0	resolved
122	F	56	128/71	133/74	117/63	5.0	3	142	4.3	75	4.3	446	141	11.1	9.5	resolved
126	F	48	144/77	148/79	131/68	1.2	2	140	4.5	76	4.5	100	230	14.2	6.0	resolved
142	M	55	141/95	145/99	125/80	0.0	0	140	4.6	104	4.1	188	211	10.0	5.1	resolved
146	M	68	131/59	136/61	110/54	3.0	2	134	4.2	77	3.8	188	161	35.3	16.1	resolved
160	F	35	127/84	133/91	117/75	1.0	1	136	3.6	57	3.6	1568	150	6.2	2.4	resolved
162	M	51	140/76	144/78	122/65	0.0	0	139	4.2	70	4.0	368	169	16.1	12.1	resolved
182	M	57	120/78	123/80	114/71	1.0	1	139	3.9	76	3.9	449	139	3.8	1.4	resolved
195	M	58	133/89	141/95	102/65	2.0	2	138	4.6	148	4.8	241	150	19.1	14.2	resolved

Assessors (J.D., J.W.M.L., A.P., T.D.) were blinded for patient randomisation and baseline characteristics. Conversion to SI Units: Sodium mmol/l to mEq/l; Potassium mmol/l to mEq/l conversion factor 1.0; Creatinine µmol/l to mg/dl conversion factor 0.0113; Renin mU/l to pg/ml 0.63; Aldosterone pmol/l to ng/dl conversion factor 0.36, Aldosterone urine nmol/24hr to µg/24hr conversion factor 0.36.

Table S5. Adverse and Serious Adverse Events

	AVS-based treatment	CT-based treatment
Diagnostic phase		
SAE	Stroke	-
AE	-	Allergic skin reaction to contrast; Subcutaneous contrast injection
Adrenalectomy		
SAE	Ureter damage; Subtotal adrenalectomy requiring repeated surgery ; Post-operative pneumonia; Post-operative renal insufficiency; Post-operative hypokalemia	Conversion to open procedure with removal of 12th rib, complicated by wound infection; MRSA carrier status
AE	Transient post-operative hypokalemia (n=4); Sleep apnoea desaturations post-operatively	Wound infection; Transient post-operative hypokalemia; Pneumothorax ; Post-operative pneumonia; Conversion from retroperitoneoscopic to laparoscopic approach; Dental damage on extubation
Follow-up		
SAE	Stroke (n=3); Metastasised melanoma; Myelodysplastic syndrome; Hypoaldosteronism; Renal failure; Hypertensive urgency; Traumatic pneumothorax	Stroke; Coloncarcinoma (n=2); Hypertensive urgency; Suspicion gastric malignancy; Herpes Zoster /constipation; Diplopia because of pre-existent meningioma ; Pulmonary embolism
AE	Mainly mild medication side-effects n=167	Mainly mild medication side-effects n=142
Total	**187 (SAE: 12; AE: 175)**	**159 (SAE: 9; AE: 150)**

SAE = serious adverse event; AE = adverse event.

Table S6. Per protocol analysis*. Outcome at one year follow-up for the total cohort, and for the patients treated by ADX or MRA separately.

	Total cohort		ADX		MRA	
	CT (n=92)	**AVS (n=88)**	**CT (n=46)**	**AVS (n=44)**	**CT (n=46)**	**AVS (n=44)**
DDD	3.0 (1.0-5.0)	3.0 (1.1-5.9)	1.2 (0-3.0)	1.2 (0-3.0)	4.0 (2.3 – 6.7)	5.7 (3.5-8.3) §
No. of antihypertensive drugs	2 (1-3)	2 (1-3)	1 (0-2)	1 (0-2)	2 (2-3)	3 (2-4)
sABP / dABP 24-hrs - mmHg	127(120-138) /80(75-86)	128(122-137) /81(76-85)	129(121-141) /82(76-87)	128(121-137) /81(76-85)	125(120-135) /80(74-86)	128(123-134) /82(76-86)
sABP / dABP day - mmHg	131(124-141) /83(77-89)	131(125-138) /84(78-87)	133(123-143) /83(78-91)	131(123-140) /84(79-88)	128(124-142) /84(77-89)	131(125-138) /84(78-87)
sABP / dABP night - mmHg	117(109-131) /71(65-78)	120(112-128) /72(69-78)	116(111-131) /71(66-80)	120(111-128) /71(68-81)	117(109-128) /72(65-78)	120(112-129) /73(69-77)
No. at target day ABP (%)#	39 (42.9%)	39 (44.3%)	18 (40%)	20 (46%)	21 (46%)	19 (43%)
Potassium – mmol/l	4.3 (4.0-4.6)	4.2 (4.0-4.5)	4.3(3.9-4.6)	4.2 (3.9-4.4)	4.3 (4.0-4.6)	4.3 (4.0-4.6)
RAND-36 PCS	52.7 (43.9-56.8)	MV	54.3 (45.2-58.2)	MV	50.9 (40.7-56.4)	MV
RAND-36 MCS	52.7 (44.9-55.5)	MV	53.8 (48.8-55.8)	MV	51.1 (41.7-55.0)	MV
Aldosterone (pmol/l)	-	-	230 (150-360)	260 (170-354)	-	-
DRC – mU/l (n=34/n=37)	-	-	14.0 (9.2-20.3)	14.0 (9.0-22.0)	-	-
PRA – µg/l/hr (n=10/n=5)	-	-	0.63 (0.38-1.94)	2.74 (1.68-4.05)	-	-
Post SLT aldo – nmol/l (n=41/n=38)	-	-	120 (71-175)	110 (75-169)	-	-
Post SLT Urine aldo – nmol/24hr (n=5/n=4)	-	-	22.0 (12.9-46.5)	26.0 (12.5-35.0)	-	-
Post SLT aldosterone						
suppressed	-	-	24 (52%)	30 (70%)	-	-
indeterminate	-	-	17 (37%)	8 (19%)	-	-
not suppressed	-	-	5 (11%)	5 (12%)	-	-
Biochemical outcome						
persistent primary aldosteronism	-	-	9 (20%)	5 (12%)	-	-
resolved aldosteronism	-	-	37 (80%)	38 (88%)	-	-

* all patients with a failed AVS and the patient with a selectivity ratio just <3.0 were excluded. § significant difference compared to CT $p < 0.05$ CT = CT-scan; AVS = adrenal vein sampling; ADX = adrenalectomy; MRA = mineralocorticoid receptor antagonists; DDD = defined daily dosage; sABP = systolic ambulant blood pressure; dABP = diastolic ambulant blood pressure; ABP = ambulatory blood pressure; PCS = physical component summary score; MCS = mental component summary score; DRC = direct renin concentration; PRA: plasma renin activity; aldo = aldosterone

Table S7. Per protocol analysis with a selectivity ratio ≥ 5.0*. Outcome at one year follow-up for the total cohort, and for the patients treated by ADX or MRA separately.

	Total cohort		ADX		MRA	
	CT (n=92)	AVS (n=84)	CT (n=46)	AVS (n=43)	CT (n=46)	AVS (n=41)
DDD	3.0 (1.0-5.0)	3.0 (0.6-5.9)	1.2 (0-3.0)	1.0 (0-3.0)	4.0 (2.3 – 6.7)	5.7 (2.3-6.7)§
No. of antihypertensive drugs	2 (1-3)	2 (1-3)	1 (0-2)	1 (0-2)	2 (2-3)	3 (2-4)
sABP / dABP 24-hrs - mmHg	127(120-138) /80(75-86)	128(121-135) /81(76-85)	129(121-141) /82(76-87)	128(121-137) /81(77-85)	125(120-135) /80(74-86)	128(123-133) /80(76-85)
sABP / dABP day - mmHg	131(124-141) /83(77-89)	130(124-138) /84(78-87)	133(123-143) /83(78-91)	131(123-140) /84(79-88)	128(124-142) /84(77-89)	130(125-138) /84(77-87)
sABP / dABP night - mmHg	117(109-131) /71(65-78)	120(112-128) /72(69-78)	116(111-131) /71(66-80)	120(111-128) /72(69-81)	117(109-128) /72(65-78)	119(112-128) /73(69-77)
No. at target day ABP (%)#	39 (43%)	38 (45%)	18 (40%)	19 (44%)	21 (46%)	19 (46%)
Potassium – mmol/l	4.3 (4.0-4.6)	4.2 (4.0-4.5)	4.3(3.9-4.6)	4.2 (4.0-4.4)	4.3 (4.0-4.6)	4.3 (4.0-4.6)
RAND-36 PCS	52.7 (43.9-56.8)	53.4 (43.6-56.8)	54.3 (45.2-58.2)	54.4 (47.0-58.0)	50.9 (40.7-56.4)	51.8 (41.2-56.2)
RAND-36 MCS	52.7 (44.9-55.5)	50.6 (43.1-54.8)	53.8 (48.8-55.8)	50.9 (45.3-56.3)	51.1 (41.7-55.0)	49.0 (40.9-53.7)
Aldosterone (pmol/l)	-	-	230 (150-360)	265 (170-380)	-	-
DRC – mU/l (n=34/n=37)	-	-	14.0 (9.2-20.3)	14.0 (9.1-22.1)	-	-
PRA – µg/l/hr (n=10/n=5)	-	-	0.63 (0.38-1.94)	2.48 (1.25-4.70)	-	-
Post SLT aldo – nmol/l (n=41/n=38)	-	-	120 (71-175)	112 (78-164)	-	-
Post SLT Urine aldo – nmol/24hr (n=5/n=4)	-	-	22.0 (12.9-46.5)	24.5 (11.3-35.5	-	-
Post SLT aldosterone suppressed indeterminate not suppressed	 - - -	 - - -	 24 (52%) 17 (37%) 5 (11%)	 30 (70%) 8 (19%) 5 (12%)	 - - -	 - - -
Biochemical outcome persistent primary aldosteronism resolved aldosteronism	 - -	 - -	 9 (20%) 37 (80%)	 5 (12%) 38 (88%)	 - -	 - -

* all patients with a AVS with a selectivity ratio between 3.0-5.0 were excluded. § significant difference compared to CT $p < 0.05$ CT = CT-scan; AVS = adrenal vein sampling; ADX = adrenalectomy; MRA = mineralocorticoid receptor antagonists; DDD = defined daily dosage; sABP = systolic ambulant blood pressure; dABP = diastolic ambulant blood pressure; ABP = ambulatory blood pressure; PCS = physical component summary score; MCS = mental component summary score; DRC = direct renin concentration; PRA: plasma renin activity; aldo = aldosterone

Table S8. Baseline and diagnostic characteristics of patients with persistent aldosteronism and resolved aldosteronism after adrenalectomy (n=92)

	Persistent	Resolved
AVS-based adrenalectomy	N = 5	N = 41
Male – no. (%)	4 (80%)	30 (73%)
Age – years*	55.8±8.6	51.4±10.3
Baseline Sys/Dias ABP 24-hrs – mmHg	155(152-163)/92(86-98)	147(134-162)/90(85-101)
Baseline DDD	3.0 (2.3-4.0)	3.0 (2.0-3.5)
Baseline Potassium – mmol/l	3.2 (3.1-4.0)	3.5 (3.2-3.7)
Baseline Post-SLT plasma aldosterone – pmol/l (n=5/n=36)	320 (290-350)	110 970-130)
Baseline Post-SLT urine aldosterone – nmol/24hr (n=0/n=5)	-	26.0 (12.5-35.0)
AVS selectivity index RAV[1]	17.3 (13.4-28.5)	26.4 (14.4-33.6)
AVS selectivity index LAV[1]	11.1 (7.2-23.3)	13.4 (10.3-18.1)
AVS lateralisation index[1]	6.8 (4.7-27.4)	16.4 (9.6-33.4)
AVS-CT concordance[1]	0 (0%)	25 (62.5%)
CT-based adrenalectomy	N = 9	N = 37
Male – no. (%)	8 (89%)	24 (65%)
Age – years*	50.3±11.7	52.0±9.9
Baseline Sys/Dias ABP 24-hrs– mmHg	148 (132-150)/93(81-101)	149(134-158)/88(82-103)
Baseline DDD	3.0 (1.8-6.0)	2.8 (1.0-4.2)
Baseline Potassium – mmol/l	3.6 (3.3-4.0)	3.5 (3.1-4.0)
Baseline Post-SLT aldosterone – pmol/l (n=7/n=34)	289 (220-310)	100 (68-150)
Baseline Post-SLT urine aldosterone – nmol/24hr (n=2/n=3)	46.5	16.0
Age < 40 years	1 (11%)	3 (8%)
CT node-size – mm	10 (7-13)	11 (10-17)[2]

Data presented as median and interquartile range unless stated otherwise. * mean±SD. There were no significant between-group differences except for AVS-CT concordance. AVS = Adrenal Vein Sampling; ABP = Ambulatory Blood Pressure. DDD = Defined Daily Dosage[35]. SLT = Salt Loading Test. [1] Of the 46 AVS-based adrenalectomies one patient had a failed AVS and was treated based on the pre-AVS CT-scan. Data of 45 actual AVS patients are shown. [2] Node size was documented in 32 of 36 patients. Conversion to SI Units: Potassium mmol/l to mEq/l conversion factor 1.0; Aldosterone pmol/l to ng/dl 0.36, Aldosterone urine nmol/24hr to µg/24hr conversion factor 0.36.

Table S9. CT and AVS characteristics of the AVS patients with persistent hyperaldosteronism (n=5).

No.	Age (years)	Gender	CT conclusion	AVS conclusion	AVS selectivity index right	AVS selectivity index left	AVS lateralisation index	AVS suppression index
26	63	M	Bilateral	Left-sided	11.8	11.1	6.8	0.6
71	46	F	Bilateral	Left-sided	24.9	17.1	44.2	0.1
112	60	M	Bilateral	Right-sided	15.0	5.6	10.7	0.4
116	47	M	Bilateral	Right-sided	17.3	8.7	5.3	0.9
157	63	M	Bilateral	Right-sided	32.1	29.6	4.0	0.7

CT = CT-scan; AVS = adrenal vein sampling; M = Male; F = Female.

Table S10. Treatment outcome in patients below and above the age of 40 years.

	Complete cohort		ADX		MRA	
	AVS	CT	AVS	CT	AVS	CT
< 40 years	N=9	N=4	N=7	N=4	N=2	N=0
DDD	0 (0-2.8)	1.1 (0.3-1.8)	0 (0-0.5)	1.1 (0.3-1.8)	1.1 (0.3-1.8)	-
No. of antihypertensive drugs	0 (0-1)	1.5 (0.3-2.0)	0 (0-1)	1.5 (0.3-2.0)	1.5 (0.25-2.0)	-
sABP / dABP 24-hrs - mmHg	123(117-128) /78(75-83)	128(126-138) /81(73-92)	123(113-127) /76(74-82)	128(126-138) /81(73-92)	128(126-138) /81(73-92)	-
No. at target day ABP (%)#	7 (78%)	1 (25%)	6 (86%)	1 (25%)	1 (25%)	-
Potassium – mmol/l	4.3 (3.9-4.6)	3.7 (3.6-5.1)	4.2 (3.8-4.5)	3.7 (3.6-5.1)	3.7 (3.6-5.1)	-
Post SLT aldo – nmol/l	-	-	90 (58-116)	125 (47-203)	125 (47-203)	-
Post SLT aldo						
suppressed	-	-	7 (100%)	2 (50%)	-	-
indeterminate	-	-	0	2 (50%)	-	-
not suppressed	-	-	0	0 (0%)	-	-
Biochemical outcome						
persistent PA	-	-	0 (0%)	1 (25%)	-	-
resolved PA	-	-	7 (100%)	4 (75%)	-	-
RAND-36 PCS	55.8 (53.7-57.6)	55.9 (54.3-58.0)	55.8 (52.8-58.0)	55.9 (54.3 -58.0)	55.3 (53.5-na)	
RAND-36 MCS	51.1 (46.4-54.7)	54.1 (51.1-59.2)	50.4 (45.1-55.6)	54.1 (51.1-59.2)	51.6 (50.8-na)	

>>

≥ 40 years	N= 83	N=88	N=39	N=42	N=44	N=46
DDD	3.5 (1.5-6.0)	3.0 (1.0-5.0)	1.5 (0-3.0)	1.6 (0-3.1)	5.7	4.0 (2.3-6.7)
No. of antihypertensive drugs	2 (1-3)	2 (1-3)	1 (0-21)	1 (0-2)	3.0	2 (2-3.25)
sABP / dABP 24-hrs - mmHg	128 (122-137) /81(76-85)	127(120-138) /80(75-86)	131 (121-137) /81 (78-86)	131(119-142) /82(76-87)	128(122-134) /81(75-85)	125(120-135) /80(74-86)
No. at target day ABP (%)#	34 (41%)	38 (44%)	14 (36%)	17 (42%)	20 (46%)	21 (46%)
Potassium – mmol/l	4.2 (4.0-4.6)	4.3 (4.0-4.6)	4.2 (4.0-4.4)	4.3 (4.0-4.6)	4.4 (4.0-4.6)	4.3 (4.0-4.6)
Post SLT aldo – nmol/l	-	-	120 (80-184)	120 (71-175)	-	-
Post SLT urine aldo	-	-	26 (13-35)	22 (13-47)	-	-
Post SLT aldosterone						
suppressed	-	-	26 (67%)	22 (52%)	-	-
indeterminate	-	-	8 (21%)	15 (36%)	-	-
not suppressed	-	-	5 (13%)	5 (12%)	-	-
Biochemical outcome						
persistent PA	-	-	5 (13%)	8 (19%)	-	-
resolved PA	-	-	34 (87%)	34 (81%)	-	-
RAND-36 PCS	52.2 (43.1-56.7)	51.7 (42.0-56.8)	53.2 (45.4-58.0)	53.5 (44.5-58.2)	51.6 (41.3-56.0)	50.9 (40.7-56.4)
RAND-36 MCS	49.7 (51.2-54.6)	52.7 (44.2-55.3)	50.8 (42.4-56.7)	53.8 (47.0-55.6)	49.0 (41.1-53.4)	51.1 (41.7-55.0)

There were no significant differences between CT and AVS in any of the subgroups defined in the Table. PA = primary aldosteronism; CT = CT-scan; AVS = adrenal vein sampling; ADX = adrenalectomy; MRA = mineralocorticoid receptor antagonists; DDD = defined daily dosage; sABP = systolic ambulant blood pressure; dABP = diastolic ambulant blood pressure; ABP = ambulatory blood pressure; PCS = physical component summary score; MCS = mental component summary score.

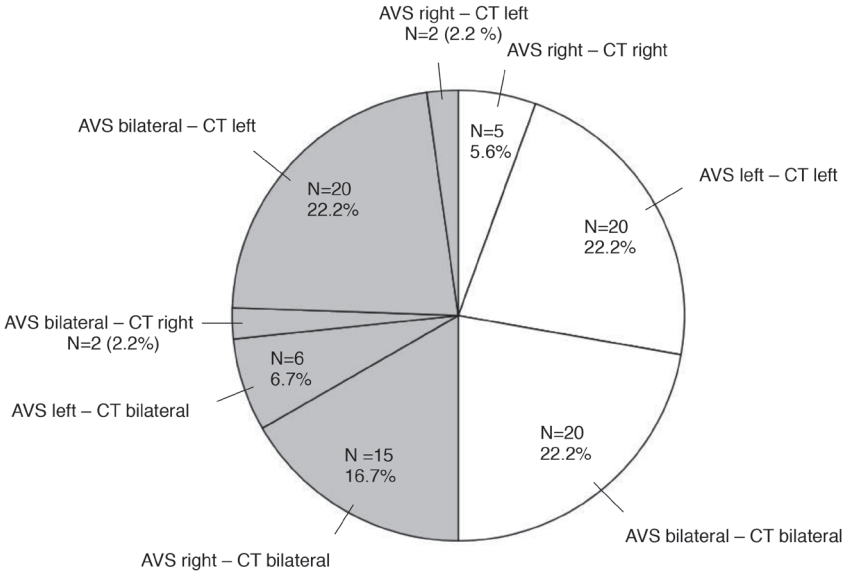


Figure S1. Concordant (white) and discordant (shaded) results between AVS and pre-AVS CT-scan (n=90).

Shown are conclusions according to AVS and CT. Right/left indicates right/left-sided adenoma. Bilateral indicates bilaterally normal, or bilaterally enlarged adrenal glands (CT) or non-lateralised aldosterone secretion (AVS). Four AVS procedures failed because the right adrenal vein could not be cannulated. Two pre-AVS CT-scans were inconclusive because of too little intra-abdominal fat and respiratory movement artefacts.

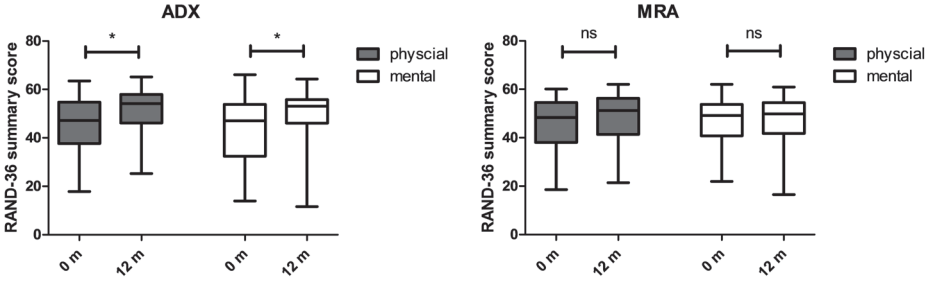


Figure S2. Health related quality of life Physical Component Summary Score (PCS) and Mental Component Summary Score (MCS) after adrenalectomy (ADX) or mineralocorticoid receptor antagonist (MRA) treatment.

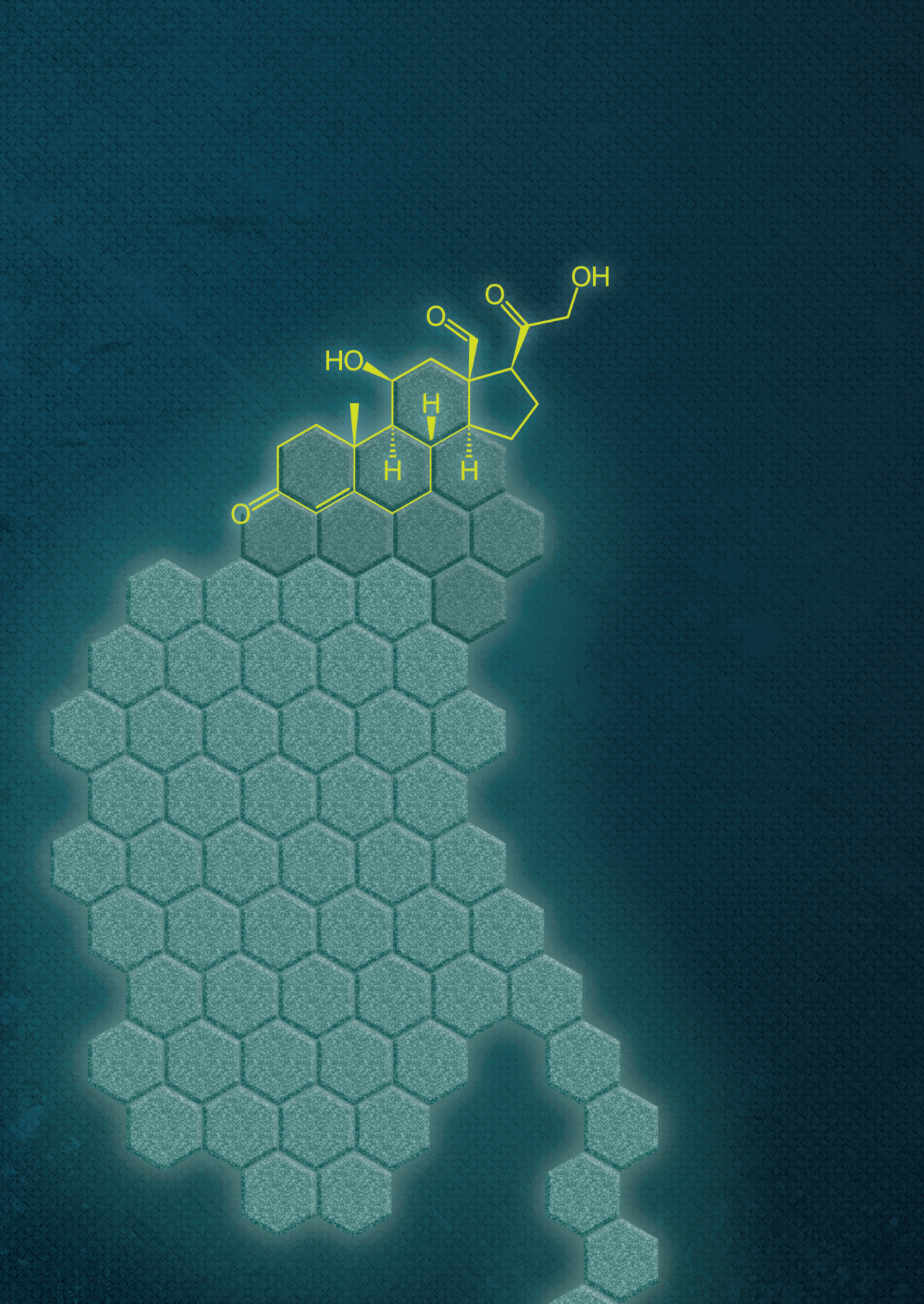
Shown are box and whiskers (10-90 percentile) plots. * $p < 0.05$ for difference between baseline (0m) and final evaluation at 12 months (12m). ns= not statistically significant for difference baseline (0m) and final evaluation (12 m).

REFERENCES

1. Funder JW, Carey RM, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. The Journal of clinical endocrinology and metabolism 2016;101:1889-916.
2. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. Journal of the American College of Cardiology 2005;45:1243-8.
3. Rossi GP, Barisa M, Allolio B, et al. The Adrenal Vein Sampling International Study (AVIS) for identifying the major subtypes of primary aldosteronism. The Journal of clinical endocrinology and metabolism 2012;97:1606-14.
4. Lim V, Guo Q, Grant CS, et al. Accuracy of adrenal imaging and adrenal venous sampling in predicting surgical cure of primary aldosteronism. The Journal of clinical endocrinology and metabolism 2014;99:2712-9.
5. Mulatero P, Bertello C, Rossato D, et al. Roles of clinical criteria, computed tomography scan, and adrenal vein sampling in differential diagnosis of primary aldosteronism subtypes. The Journal of clinical endocrinology and metabolism 2008;93:1366-71.
6. Young WF, Stanson AW, Thompson GB, Grant CS, Farley DR, van Heerden JA. Role for adrenal venous sampling in primary aldosteronism. Surgery 2004;136:1227-35.
7. Kempers MJ, Lenders JW, van Outheusden L, et al. Systematic review: diagnostic procedures to differentiate unilateral from bilateral adrenal abnormality in primary aldosteronism. Annals of internal medicine 2009;151:329-37.
8. Stewart PM, Allolio B. Adrenal vein sampling for Primary Aldosteronism: time for a reality check. Clinical endocrinology 2010;72:146-8.
9. Zee vd, K. I., Sanderman R. Het meten van de algemene gezondheidstoestand met de RAND-36: een handleiding. Groningen: Noordelijk Centrum voor Gezondheidsvraagstukken, NCG.
10. Ware JE, Jr., Kosinski M, Bayliss MS, McHorney CA, Rogers WH, Raczek A. Comparison of methods for the scoring and statistical analysis of SF-36 health profile and summary measures: summary of results from the Medical Outcomes Study. Medical care 1995;33:As264-79.
11. Vincent JM, Morrison ID, Armstrong P, Reznek RH. The size of normal adrenal glands on computed tomography. Clinical radiology 1994;49:453-5.

12. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of hypertension 2013;31:1281-357.
13. Monticone S, Viola A, Rossato D, et al. Adrenal vein sampling in primary aldosteronism: towards a standardised protocol. The lancet Diabetes & endocrinology 2015;3:296-303.
14. Rossi GP, Auchus RJ, Brown M, et al. An expert consensus statement on use of adrenal vein sampling for the subtyping of primary aldosteronism. Hypertension 2014;63:151-60.
15. Gordon RD. Diagnostic investigations in primary aldosteronism. In: A Z, ed. Clinical medicine series on hypertension Maidenhead: McGraw-Hill International; 2001:101-11.
16. Nwariaku FE, Miller BS, Auchus R, et al. Primary hyperaldosteronism: effect of adrenal vein sampling on surgical outcome. Archives of surgery (Chicago, Ill : 1960) 2006;141:497-502; discussion -3.
17. Schneller J, Reiser M, Beuschlein F, et al. Linear and volumetric evaluation of the adrenal gland--MDCT-based measurements of the adrenals. Academic radiology 2014;21:1465-74.
18. Satoh F, Morimoto R, Ono Y, et al. 8D.04: CLINICAL BENEFITS OF ADMINISTERING SUPER-SELECTIVE SEGMENTAL ADRENAL VENOUS SAMPLING AND PERFORMING ADRENAL SPARING SURGERY IN THE PATIENTS WITH PRIMARY ALDOSTERONISM. Journal of hypertension 2015;33 Suppl 1:e114.
19. Kline GA, Dias VC, So B, Harvey A, Pasieka JL. Despite limited specificity, computed tomography predicts lateralization and clinical outcome in primary aldosteronism. World journal of surgery 2014;38:2855-62.
20. Kline GA, So B, Dias VC, Harvey A, Pasieka JL. Catheterization during adrenal vein sampling for primary aldosteronism: failure to use (1-24) ACTH may increase apparent failure rate. Journal of clinical hypertension (Greenwich, Conn) 2013;15:480-4.
21. Seccia TM, Miotto D, De Toni R, et al. Adrenocorticotropic hormone stimulation during adrenal vein sampling for identifying surgically curable subtypes of primary aldosteronism: comparison of 3 different protocols. Hypertension 2009;53:761-6.
22. Carr CE, Cope C, Cohen DL, Fraker DL, Trerotola SO. Comparison of sequential versus simultaneous methods of adrenal venous sampling. Journal of vascular and interventional radiology : JVIR 2004;15:1245-50.
23. Lethielleux G, Amar L, Raynaud A, Plouin PF, Steichen O. Influence of diagnostic criteria on the interpretation of adrenal vein sampling. Hypertension 2015;65:849-54.

24. Mulatero P, Bertello C, Sukor N, et al. Impact of different diagnostic criteria during adrenal vein sampling on reproducibility of subtype diagnosis in patients with primary aldosteronism. Hypertension 2010;55:667-73.
25. Auchus RJ, Wians FH, Jr., Anderson ME, et al. What we still do not know about adrenal vein sampling for primary aldosteronism. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2010;42:411-5.
26. Dekkers T, ter Meer M, Lenders JW, et al. Adrenal nodularity and somatic mutations in primary aldosteronism: one node is the culprit? The Journal of clinical endocrinology and metabolism 2014;99:E1341-51.
27. Nishimoto K, Tomlins SA, Kuick R, et al. Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands. Proceedings of the National Academy of Sciences of the United States of America 2015;112:E4591-9.
28. Goupil R, Wolley M, Ahmed AH, Gordon RD, Stowasser M. Does concomitant autonomous adrenal cortisol overproduction have the potential to confound the interpretation of adrenal venous sampling in primary aldosteronism? Clinical endocrinology 2015;83:456-61.
29. Fallo F, Bertello C, Tizzani D, et al. Concurrent primary aldosteronism and subclinical cortisol hypersecretion: a prospective study. Journal of hypertension 2011;29:1773-7.
30. Wilms S, Dekkers T, Hermus AR, schultze Kool L, Lenders JW, Deinum J. Should Management of Primary Aldosteronism Be based on Adrenal Vein Sampling or Adrenal CT-scan? A Retrospective Study of Blood Pressure, Medication Use, Potassium and Aldosterone. 20th Scientific Meeting of the European Society of Hypertension 2010. Oslo: Journal of Hypertension; 2010:p.e304.
31. Kerstens MN, Kobold AC, Volmer M, Koerts J, Sluiter WJ, Dullaart RP. Reference values for aldosterone-renin ratios in normotensive individuals and effect of changes in dietary sodium consumption. Clinical chemistry 2011;57:1607-11.
32. Bravo EL, Tarazi RC, Dustan HP, et al. The changing clinical spectrum of primary aldosteronism. The American journal of medicine 1983;74:641-51.
33. Johnson PT, Horton KM, Fishman EK. Adrenal imaging with multidetector CT: evidence-based protocol optimization and interpretative practice. Radiographics : a review publication of the Radiological Society of North America, Inc 2009;29:1319-31.
34. Aaronson NK, Muller M, Cohen PD, et al. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. Journal of clinical epidemiology 1998;51:1055-68.
35. WHO Collaborating Centre for Drug Statistics Methodology (WHOCC). at http://www.whocc.no/atc_ddd_index/.)


OH
O
O
HO
H
H
H
O

Single versus duplicate blood samples in ACTH stimulated adrenal vein sampling

Tanja Dekkers[1] | Mark J. Arntz[2] | Gert Jan van der Wilt[3] | Leo J. Schultze Kool[2]
Fred C.G.J. Sweep[4] | Ad R.M.M. Hermus[5] | Jacques W.M. Lenders[1,6] | Jaap Deinum[1]

[1] Department of General Internal Medicine, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands
[2] Department of Radiology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands
[3] Department of Epidemiology, Biostatistics and HTA, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands
[4] Department of Laboratory Medicine, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands
[5] Department of Endocrinology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands
[6] Department of Internal Medicine III, University Hospital Carl Gustav Carus Dresden, Germany

Clin Chim Acta. 2013 Aug 23;423:15-7.

ABSTRACT

Background. Adrenal vein sampling (AVS) is the preferred test for subtyping primary aldosteronism. However, the procedure is technically demanding and costly. In AVS it is common practice to take duplicate blood samples at each location. In this paper we explore whether a single sample procedure leads to a different conclusion concerning the location of adrenal aldosterone secretion than a duplicate sample procedure.

Methods. AVS procedures with duplicate measurements performed in our university medical centre between 2005 and 2010 were evaluated retrospectively. We compared the conclusions regarding selectivity and lateralization based on the first sample taken (A) to the conclusions based on the average of duplicate samples (AB). We also calculated the number needed to be sampled in duplicate to prevent one misclassification.

Results. Ninety-six AVS procedures of 82 patients were included. The concordance in AVS conclusions between samples A and AB was 98–100%, depending on the criteria used for selectivity and lateralization. With permissive and strict criteria the number needed to be sampled in duplicate were infinite and 48, respectively.

Conclusions. The incremental benefit of duplicate sampling compared to single sampling is low. Therefore, in the case of technical difficulties during AVS, conclusions can also be reliably drawn from a single blood sample.

INTRODUCTION

Primary aldosteronism (PA) is the most common form of secondary hypertension.[1-5] PA manifests itself in hypertension, high plasma aldosterone levels, suppressed plasma renin levels and frequently hypokalemia. PA is usually caused by either a unilateral aldosterone-producing adenoma (APA) or bilateral adrenal hyperplasia.[6] Distinction between the two is crucial, since the former is treated by adrenalectomy and the latter by mineralocorticoid receptor antagonists.[1] Adrenal vein sampling (AVS) is considered the preference test for subtyping PA.[1,7] The selectivity index, used to define successful sampling, is defined as the cortisol ratio between the adrenal vein (AV) and the inferior vena cava (IVC). Lateralization of aldosterone production is defined by the ratio of the dominant over the non-dominant aldosterone/cortisol ratios of the two adrenal vein samples. A higher aldosterone/cortisol ratio of one side over the other side is indicative for unilateral aldosterone production.[8] AVS is a technically demanding procedure, which is relatively costly and burdensome to the patient. In several medical centres, including our university medical centre, it is common practice to take duplicate blood samples at three locations, adding up to a total of at least six samples. The aim of this study is to assess whether the conclusion concerning adrenal aldosterone secretion is different between a single sample AVS and a duplicate sample AVS.

MATERIALS AND METHODS

AVS procedures were performed in consecutive PA patients attending our university medical centre from 2005 to 2010. PA had been confirmed in all cases using a saline infusion test (SIT) and interfering medication was discontinued prior to AVS in accordance with current guidelines.[1] AVS was performed after 3 h of bed rest under continuous ACTH (adrenocorticotropic hormone) stimulation (5 µg/h) [9] with sequential catheterization of both adrenal veins. From 2008 onward we performed rapid cortisol assays during the AVS procedure to confirm correct catheter placement.[10] Duplicate 5 ml blood samples (samples A and B) were taken consecutively at each of three locations: right AV, left AV and IVC. Duplicate blood samples A and B were taken at exactly the same catheter position by gravity

or with very gentle negative pressure within 5 min. The pair of duplicate samples from the IVC was taken subsequently to the samples from the adrenal veins and was used to compare with both pairs of adrenal vein samples.

We measured serum aldosterone by radioimmunoassay after extraction with dichloromethane and subsequent paper chromatography (within-assay coefficient of variation (CV) of 4.8% and between-assay CV of 12.3% at a level of 0.32 nmol/l). Until January 2009 serum cortisol was measured by fluorescence polarization immunoassay on a TDX batch analyzer (Abbott, Hoofddorp, The Netherlands; within-assay CVs: 4.6% at 0.22 µmol/l, 5.8% at 0.52 µmol/l and 4.6% at 1.06 µmol/l; between-assay CVs: 9.1%, 7.7% and 6.6% at these concentration levels). From January 2009 to November 2009 cortisol was measured by luminescence immunoassay on an Architect random access analyzer (Abbott, Hoofddorp, The Netherlands; within-assay CVs: 3.9% at 0.16 µmol/l, 4.8% at 0.44 µmol/l; between-assay CVs: 4.5% and 6.2% at these concentration levels) and from November 2009 onwards by an electrochemoluminescence immuno assay on a Modular E170 analyzer (Roche; within-assay CVs: 3.0 at 0.188 µmol/l and 1.8% at 0.509 µmol/l; between-assay CVs: 3.8% and 2.3% at these concentrations).

We evaluated all AVS procedures with duplicate sampling. We compared selectivity and lateralization indices based on a single sample A (A) with those based on the average of a duplicate sample A and B (AB) and calculated discordance. The averages of AB for the cortisol (C) ratio and the aldosterone/cortisol (ALD/C) ratio were calculated using the following formulas: cortisol ratio$_{AB}$ = (($C_{AV\text{-}A}$ + $C_{AV\text{-}B}$) / 2) / (($C_{IVC\text{-}A}$ + $C_{IVC\text{-}B}$) / 2); aldosterone/cortisol ratio$_{AB}$ = ((($ALD_{AV\text{-}A\ dominant}$ + $ALD_{AV\text{-}B\ dominant}$) / 2) / (($C_{AV\text{-}A\ dominant}$ + $C_{AV\text{-}B\ dominant}$) / 2)) / ((($ALD_{AV\text{-}A\ non\text{-}dominant}$ + $ALD_{AV\text{-}B\ non\text{-}dominant}$) / 2) / (($C_{AV\text{-}A\ non\text{-}dominant}$ + $C_{AV\text{-}B\ non\text{-}dominant}$) / 2)). We applied two sets of selectivity and lateralization criteria which have been reported in the literature: Permissive criteria using a selectivity index (cortisol ratio between the AV and the IVC) of ≥ 2.0 and a lateralization index (ratio of the aldosterone/cortisol ratio of the dominant adrenal over non-dominant adrenal) of ≥ 4.0 [1] and strict criteria using a selectivity index of ≥ 5.0 and a lateralization index of ≥ 4.0.[6] We calculated the average number of duplicate sampling procedures to obtain one change in diagnosis (number needed to be sampled in duplicate, NNSD). We assessed treatment outcome and clinical features of patients with a discordant conclusion between single and duplicate sampling procedures to evaluate which approach leads to the correct diagnosis. The reference standard for correct diagnosis of APA was based

on the four corner approach, requiring the following aspects: 1) biochemical diagnosis of PA; 2) lateralization of aldosterone secretion on AVS; 3) evidence of adrenocortical adenoma at imaging and/or pathology and 4) correction of hyperaldosteronism and unequivocal fall of blood pressure post-operatively.[3]

The study was performed in accordance with the requirements of the medical ethical committee of the Radboud University Nijmegen Medical Center. SPSS V16.0 was used for the statistical analyses.

RESULTS

We included 96 AVS procedures from 82 patients. Patients had a mean age of 53.8 years (range: 24–75 years) and 68.3% of them were male. Sampling outcome for the different selectivity and lateralization criteria based upon sample A or AB are shown in Table 1. Using the permissive criteria, there was a 100% concordance in the conclusions of samples A and AB. With the use of the strict criteria the concordance between single and duplicate sampling in the AVS conclusion was 98%. Using the strict criteria, the AVS procedure gave different conclusions in two patients when a single sample instead of a duplicate sample was used. Assuming that the duplicate sampling procedure was correct, the NNSD would be infinite (∞) for the permissive criteria and 48 for the strict criteria. In other words, when using the strict criteria, 48 patients would have to undergo a duplicate instead of a single sampling procedure to obtain one extra correctly diagnosed patient.

The two patients who had discordant results, according to the strict criteria, both underwent adrenalectomy. The resected adrenal gland of one patient, in whom the decision to perform adrenalectomy was in accordance with the conclusion of the duplicate (AB) sampling and CT-scan, showed nodular hyperplasia. Eighteen months after surgery, this patient had a blood pressure of 121/78 mm Hg without medication and a plasma potassium level of 4.5 mmol/l. A SIT showed a post-test aldosterone value of 0.18 nmol/l and a post-test renin value of 31 mE/l, compared to pre-operative post-test values of 0.54 nmol/l and 4.1 mE/l, respectively. Hence, renin levels were no longer suppressed. In the other patient, in whom the decision to perform adrenalectomy was in accordance with the single (A) sampling, the

resected adrenal gland showed a solitary adenoma. Pre-operatively, the CT-scan did not show any abnormalities. Eighteen months after surgery, this patient had a blood pressure of 142/82 mm Hg and a plasma potassium level of 3.3 mmol/l with 5 mg amlodipine. In this patient the SIT showed post-test plasma aldosterone and renin values of 0.27 nmol/l and 20 mE/l, respectively, compared to pre-operative post-test aldosterone and renin values of 0.50 nmol/l and 3.3 mE/l, respectively. Also in this patient renin was no longer suppressed. Both patients met our reference standard for correct diagnosis of APA and we therefore assume that surgery was successful in both cases.

Table 1. AVS conclusion based on permissive and strict selectivity and lateralization criteria. N = 96.

Criterion	Sample	Not selective	Selective: lateralization	Selective: no lateralization	Discordance A – AB	NNSD
Permissive	A	31	44	21	0 (0%)	∞
	AB	31	44	21		
Strict	A	37	40	19	2 (2%)	48
	AB	37	40	19		

Permissive criteria: $C_{AV}/C_{IVC} \geq 2.0$ and $[ALD/C]_{dominant\ AV} / [ALD/C]_{non\text{-}dominant\ AV} \geq 4.0$; strict criteria: $C_{AV}/C_{IVC} \geq 5.0$ and $[ALD/C]_{dominant\ AV} / [ALD/C]_{non\text{-}dominant\ AV} \geq 4.0$. NNSD = number needed to be sampled in duplicate. A = sample A, AB = average of sample A and sample B. ∞ = infinite.

DISCUSSION

Our results show that there is a high concordance between single and duplicate sampling regarding AVS selectivity and lateralization. In the two cases with discordant results, follow-up data suggest successful surgery based on the four corner approach. This favours the conclusion that a single sample A would have sufficed in one patient but that duplicate sampling would have been essential in the other.

Theoretically it is to be expected that two samples taken at the same position within a short time span during an ACTH stimulated adrenal venous sampling procedure give similar results and that the variances found only express the coefficient of variation of the assays. In this light our findings are not surprising. However, many centres, including our own centre, still use duplicate samples in their procedure. This is probably due to the fact that

in daily practice, clinicians heed for unsteady sampling conditions such as fluctuations in cortisol and aldosterone secretion, despite ACTH stimulation, because of unknown factors. To prevent possible distortions in sampling results because of such factors, clinicians often resort to duplicate sampling. Our study shows that these factors cause less variation and, hence, interpretation problems, than clinicians might expect. Therefore, duplicate sampling is not a prerequisite for a reliable result.

AVS is a technically demanding procedure and when the adrenal vein orifice is of low calibre sampling can be very difficult. Taking duplicate samples enhances the complexity of the procedure, which could result in a more time-consuming procedure with a higher chance of catheter displacement and complications like adrenal vein dissection or rupture. Duplicate sampling procedures are also more expensive, as they double the laboratory costs of AVS. However, in a single sample procedure the absence of spare blood samples could make the AVS procedure more vulnerable to loss of samples or uncertainty about results. Processing and analyzing adrenal blood samples is a delicate process which is prone to mistakes or mix-ups. In the case of such a processing error the presence of additional blood samples can be important.

We had a seemingly high AVS failure rate (unselective samplings according to the cortisol ratio) in our study. This is due to the inclusion of failed first attempts of AVS in patients in whom the second attempt was successful. Secondly, there was a learning curve for the radiologist. Eventually, 64 of the 82 patients (78%) had a selective AVS after one or two attempts (using the permissive criteria), with a success rate of 50% in the first three years and 90% thereafter.

One of the limitations of the study is that it is retrospective. There was no explicit instruction for the interventional radiologist to mention a change of catheter position between samples A and B. In this study all AVS procedures were performed under continuous ACTH stimulation with sequential catheterization of the adrenal veins. This enhances the cortisol and aldosterone secretion of the adrenal gland and abrogates the pulsatile character of cortisol secretion.[9] This is expected to improve the concordance in cortisol and aldosterone measurements between samples A and B.[1,11-14] However, in the current literature there is no consensus on the use of ACTH stimulation in AVS.[11,15,16] In some centres AVS is performed without ACTH stimulation.[17] In that case differences between the two samples may be

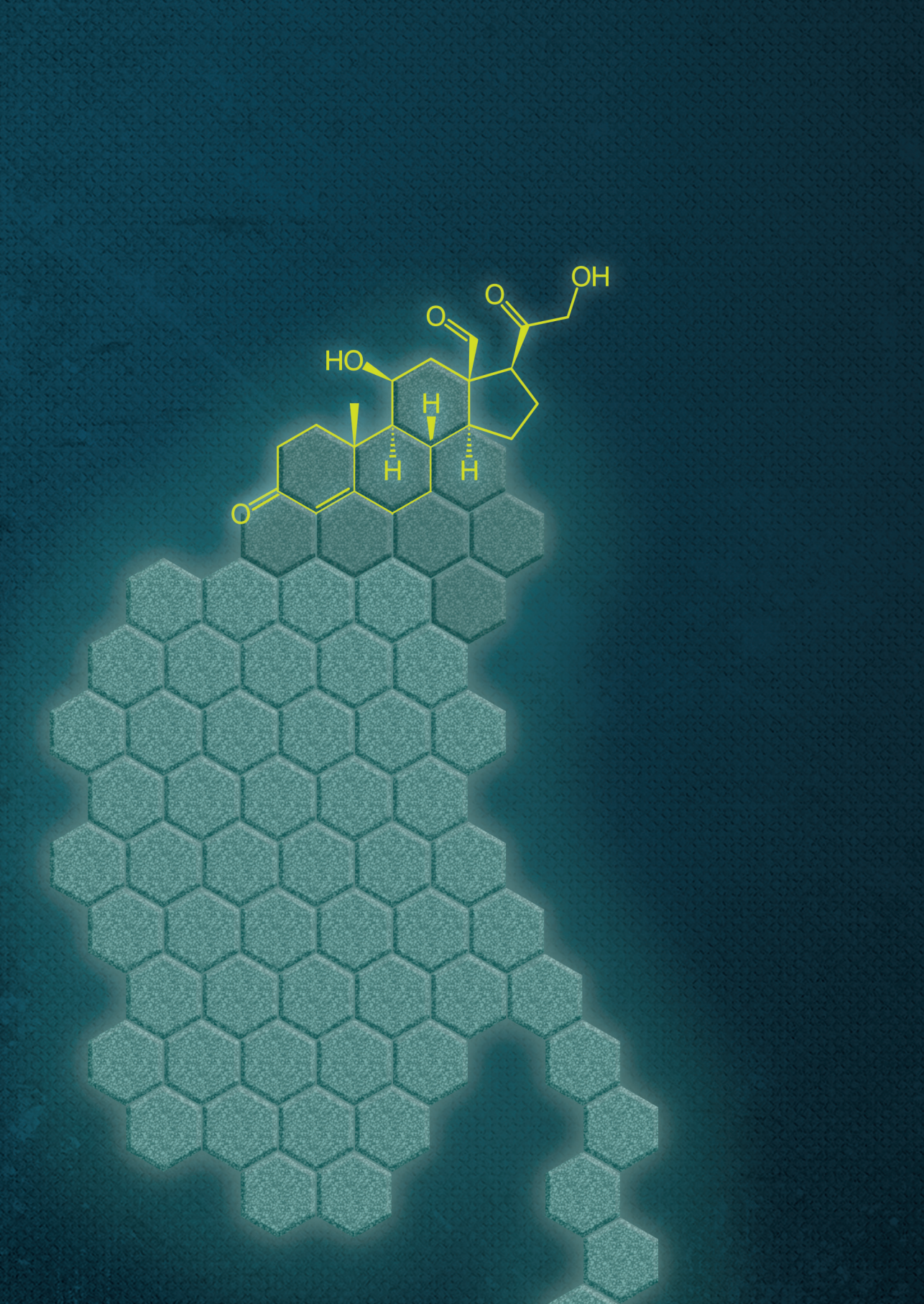
larger.[18] Therefore, our results may not be applicable to AVS procedures without ACTH stimulation.

In conclusion, this study shows that the incremental benefit of duplicate sampling is low. Besides the fact that it renders the AVS procedure less vulnerable to errors, taking duplicate samples at each catheter position seems of little value, whereas taking only one sample at each position is usually sufficient. In case the collection of a second blood sample fails, because of technical difficulties or unintentional catheter displacement, AVS conclusions can be reliably drawn from the result of a single adrenal blood sample.

REFERENCES

1. Funder JW, Carey RM, Fardella C, et al. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. The Journal of clinical endocrinology and metabolism 2008;93:3266-81.
2. Mosso L, Carvajal C, Gonzalez A, et al. Primary aldosteronism and hypertensive disease. Hypertension 2003;42:161-5.
3. Rossi GP, Bernini G, Caliumi C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. Journal of the American College of Cardiology 2006;48:2293-300.
4. Mulatero P, Stowasser M, Loh KC, et al. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. The Journal of clinical endocrinology and metabolism 2004;89:1045-50.
5. Lim PO, Rodgers P, Cardale K, Watson AD, MacDonald TM. Potentially high prevalence of primary aldosteronism in a primary-care population. Lancet (London, England) 1999;353:40.
6. Young WF, Stanson AW, Thompson GB, Grant CS, Farley DR, van Heerden JA. Role for adrenal venous sampling in primary aldosteronism. Surgery 2004;136:1227-35.
7. Kempers MJ, Lenders JW, van Outheusden L, et al. Systematic review: diagnostic procedures to differentiate unilateral from bilateral adrenal abnormality in primary aldosteronism. Annals of internal medicine 2009;151:329-37.
8. Kahn SL, Angle JF. Adrenal vein sampling. Techniques in vascular and interventional radiology 2010;13:110-25.
9. Arvat E, Di Vito L, Lanfranco F, et al. Stimulatory effect of adrenocorticotropin on cortisol, aldosterone, and dehydroepiandrosterone secretion in normal humans: dose-response study. The Journal of clinical endocrinology and metabolism 2000;85:3141-6.
10. Mengozzi G, Rossato D, Bertello C, et al. Rapid cortisol assay during adrenal vein sampling in patients with primary aldosteronism. Clinical chemistry 2007;53:1968-71.
11. Monticone S, Satoh F, Giacchetti G, et al. Effect of adrenocorticotropic hormone stimulation during adrenal vein sampling in primary aldosteronism. Hypertension 2012;59:840-6.
12. Carr CE, Cope C, Cohen DL, Fraker DL, Trerotola SO. Comparison of sequential versus simultaneous methods of adrenal venous sampling. Journal of vascular and interventional radiology : JVIR 2004;15:1245-50.

13. Auchus RJ, Wians FH, Jr., Anderson ME, et al. What we still do not know about adrenal vein sampling for primary aldosteronism. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2010;42:411-5.
14. Kline GA, Harvey A, Jones C, et al. Adrenal vein sampling may not be a gold-standard diagnostic test in primary aldosteronism: final diagnosis depends upon which interpretation rule is used. Variable interpretation of adrenal vein sampling. International urology and nephrology 2008;40:1035-43.
15. Seccia TM, Miotto D, De Toni R, et al. Adrenocorticotropic hormone stimulation during adrenal vein sampling for identifying surgically curable subtypes of primary aldosteronism: comparison of 3 different protocols. Hypertension 2009;53:761-6.
16. Tanemoto M, Suzuki T, Abe M, Abe T, Ito S. Physiologic variance of corticotropin affects diagnosis in adrenal vein sampling. European journal of endocrinology / European Federation of Endocrine Societies 2009;160:459-63.
17. Rossi GP, Barisa M, Allolio B, et al. The Adrenal Vein Sampling International Study (AVIS) for identifying the major subtypes of primary aldosteronism. The Journal of clinical endocrinology and metabolism 2012;97:1606-14.
18. Seccia TM, Miotto D, Battistel M, et al. A stress reaction affects assessment of selectivity of adrenal venous sampling and of lateralization of aldosterone excess in primary aldosteronism. European journal of endocrinology / European Federation of Endocrine Societies 2012;166:869-75.


OH
O
O
HO
H
H
H
O

Plasma metanephrine for assessing the selectivity of adrenal venous sampling

Tanja Dekkers[1] | Jaap Deinum[1] | Leo J. Schultze Kool[2] | Dirk Blondin[4] | Oliver Vonend[5]
Ad R.R.M. Hermus[1] | Mirko Peitzsch[7] | Lars C. Rump[5] | Gerald Antoch[4]
Fred C.G.J. Sweep[3] | Stefan R. Bornstein[8] | Jacques W.M. Lenders[1,8]
Holger S. Willenberg[6] | Graeme Eisenhofer[8]

[1] Department of General Internal Medicine, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands
[2] Department of Radiology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands
[3] Department of Laboratory Medicine, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands
[4] Department of Diagnostic and Interventional Radiology, University Dusseldorf, Medical Faculty, Dusseldorf, Germany
[5] Department of Nephrology, University Dusseldorf, Medical Faculty, Dusseldorf, Germany
[6] Department of Endocrinology, Diabetes, and Metabolism, University Dusseldorf, Medical Faculty, Dusseldorf, Germany
[7] Institute of Clinical Chemistry and Laboratory Medicine
[8] Department of Internal Medicine III, University Hospital Carl Gustav Carus, Dresden, Germany

Hypertension. 2013 Dec;62(6):1152-7

ABSTRACT

Adrenal vein sampling is used to establish the origins of excess production of adrenal hormones in primary aldosteronism. Correct catheter positioning is confirmed using adrenal vein measurements of cortisol, but this parameter is not always reliable. Plasma metanephrine represents an alternative parameter. The objective of our study was to determine the use of plasma metanephrine concentrations to establish correct catheter positioning during adrenal vein sampling with and without cosyntropin stimulation. We included 52 cosyntropin-stimulated and 34 nonstimulated sequential procedures. Plasma cortisol and metanephrine concentrations were measured in adrenal and peripheral venous samples. Success rates of sampling, using an adrenal to peripheral cortisol selectivity index of 3.0, were compared with success rates of metanephrine using a selectivity index determined by receiver operating characteristic curve analysis. Among procedures assessed as selective using cortisol, the adrenal to peripheral vein ratio of metanephrine was 6-fold higher than that of cortisol (94.0 versus 15.5; $P<0.0001$). There were significant positive relationships between adrenal to peripheral vein ratios of cortisol and metanephrine for cosyntropin-stimulated samplings but not for nonstimulated samplings. Receiver operating characteristic curve analysis indicated a plasma metanephrine selectivity index cut-off of 12. Using this cut-off, concordance in sampling success rates determined by cortisol and metanephrine was substantially higher in cosyntropin-stimulated than in nonstimulated samplings (98% versus 59%). For the latter procedures, sampling success rates determined by metanephrine were higher ($P<0.01$) than those determined by cortisol (91% versus 56%). In conclusion, metanephrine provides a superior analyte compared with cortisol in assessing the selectivity of adrenal vein sampling during procedures without cosyntropin stimulation.

INTRODUCTION

Adrenal hypertension caused by primary aldosteronism comprises the most common curable form of secondary hypertension. In the analytic workup of patients with primary aldosteronism, adrenal venous sampling (AVS) is recommended for establishing the origins of excess production of hormones.[1] AVS is a technically demanding procedure in which correct cannulation of the adrenal veins, especially the right, can pose significant difficulty.[2,3] Correct positioning of the catheter is verified by measurement of plasma cortisol concentrations. High cortisol concentrations in adrenal blood compared with peripheral blood ascertain correct catheter placement and thus selective sampling. Because cortisol has a long circulating half-life (100 minutes), increases in adrenal vein (AV) blood above levels of peripheral venous (PV) blood are relatively minor and subsequently subject to interpretative error. Furthermore, as a result of physiological corticotropin fluctuations, cortisol is secreted in a variable fashion so that fluctuating levels can interfere with the interpretation of AVS selectivity.[4-6] This problem can be overcome using cosyntropin stimulation.[7] Cosyntropin stimulation, however, adds to the complexity of the procedure and for this reason is not always used.

With the above considerations in mind, there seems a need for more reliable parameters than cortisol in assessing the correct positioning of catheters during AVS.[8] Plasma metanephrine, the O-methylated metabolite of epinephrine, represents one such alternative analyte. More than 90% of plasma metanephrine is produced within the adrenal medulla, with <10% produced from epinephrine after release from the adrenals.[7] Compared with cortisol, plasma metanephrine has a short circulating half-life of 3 to 6 minutes, resulting in close to 90-fold increases of AV compared with PV concentrations in situations where catheters are correctly positioned.[7] Such large gradients should provide more accurate and sensitive means to detect the correct AV site of sampling than the smaller gradients of plasma cortisol. Importantly, adrenal production of metanephrine occurs as a result of leakage of adrenaline from storage vesicles into the cytoplasm where the amine is metabolized by catechol-O-methyltransferase.[9] This process occurs continuously and independently of adrenaline release. Hence, plasma concentrations of metanephrine show relatively little increase in response to stress.[9,10]

We hypothesized that the continuous adrenal production and rapid circulatory clearance of metanephrine might provide advantages for measurements of the metabolite compared with cortisol in assessing the correct positioning of catheters during AVS. We further hypothesized that any advantage would be most apparent for procedures conducted without cosyntropin stimulation. The purpose of this study was to, therefore, determine the usefulness of AV measurements of metanephrine compared with cortisol concentrations to establish selective cannulation in AVS with and without cosyntropin stimulation.

METHODS

An expanded Methods section is available in the Data Supplement.

SUBJECTS

We included 83 consecutive patients who underwent a total of 86 AVS procedures between 2010 and 2012 at the Radboud University Nijmegen Medical Center and the University Hospital Düsseldorf (Table 1). At the Radboud University Nijmegen Medical Center all AVS procedures were performed under continuous cosyntropin stimulation of 50 µg/hr with sequential catheterization of adrenal veins (N=52). At the University Hospital Düsseldorf all procedures were performed without cosyntropin stimulation with sequential catheterization of adrenal veins. PV samples were collected simultaneously with each AV sample to account for cortisol fluctuations (n=34). Blood was collected with gentle negative pressure and heparinised blood samples were directly stored on ice.

Informed consent was obtained under approved clinical protocols from all patients at Düsseldorf and 35 patients at Nijmegen. In 14 patients at Nijmegen, consent was waived by the local ethics committee. This was in accordance with the applicable rules on reviews by research ethics committees and informed consent.

MEASUREMENTS OF CORTISOL, METANEPHRINES AND CATECHOLAMINES

At the Radboud University Nijmegen Medical Center, cortisol measurements were performed by electrochemiluminescence immunoassays using a Modular E170 analyzer (Roche diagnostics Woerden, the Netherlands). At the University Hospital Düsseldorf

cortisol measurements were performed by an Elecsys analyzer (Roche Diagnostics, Mannheim, Germany). Plasma concentrations of metanephrines and catecholamines were measured at a single central laboratory (Hospital Carl Gustav Carus, Dresden) using liquid chromatography with tandem mass spectrometry[11] or electrochemical detection[12].

DATA AND STATISTICAL ANALYSIS

The cortisol-derived selectivity index was calculated as the concentration of cortisol in AV samples divided by that in PV samples. A cortisol SI of ≥ 3.0 was used to determine successful catheterization.[1] In addition, the effect of lowering this cut-off to ≥ 2.0 was analyzed. The metanephrine-derived selectivity index was calculated from the ratio of AV to PV plasma metanephrine concentrations and the selectivity index cut-off established by receiver operating characteristic (ROC) curve analyses. Ratios of concentrations of metanephrine to normetanephrine and of epinephrine to norepinephrine in AV and PV plasma were also calculated to assess use of these parameters for establishing correct AV catheter positioning. Data are expressed as means and standard deviations or, in case of skewed distributions, as medians and ranges. $P<0.05$ was considered significant.

Table 1. Patient Characteristics

Characteristics	Cosyntropin Stimulated (n=49)	Nonstimulated (n=34)	*P* Value
Male sex	64%	50%	NS
Age, y	52±11	53±13	NS
MAP, mmHg	113±13	114±13	NS
DDD*	3.0 (0–14.0)	3.3 (0–15.0)	NS
Potassium, nmol/L	3.5±0.5	3.4±0.6	NS

Before adrenal vein sampling, interfering medication was stopped according to Endocrine Society guidelines. DDD indicates defined daily dosage (http://www.whocc.no/atc_ddd_index/) at study enrolment; MAP, mean arterial pressure; and NS, nonsignificant. * Median (range).

RESULTS

AV CORTISOL, METANEPHRINE, AND EPINEPHRINE FOR SELECTIVE SAMPLINGS

With a cortisol-derived selectivity index of ≥3.0 to define selective samplings, plasma concentrations of metanephrine and epinephrine were considerably higher (P<0.0001) in right and left AV samples than in PV samples with and without cosyntropin stimulation (Table 2). AV and PV concentrations of cortisol and right AV and PV concentrations of epinephrine were higher (P<0.05) in samplings with than without cosyntropin stimulation. As indicated by ratios of AV to PV concentrations of cortisol, metanephrine, and epinephrine, PV to AV increases in plasma metanephrine and epinephrine were, respectively, 6.1- and 19.0-fold higher (P<0.0001) than those in cortisol (Table 2). The difference in combined left and right AV/PV ratios for metanephrine compared with cortisol was larger (P=0.001) in studies without than with cosyntropin stimulation (9.9 versus 5.4), whereas no difference was present for epinephrine (19.6 versus 18.5).

RATIOS OF METANEPHRINE TO NORMETANEPHRINE AND EPINEPHRINE TO NOREPINEPHRINE

Selective AV samples showed metanephrine to normetanephrine ratios and epinephrine to norepinephrine ratios that were, respectively, 10- and 41-fold higher (P<0.0001) than the ratios in PV samples (Figure S1 in the online-only Data Supplement). The 2.5 and 97.5 percentiles of these ratios in AV samples showed no overlap with those of PV samples.

AV CORTISOL AND METANEPHRINES FOR NONSELECTIVE SAMPLINGS

For AV samples in which a cortisol selectivity index of 3.0 did not confirm correct catheter positioning (Table S1), metanephrine to normetanephrine ratios were within the 2.5 and 97.5 percentiles of ratios for confirmed AV samples in more (P<0.0001) samplings without than with cosyntropin stimulation (89% versus 22%). Similarly, AV/PV ratios of metanephrine were on average 37-fold higher (P<0.0001) without than with cosyntropin stimulation.

Table 2. Adrenal and Peripheral Venous Plasma Concentrations and Adrenal Venous to Peripheral Venous Ratios of Cortisol, Metanephrine, and Epinephrine

	Cosyntropin stimulated		Nonstimulated	
Parameter	**n**	**Median (Range)**	**n**	**Median (Range)**
Cortisol, μg/dL				
PV	52	30 (14–62)*	31	16 (5–37)*†
RAV	44	815 (75–1863)‡	26	251 (32–1265)‡†
LAV	51	451 (114–1403)	24	197 (28–576)†
Metanephrine, pg/mL				
PV	52	30 (13–81)*	31	30 (8–74)*
RAV	44	3276 (174–12 720)‡	26	3493 (189–18 850)
LAV	51	2100 (640–5960)	24	3745 (970–9101)†
Epinephrine, pg/mL				
PV	50	23 (4–369)*	16	13 (3–107)*†
RAV	43	9725 (917–221 104)‡	12	5081 (729–15 678)†
LAV	50	5237 (859–28 765)	14	5837 (801–13 180)
Cortisol AV/PV ratios				
RAV	44	25.3 (3.1–59.2)‡	26	16.7 (4.2–47.6)‡†
LAV	51	12.9 (4.5–54.9)	24	10.4 (3.8–28.5)
Metanephrine AV/PV ratios				
RAV	44	128 (5–551)‡	26	134 (5–582)
LAV	51	73 (17–166)	24	150 (24–324)†
Epinephrine AV/PV ratios				
RAV	43	587 (14–10 858)‡	12	349 (69–1574)
LAV	49	215 (24–912)	13	375 (86–1405)†

To convert to SI units of nmol/L, multiply by 27.59 for cortisol and divide by the molecular weight for metanephrine (197.2) and epinephrine (183.2). Data are shown with and without cosyntropin stimulation for selective samplings according to a cortisol-derived SI of 3.0. AV indicates adrenal vein; LAV, left adrenal vein; PV, peripheral vein; and RAV, right adrenal vein. * $P<0.0001$ different from RAV and LAV. † $P<0.05$ different from corresponding sampling site in cosyntropin-treated patients. ‡ $P<0.0166$ different from LAV.

RELATIONSHIPS OF PLASMA CORTISOL, METANEPHRINE, AND EPINEPHRINE

There were positive ($P<0.01$) relationships of right and left AV plasma cortisol concentrations with both metanephrine and epinephrine concentrations in respective right and left AV samples for procedures with cosyntropin stimulation (Figure 1A and 1B). In contrast,

there were no relationships between plasma cortisol with metanephrine or epinephrine for procedures without cosyntropin stimulation (Figure 1C and 1D). Nevertheless, for both procedures, positive relationships were observed between plasma epinephrine and metanephrine (Figure S2).

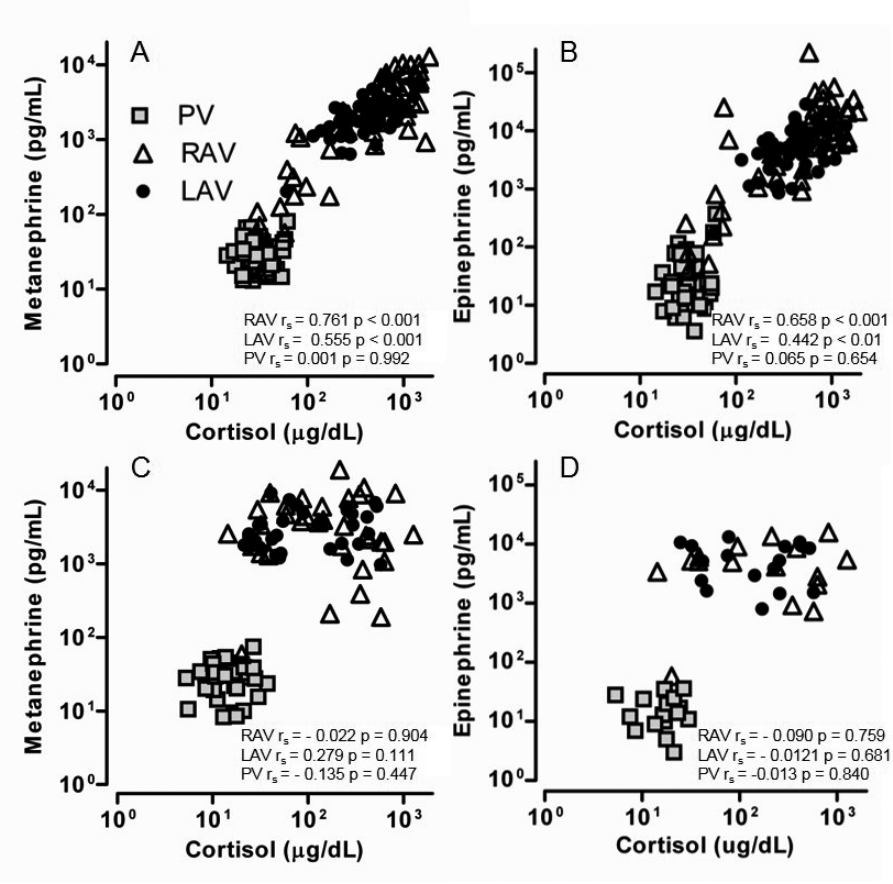


Figure 1. Correlation of plasma metanephrine, plasma epinephrine, and plasma cortisol for cosyntropin-stimulated (upper row: A and B) and nonstimulated (bottom row: C and D) adrenal vein sampling.

Spearman correlation coefficient (rs) is given for each sampling location. Conversion factor to SI units—cortisol (nmol/L): 27.59; epinephrine (pmol/L): 5.454; metanephrine (pmol/L): 5.07. LAV indicates left adrenal vein; PV, peripheral vein; and RAV, right adrenal vein.

RELATIONSHIPS OF AV/PV RATIOS FOR PLASMA METANEPHRINE VERSUS CORTISOL

Significant positive relationships between AV/PV ratios for metanephrine and cortisol were observed for right AV (rs=0.764; 95% confidence interval, 0.62–0.86; P<0.001) and left AV (rs=0.577; 95% confidence interval, 0.36–0.73; P<0.001) samplings with cosyntropin stimulation (Figure 2A). In contrast, there were no relationships between AV/PV ratios for metanephrine and cortisol for right AV (rs=−0.040; 95% confidence interval, −0.30 to 0.37; P=0.41) and left AV (rs=0.229; 95% confidence interval, −0.13 to 0.53; P=0.096) samplings without cosyntropin stimulation (Figure 2B).

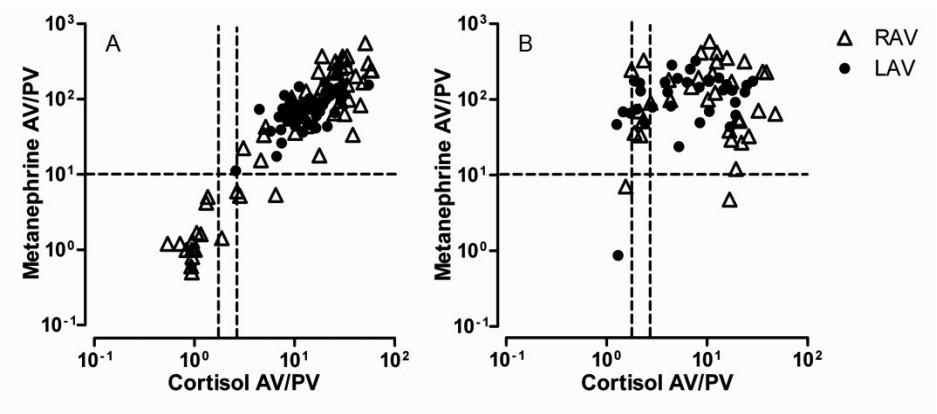


Figure 2. Correlation between cortisol ratio and metanephrine ratio for the cosyntropin-stimulated (A) and nonstimulated (B) samplings.

The cut-off for the cortisol ratio (≥2 and ≥3) and the metanephrine ratio (≥12) is represented by the vertical and horizontal dashed lines, respectively. AV indicates adrenal vein; LAV, left adrenal vein; PV, peripheral vein; and RAV, right adrenal vein.

AVS SELECTIVITY DETERMINED BY PLASMA CORTISOL

AV/PV cortisol ratios ≥3.0 indicated successful final positioning of catheters at both AVS sites in 83% of studies with cosyntropin stimulation, substantially more (P<0.01) than the 56% of studies without stimulation (Table 3). A lower SI cut-off of 2.0 increased (P<0.05) success rates of selective AV catheterizations for nonstimulated samplings to 79% but was without significant effect for cosyntropin-stimulated samplings.

Table 3. Success Rates of Selective AV Samplings With and Without Cosyntropin Stimulation According to Cortisol-Derived and Metanephrine-Derived SI Cut-offs

AVS Procedures	No. (%) Based on Cortisol (Cut-off 3.0)	No. (%) Based on Cortisol (Cut-off 2.0)	No. (%) Based on Metanephrine (Cut-off 12.0)
Cosyntropin stimulated			
RAV	44/52 (85)	46/52 (89)	43/52 (83)
LAV	51/52 (98)	52/52 (100)	51/52 (98)
Bilateral	43/52 (83)	46/52 (89)	43/52 (83)
Nonstimulated			
RAV	26/34 (76)	31/34 (91)*	32/34 (94)*
LAV	24/34 (71)†	29/34 (85)†*	33/34 (97)*
Bilateral	19/34 (56)†	27/34 (79)*	31/34 (91)*

AV indicates adrenal vein; AVS, AV sampling; LAV, left adrenal vein; and RAV, right adrenal vein. * $P<0.05$ higher than corresponding success rates determined by a cortisol-derived cut-off of 3.0. † $P<0.05$ lower than corresponding success rate in cosyntropin-stimulated samplings.

RECEIVER OPERATING CHARACTERISTIC CURVE ANALYSIS TO DETERMINE THE AVS SELECTIVITY INDEX OF METANEPHRINE

Receiver operating characteristic curve analyses exploring the performance of cosyntropin-stimulated AV/PV ratios of metanephrine to assess the selectivity of AVS sampling, with a cortisol selectivity index of 3.0 as the reference index, established an area under the curve of 0.999 (Figure S3). In contrast, the area under the curve for nonstimulated samplings was only 0.673 and not significantly improved using a cortisol selectivity index of 2.0 (0.702). Using the receiver operating characteristic curve for stimulated samplings, an AV/PV selectivity index of between 11.3 and 15.3 for metanephrines provided optimal sensitivity (99%) and specificity (100%), with no difference in either sensitivity or specificity within this selectivity index range to establish selective sampling. A selectivity index of 12 was, therefore, chosen to maintain high sensitivity (Figure S3).

AVS SELECTIVITY DETERMINED BY PLASMA METANEPHRINE VERSUS CORTISOL

Using the selectivity index cut-offs of ≥3 for cortisol and ≥12 for metanephrine, there was disagreement in the assessment of correct catheter positioning in only 1 of the total 113 left

and right AV samples obtained with consyntropin stimulation (Figure 2A). This translated to a concordance rate for bilateral successful catheterization of 98% (51/52), reflecting no difference in the overall success of AV samplings determined by cortisol (83%) or metanephrine (83%) for studies with consyntropin stimulation (Table 3).

For procedures without cosyntropin stimulation, there was disagreement in the assessment of catheter positioning according to cortisol and metanephrine in 17 of the 68 samplings (Figure 2B); in all except 1 case, this involved AV/PV ratios below the cut-off of 3.0 for cortisol and >12.0 for metanephrine. This translated to a concordance rate of only 59% (20/34) for establishing bilateral success of AVS, substantially lower ($P<0.0001$) than that of 98% for cosyntropin-stimulated samplings. Using metanephrine, AVS was assessed as bilaterally successful in 91% of samplings, considerably more ($P<0.01$) than the 56% (19/34) using the cut-off of 3.0 for cortisol (Table 3). Using a lower cut-off of 2.0 improved successful bilateral selectivity to 79%; nevertheless, for 5 of the 7 samplings with AV/PV ratios of cortisol <2.0, AV/PV ratios of metanephrine were between 36 and 244, well above the cut-off of 12 (Figure 2B and Table S1).

DISCUSSION

This study establishes novel use of plasma metanephrine as a more sensitive alternative to cortisol to assess the selectivity of AVS. Plasma metanephrine is particularly useful during AVS performed without cosyntropin stimulation for several reasons: (1) excellent agreement between use of cortisol and metanephrine in samplings performed with but not without cosyntropin stimulation; (2) larger step-ups in PV to AV plasma concentrations of metanephrine relative to cortisol; and (3) higher rates of success for establishing AVS selectivity using metanephrine than cortisol in nonstimulated samplings.

In agreement with emerging findings from other groups,[5,6,13,14] the above considerations conversely imply that cortisol provides a less than optimal parameter to establish selective catheterization in nonstimulated sequential AVS procedures. This conclusion is reinforced by our findings that metanephrine to normetanephrine and epinephrine to norepinephrine ratios in most nonstimulated AV samples designated nonselective, based on a cortisol-derived selectivity index of 3.0, were well above the range for ratios in PV samples and within the range for the AV samples designated as selective. These shortcomings in use of cortisol to

indicate the selectivity of AVS are further indicated by the complete lack of relationships between AV plasma cortisol with metanephrine or epinephrine during procedures without cosyntropin stimulation.

There are several reasons why the advantages of plasma metanephrine compared with cortisol for confirming correct positioning of AV catheters are most apparent for procedures without cosyntropin stimulation. First, as demonstrated by others,[4-6] adrenal secretion of cortisol fluctuates so that AV plasma concentrations during periods of low secretion may be only slightly higher than those in peripheral plasma, providing the rationale for cosyntropin stimulation. In contrast, metanephrine is produced continuously within adrenal medullary cells from epinephrine leaking from storage vesicles, a process that is independent of fluctuations in epinephrine release.[7,9,10] Second, without cosyntropin stimulation, up to a third of circulating cortisol may be produced and released from extra-adrenal locations, particularly hepatosplanchnic sites.[15,16] This extra-adrenal source contributes to peripheral cortisol levels and potentially affects the selectivity index. Furthermore, a previous study showed that admixture of blood from the accessory hepatic veins into AVs lowers the selectivity index of cortisol.[17] In contrast, >90% of all circulating metanephrine is produced within the adrenals, with <10% produced from epinephrine after release.[7,10] Third, cortisol is cleared from the circulation slowly, resulting in high peripheral plasma concentrations relative to rates of secretion and consequently relatively small step-ups in concentrations from PV to AV sites of release that are more easily detected by stimulating secretion with cosyntropin.[18,19] In contrast, metanephrine is cleared rapidly from the circulation so that PV concentrations are maintained at much lower levels compared with those at AV sites where most of the metabolite enters the systemic circulation.[7,10]

All the above factors likely contribute to the consistently high gradients in PV to AV plasma concentrations of metanephrine, which provide an opportunity for more accurate and sensitive detection of selective AV catheterization than the smaller gradients for cortisol or other substances evaluated for this purpose, such as chromogranin.[8,20] Additional consideration of the much higher ratios of metanephrine to normetanephrine in AV than PV plasma provides a further means for confirming correct positioning of AV catheters. Because measurements of metanephrine are commonly performed together with normetanephrine, the additional use of metanephrine to normetanephrine ratios offers another advantage of measuring these metabolites not possible with measurements of cortisol.

Although others have proposed measurements of epinephrine to assess the selectivity of AVS and although PV to AV gradients in plasma epinephrine are larger than those in metanephrine,

we nevertheless recommend metanephrine for two reasons. First, epinephrine, like cortisol, is a stress hormone that exhibits extreme physiological fluctuations, whereas metanephrine does not.[5,9,10,21] Second, metanephrines are more stable than catecholamines, so that more care must be taken with blood collections for the latter than the former.[22,23]

In addition to stimulating release of cortisol, cosyntropin increases adrenal blood flow and release of epinephrine,[24,25] which could influence adrenal medullary and cortical-derived indices of AV selectivity. Our findings of higher plasma concentrations of epinephrine with cosyntropin stimulation are consistent with effects on adrenal medullary function. Nevertheless, lack of influence of cosyntropin on metanephrine indicates that the influence does not extend to the metabolite, an expected observation given the independent nature of chromaffin cell epinephrine metabolism and exocytotic release.

The present study had some methodological limitations. First, the study did not incorporate a prospective, randomized design comparing therapeutic outcomes according to cortisol with metanephrine because this was not possible without proof that metanephrine was at least as good as cortisol in indicating the selectivity of AVS. Second, stimulated and nonstimulated AVS procedures were performed at two different centres with measurements of cortisol by different methods. Nevertheless, this was unlikely to have influenced results because the two methods yield comparable results.[26] A third limitation was the use of plasma cortisol selectivity ratios as the reference standard. All AVS selectivity indices used in both research and clinical practice are arbitrary because they have not been formally linked to outcome data in an evidence-based manner. However, the cut-offs used in our study are commonly used and recommended in the literature.[2,27]

PERSPECTIVES

In view of the importance of primary aldosteronism as a cause of hypertension, an accurate diagnosis of the site of excess aldosterone production is pivotal. AV sampling is recommended as the reference test to differentiate between unilateral and bilateral excess aldosterone production. However, technical success depends on correct positioning of sampling catheters in AVs, verified using measurements of cortisol. This study shows that these measurements fail to verify correct positioning of catheters in a substantial number of procedures performed without cosyntropin, a failing that can be overcome by measurements of plasma metanephrine. Should improved therapeutic outcomes using metanephrine be established in a prospective study, cosyntropin stimulation may become redundant and AV sampling less laborious and more diagnostically accurate than currently practiced.

Nevertheless, in this event it must be recognized that the wider availability of measurements of plasma metanephrines is required for their routine use in AVS to be fully realized.

ACKNOWLEDGMENTS

We express our gratitude to Daniela Pelzel for technical assistance. We also thank Martin Reincke and the Else-Kröner-Fresenius German Conn Registry.

SOURCES OF FUNDING

This study was supported by a grant from the Deutsche Forschungsgemeinschaft to H.S. Willenberg, J.W.M. Lenders, and G. Eisenhofer (WI 3660/1-1, KFO252) and a grant from ZonMW DoelmatigheidsOnderzoek 2010–2012 E&K (171002102) to J. Deinum. Grant providers had no influence on the content of the article.

SUPPLEMENTAL DATA

EXPANDED METHODS

SUBJECTS

We included 83 consecutive patients who underwent a total of 86 AVS procedures between 2010 and 2012 at the Radboud University Nijmegen Medical Centre and the University Hospital Düsseldorf. AVS was performed to differentiate between unilateral and bilateral primary aldosteronism (n=77), to examine the functional state of an incidentaloma (n=4), to evaluate bilateral adrenal masses in subclinical Cushing's syndrome (n=1) and to assess non-classic (late-onset) congenital adrenal hyperplasia (n=1). Informed consent was obtained under approved clinical protocols from all patients at Düsseldorf and 35 patients at Nijmegen. In 14 patients at Nijmegen consent was waived by the local ethics committee. This was in accordance with the applicable rules concerning reviews by research ethics committees and informed consent.

ADRENAL VENOUS SAMPLING

Prior to AVS, interfering medications were discontinued in accordance with current guidelines.[1] AVS was performed after an overnight fast and at least three hours of bed rest. In cases of primary aldosteronism, hypokalemia, if present, was corrected with oral or intravenous potassium supplementation before AVS. At the Radboud University Nijmegen Medical Centre all AVS procedures were performed under continuous ACTH stimulation of 50 µg/hr with sequential catheterization of both adrenal veins (N=52). At the University Hospital Düsseldorf all procedures were performed without ACTH stimulation, with sequential catheterization of adrenal veins and simultaneous collection of PV and AV samples (N=34). Blood was collected by gravity or with gentle negative pressure. Cortisol assays were performed during procedures using rapid measurements to confirm correct catheter placement, with measurements subsequently repeated according the methods outlined below for more accurate measurement. Blood samples were immediately stored on ice in lithium-heparin tubes and within 1 hour centrifuged at 3500g at 4°C for 10 minutes. Thereafter plasma was stored at -80°C.

MEASUREMENTS OF CORTISOL, METANEPHRINES AND CATECHOLAMINES

At the Radboud University Nijmegen, cortisol measurements were performed by electro-chemiluminescence immunoassays using a Modular E170 analyzer (Roche diagnostics Woerden, the Netherlands). Inter-assay coefficients of variation (CVs) were 2.3-3.8%. At the UHD cortisol measurements were performed by an Elecsys analyzer (Roche Diagnostics, Mannheim, Germany) with an inter-assay CV 6.1 %. Sample dilutions, performed to bring cortisol concentrations within the assay range, were carried out using the kit buffers with maintained CVs. Plasma concentrations of metanephrine and normetanephrine were measured by liquid chromatography with tandem mass spectrometry following sample purification using a solid phase extraction 96 well plate format.[11] Inter-assay CVs for metanephrines ranged from 3.7% at high plasma concentrations to 13.5% at low concentrations. Sample dilutions were not required for these measurements.

Plasma concentrations of norepinephrine and epinephrine — along with additional measurements of the catecholamine precursor, dihydroxyphenylalanine (DOPA), and the metabolites dihydroxyphenylglycol (DHPG) and dihydroxyphenylacetic acid (DOPAC) — were measured by liquid chromatography with electrochemical detection after batch alumina extraction.[12] DHPG and DOPAC are present in plasma at higher concentrations than the catecholamines, have a relatively narrower concentration range, and are similarly sensitive to oxidative degradation as the catecholamines. Their measurement in this study thereby enabled assessment of this potential source of artefact and exclusion of catecholamine measurements in 2 out of 52 cosyntropin-stimulated and 17 out of 34 non-stimulated samplings. Inter-assay CVs for plasma catecholamines ranged from 2.5% to 11.0%. Sample dilutions were also not required for these measurements.

In studies involving cosyntropin stimulation, additional data from 9 non-selective (SI < 3.0) blood samples (8 right AV and 1 left AV) obtained from AVS procedures in which further searching for the adrenal vein subsequently yielded selective sampling results, were included in the analysis. These additional non-selective samplings were included to delineate analyte concentrations at non selective sampling sites and establish relationships between AV concentrations and AV:PV ratios of cortisol and metanephrine.

DATA ANALYSIS

The cortisol-derived selectivity index was calculated as the concentration of cortisol in AV samples divided by that in PV samples. For both cosyntropin-stimulated and non-

stimulated samplings, a cortisol selectivity index of ≥ 3.0 was used to determine successful catheterization. In addition, the effect of lowering this cut-off to ≥ 2.0 was analyzed. The metanephrine-derived selectivity index was similarly calculated as the ratio of the AV to PV plasma concentrations of metanephrine, with the selectivity index cut-off cut-off established by receiver operating characteristic (ROC) curve analyses according to established procedures.[28,29] A cortisol derived SI of ≥3.0 was utilized as the gold standard to establish which samples were taken from correctly positioned catheters. Ratios of concentrations of metanephrine to normetanephrine and of epinephrine to norepinephrine in AV and PV plasma were also calculated to assess use of these parameters for additionally establishing correct AV catheter positioning.

STATISTICAL ANALYSIS

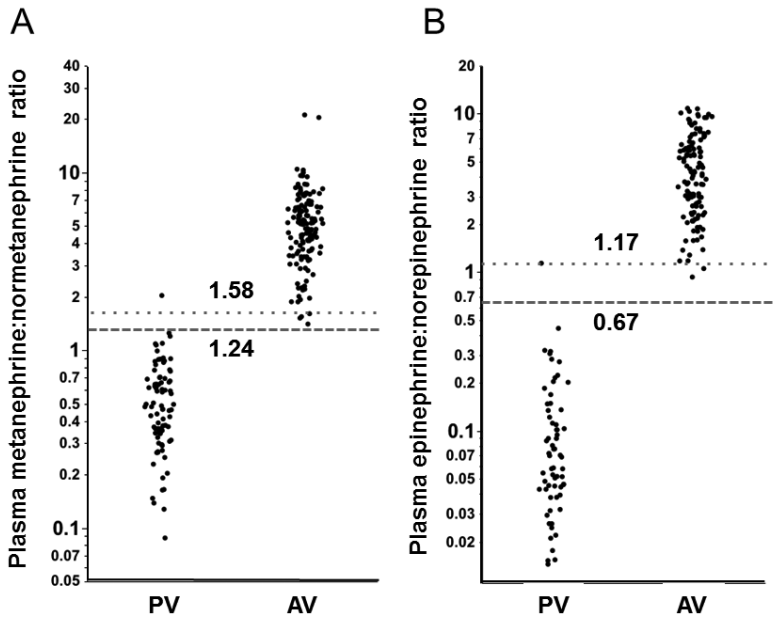
Data are expressed as means and standard deviations or, in case of skewed distributions, as medians and ranges. Mann-Whitney U, Kruskal-Wallis or Wilcoxon matched paired sign-rank tests were used to assess significance of differences in variables at the three sampling sites, or between groups. A Bonferroni-adjusted P-value ($P_{adjusted} = 0.05/3 = 0.0167$) was used to determine significance for differences among the three sampling sites. For other differences, a $P<0.05$ was considered significant. Relationships between cortisol, metanephrine and epinephrine were assessed by one tailed Spearman's correlation coefficient (rs).Differences between AVS success rates determined by cortisol and metanephrine derived SIs in cosyntropin-stimulated and non-stimulated AVS were determined by McNemar and Chi-square tests according to whether comparisons were paired or non-paired. Corresponding 95% confidence intervals (95% CI) for non-paired comparisons were calculated using the Wilson Score Method without continuity correction. Statistical analyses utilised the JMP statistics software package (SAS Institute Inc, Cary, NC), GraphPad Prism 4.0 and SPSS 18.0 for windows.

EXPANDED RESULTS

Supplemental table S1. Adrenal Venous to Peripheral Venous Cortisol and Metanephrine Ratios and Adrenal Venous Metanephrine to Normetanephrine Ratios for Non-selective Samplings (AV:PV cortisol ratio <3.0)

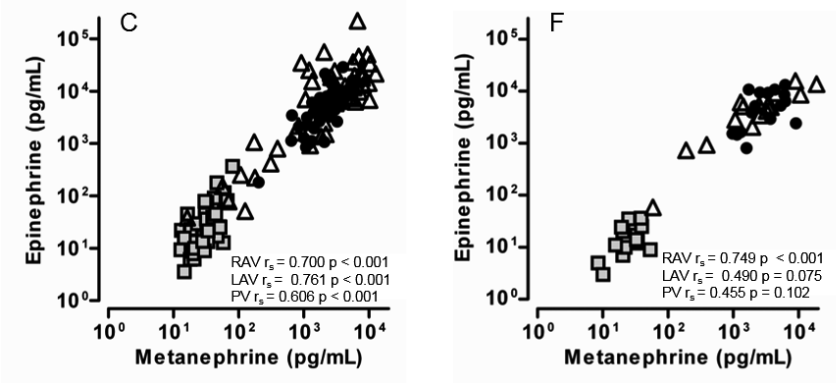
Subject	AV sampling side	AV:PV cortisol ratio	AV MN: NMN ratio	AV:PV metanephrine ratio
Cosyntropin stimulated samplings				
1	L	2.59	2.13 *	11.2
2	R	0.83	0.44	1.0
3	R	2.85	2.35 *	5.2
4	R	1.88	0.59	1.4
5	R	2.66	3.62 *	6.0
6	R	1.05	0.91	1.7
7	R	1.36	1.12	5.0
8	R	1.16	1.36	1.6
9†	R	0.54	0.37	1.2
10†	R	0.95	1.11	1.3
11	R	1.31	2.47 *	4.2
12†	R	0.94	0.44	1.0
13†	R	0.94	0.49	0.5
14†	R	0.95	0.43	0.8
15†	R	0.72	1.37	1.2
16†	R	1.01	0.65	1.0
17†	L	0.90	0.90	0.9
18	R	0.93	0.42	0.6
Non-stimulated samplings				
1	R	2.74	6.64 *	90.5
2	R	2.28	3.40 *	51.4
2	L	1.46	3.02 *	68.6
3	R	2.11	2.00 *	69.6
4	L	2.18	6.24 *	129.0
5	L	2.13	5.52 *	164.8
6	R	1.76	4.72 *	244.3
7	L	1.88	1.92 *	174.7
8	R	1.55	1.06	7.1
9	L	1.72	4.71 *	65.8
10	L	2.85	7.37 *	79.1
11	R	1.89	2.87 *	36.2
11	L	1.26	3.39 *	46.8
12	L	2.41	1.81 *	48.5
13	R	2.18	3.99 *	33.5
14	L	1.30	0.56	0.9
15	R	2.33	7.00 *	326.9
16	L	2.00	4.57 *	74.4

Abbreviations: AV, adrenal venous; PV, peripheral venous; MN, metanephrine; NMN, normetanephrine, R, right; L, left. * Indicates an AV MN:NMN ratio within the 2.5 to 97.5 percentiles of those determined for selectively positioned AV catheters (see Figure S1). † Indicates a subject in whom initial non-selective sampling was followed by a selective sampling result.



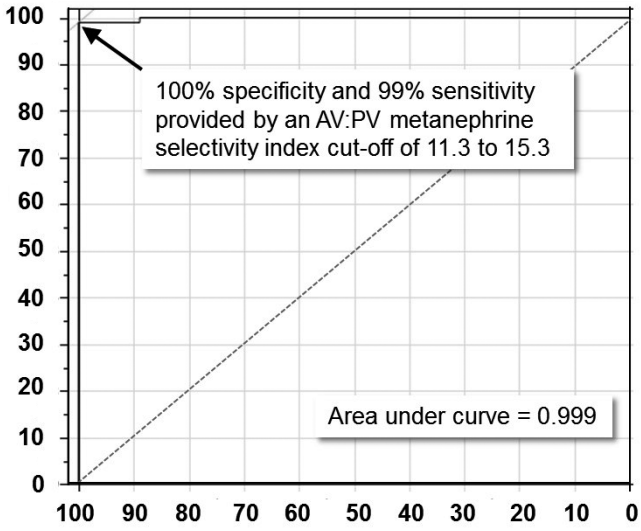
Supplemental Figure S1

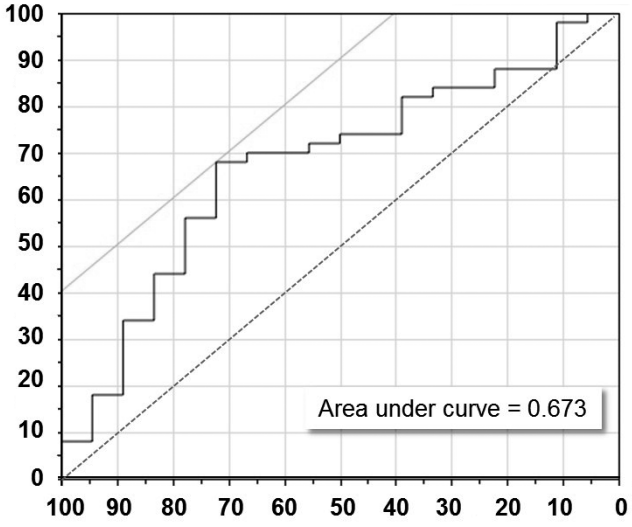
Legend to supplemental figure S1. Peripheral venous (PV) and adrenal venous (AV) plasma metanephrine to normetanephrine ratios (panel A) and plasma epinephrine to norepinephrine ratios (panel B) for selective samplings. Dotted line: 2.5 percentile of the AV samples. Dashed line: 97.5 percentile of the PV samples. The 2.5 and 97.5 percentiles of these ratios in adrenal venous samples showed no overlap with those for peripheral venous samples.



Supplemental Figure S2

Legend to supplemental figure S2. Correlation of plasma metanephrine and plasma epinephrine for cosyntropin-stimulated (panel A) and non-stimulated (Panel B) adrenal venous samplings. Spearman correlation coefficients (r_s) are shown for each sampling location. RAV = right adrenal vein; LAV = left adrenal vein; PV = peripheral vein. Conversion factor to SI units: Epinephrine (pmol/l): 5.45: Metanephrine (pmol/l): 5.07.





Supplemental Figure S3

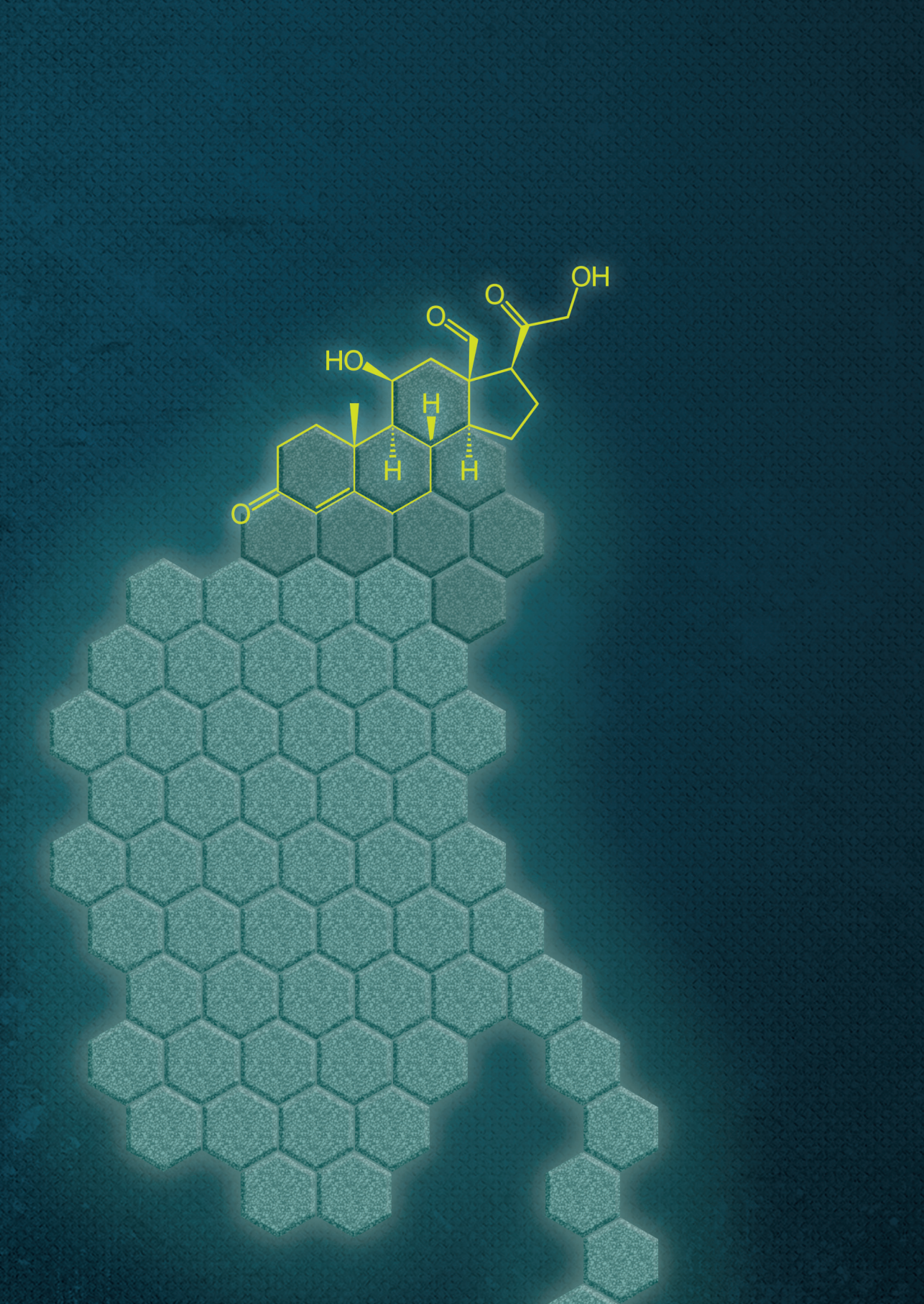
Legend to supplemental figure S3. ROC curve analysis exploring the diagnostic performance of cosyntropin-stimulated (Panel A) and non-stimulated (Panel B) AV:PV ratios of metanephrine to assess selectivity of AVS sampling, according to a cortisol selectivity index of ≥ 3.0. The AV:PV metanephrine ratio most appropriate to indicate selective adrenal venous sampling was established for the point on the ROC curve for cosyntropin-stimulated sampling that provided both optimal diagnostic sensitivity (99%) and specificity (100%). This point corresponded to an AV:PV ratio between 11.3 and 15.3.

REFERENCES

1. Funder JW, Carey RM, Fardella C, et al. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. The Journal of clinical endocrinology and metabolism 2008;93:3266-81.
2. Daunt N. Adrenal vein sampling: how to make it quick, easy, and successful. Radiographics : a review publication of the Radiological Society of North America, Inc 2005;25 Suppl 1:S143-58.
3. Vonend O, Ockenfels N, Gao X, et al. Adrenal venous sampling: evaluation of the German Conn's registry. Hypertension 2011;57:990-5.
4. Spark RF, Kettyle WR, Eisenberg H. Cortisol dynamics in the adrenal venous effluent. The Journal of clinical endocrinology and metabolism 1974;39:305-10.
5. Seccia TM, Miotto D, Battistel M, et al. A stress reaction affects assessment of selectivity of adrenal venous sampling and of lateralization of aldosterone excess in primary aldosteronism. European journal of endocrinology / European Federation of Endocrine Societies 2012;166:869-75.
6. Tanemoto M, Suzuki T, Abe M, Abe T, Ito S. Physiologic variance of corticotropin affects diagnosis in adrenal vein sampling. European journal of endocrinology / European Federation of Endocrine Societies 2009;160:459-63.
7. Eisenhofer G, Rundquist B, Aneman A, et al. Regional release and removal of catecholamines and extraneuronal metabolism to metanephrines. The Journal of clinical endocrinology and metabolism 1995;80:3009-17.
8. Seccia TM, Miotto D, De Toni R, et al. Chromogranin a measurement for assessing the selectivity of adrenal venous sampling in primary aldosteronism. The Journal of clinical endocrinology and metabolism 2011;96:E825-9.
9. Eisenhofer G, Keiser H, Friberg P, et al. Plasma metanephrines are markers of pheochromocytoma produced by catechol-O-methyltransferase within tumors. The Journal of clinical endocrinology and metabolism 1998;83:2175-85.
10. Eisenhofer G, Friberg P, Pacak K, et al. Plasma metadrenalines: do they provide useful information about sympatho-adrenal function and catecholamine metabolism? Clinical science (London, England : 1979) 1995;88:533-42.
11. Peitzsch A, Prejbisz A, Kroiß M, et al. Analysis of plasma 3-methoxytyramine, normetanephrine and metanephrine by ultra performance liquid chromatography - tandem mass spectrometry: utility for diagnosis of dopamine-producing metastatic phaeochromocytoma. Ann Clin Biochem 2012;„in press".

12. Eisenhofer G, Goldstein D, Kopin I. Simultaneous liquid-chromatographic determination of plasma catecholamines and metabolites. Clinical chemistry 2009;55:2223-4.
13. Hiraishi K, Yoshimoto T, Tsuchiya K, et al. Clinicopathological features of primary aldosteronism associated with subclinical Cushing's syndrome. Endocrine journal 2011;58:543-51.
14. Baba Y, Hayashi S, Nakajo M. Are catecholamine-derived indexes in adrenal venous sampling useful for judging selectivity and laterality in patients with primary aldosteronism? Endocrine 2012.
15. Basu R, Singh RJ, Basu A, et al. Splanchnic cortisol production occurs in humans: evidence for conversion of cortisone to cortisol via the 11-beta hydroxysteroid dehydrogenase (11beta-hsd) type 1 pathway. Diabetes 2004;53:2051-9.
16. Basu R, Basu A, Grudzien M, et al. Liver is the site of splanchnic cortisol production in obese nondiabetic humans. Diabetes 2009;58:39-45.
17. Miotto D, De Toni R, Pitter G, et al. Impact of accessory hepatic veins on adrenal vein sampling for identification of surgically curable primary aldosteronism. Hypertension 2009;54:885-9.
18. Seccia TM, Miotto D, De Toni R, et al. Adrenocorticotropic hormone stimulation during adrenal vein sampling for identifying surgically curable subtypes of primary aldosteronism: comparison of 3 different protocols. Hypertension 2009;53:761-6.
19. Rossi GP, Pitter G, Bernante P, Motta R, Feltrin G, Miotto D. Adrenal vein sampling for primary aldosteronism: the assessment of selectivity and lateralization of aldosterone excess baseline and after adrenocorticotropic hormone (ACTH) stimulation. Journal of hypertension 2008;26:989-97.
20. Seccia TM, Miotto D, De Toni R, et al. Subtyping of primary aldosteronism by adrenal vein sampling: effect of acute D(2) receptor dopaminergic blockade on adrenal vein cortisol and chromogranin A levels. European journal of endocrinology / European Federation of Endocrine Societies 2011;165:85-90.
21. Raber W, Raffesberg W, Bischof M, et al. Diagnostic efficacy of unconjugated plasma metanephrines for the detection of pheochromocytoma. Archives of internal medicine 2000;160:2957-63.
22. Ross HA, Lenders JW, Sweep FC. A study of longer-time stability of plasma free metanephrines. Ann Clin Biochem 2011;48:270-1.
23. Willemsen JJ, Sweep CG, Lenders JW, Ross HA. Stability of plasma free metanephrines during collection and storage as assessed by an optimized HPLC method with electrochemical detection. Clinical chemistry 2003;49:1951-3.
24. Fenske M, Fuchs E, Probst B. Corticosteroid, catecholamine and glucose plasma levels in rabbits after repeated exposure to a novel environment or administration of (1-24) ACTH or insulin. Life sciences 1982;31:127-32.

25. Valenta LJ, Elias AN, Eisenberg H. ACTH stimulation of adrenal epinephrine and norepinephrine release. Horm Res 1986;23:16-20.
26. Bieglmayer C, Chan DW, Sokoll L, et al. Multicentre performance evaluation of the E170 module for modular analytics. Clinical chemistry and laboratory medicine : CCLM / FESCC 2004;42:1186-202.
27. Stowasser M, Gordon RD, Rutherford JC, Nikwan NZ, Daunt N, Slater GJ. Diagnosis and management of primary aldosteronism. Journal of the renin-angiotensin-aldosterone system : JRAAS 2001;2:156-69.
28. Florkowski CM. Sensitivity, specificity, receiver-operating characteristic (ROC) curves and likelihood ratios: communicating the performance of diagnostic tests. The Clinical biochemist Reviews 2008;29 Suppl 1:S83-7.
29. Receiver operating characteristic (ROC) analysis. Evaluating discriminance effects among descision support systems 2001. at https://www8.cs.umu.se/research/reports/show.cgi?year=2001&nr=018.)


OH
O
O
HO
H
H
H
O

Adrenal nodularity and somatic mutations in primary aldosteronism: one node is the culprit?

Tanja Dekkers[1] | Marja ter Meer[2] | Jacques W.M. Lenders[1] | Ad R.M.M. Hermus[3]
Leo J. Schultze Kool[4] | Johannes F. Langenhuijsen[5] | Koshiro Nishimoto[6]
Tadashi Ogishima[8] | Kuniaki Mukai[7] | Elena Aisha Azizan[9] | Bas Tops[2] | Jaap Deinum J[1]
Benno Kusters[2,10]

[1] Department of Internal Medicine, Radboud University Nijmegen Medical Center, the Netherlands
[2] Department of Pathology, Radboud University Nijmegen Medical Center, the Netherlands
[3] Department of Endocrinology, Radboud University Nijmegen Medical Center, the Netherlands
[4] Departement of Radiology, Radboud University Nijmegen Medical Center, the Netherlands
[5] Department of Urology, Radboud University Nijmegen Medical Center, the Netherlands;
[6] Department of Urology, School of Medicine, Keio University, Tokyo, Japan
[7] Department of Biochemistry, School of Medicine, Keio University, Tokyo, Japan;
[8] Department of Chemistry, Faculty of Sciences, Kyushu University, Fukuoka, Japan;
[9] Clinical Pharmacology Unit, Department of Medicine, University of Cambridge, Addenbrooke's Centre for Clinical Investigation, United Kingdom;
[10] Department of Pathology, Maastricht University Medical Center, the Netherlands.

J Clin Endocrinol Metab. 2014 Jul;99(7):E1341-51.

ABSTRACT

Context. Somatic mutations in genes that influence cell entry of calcium have been identified in aldosterone-producing adenomas (APAs) of adrenal cortex in primary aldosteronism (PA). Many adrenal glands removed for suspicion of APA do not contain a single adenoma but nodular hyperplasia.

Objective. The objective of the study was to assess multinodularity and phenotypic and genotypic characteristics of adrenals removed because of the suspicion of APAs.

Design and Methods. We assessed the adrenals of 53 PA patients for histopathological characteristics and immunohistochemistry for aldosterone (P450C18) and cortisol (P450C11) synthesis and for KCNJ5, ATP1A1, ATP2B3, and CACNA1D mutations in microdissected nodi.

Results. Glands contained a solitary adenoma in 43% and nodular hyperplasia in 53% of cases. Most adrenal glands contained only one nodule positive for P450C18 expression, with all other nodules negative. KCNJ5 mutations were present in 22 of 53 adrenals (13 adenoma and nine multinodular adrenals). An ATP1A1 and a CACNA1D mutation were found in one multinodular gland each and an ATP2B3 mutation in five APA-containing glands. Mutations were always located in the P450C18-positive nodule. In one gland two nodules containing two different KCNJ5 mutations were present. Zona fasciculata-like cells were more typical for KCNJ5 mutation-containing nodules and zona glomerulosa-like cells for the other three genes.

Conclusions. Somatic mutations in KCNJ5, ATP1A1, or CACNA1D genes are not limited to APAs but are also found in the more frequent multinodular adrenals. In multinodular glands, only one nodule harbours a mutation. This suggests that the occurrence of a mutation and nodule formation are independent processes. The implications for clinical management remain to be determined.

INTRODUCTION

Classically, endocrinologists consider the cause of primary aldosteronism to be either a unilateral aldosterone producing (micro) adenoma (APA) or bilateral adrenal hyperplasia (BAH). The first is best treated by laparoscopic adrenalectomy, while the latter requires therapy with mineralocorticoid receptor antagonists.[1] Correct preoperative diagnosis of an APA is confirmed by improvement or cure of hypertension and hypokalemia, the hallmarks of aldosteronism, after unilateral adrenalectomy. Numerous authors also regard the presence of an APA at pathological examination proof of a correct preoperative diagnosis and claim to find single adenomas in all excised glands.[2-5] However, in our experience and that of others, in many cases the removed gland does not contain a single adenoma, but demonstrates various patterns of macronodular or micronodular hyperplasia.[6-16]

Adrenal glands removed because of suspicion of APA have other remarkable features. Many nodules do not have the appearance of aldosterone producing zona glomerulosa (ZG) cells, as would be expected, but of zona fasciculata (ZF) cells, which normally produce cortisol.[6,17] Immunohistochemically, adrenal nodules may express both p450C11, or cortisol synthase, encoded by *CYP11B1*, and p450C18, or aldosterone synthase, encoded by *CYP11B2*, suggesting that they are capable to produce both cortisol and aldosterone.[13,18-20] Furthermore, in the surrounding pre-existent cortical tissue, small extra-nodular cell clusters are observed with strong p450C18 and no p450C11 expression, which leave normal cortex zonation intact.[18] The function of these so called aldosterone producing cell clusters (APCCs), which are present in both normal and pathological conditions, is unknown.[13,18] Another striking finding in the surrounding pre-existent adrenal tissue in APAs is the almost ubiquitous presence of zona glomerulosa thickening where atrophy would be expected.[21]

An explanation for some histopathological findings might be found in the recently discovered somatic mutations of *KCNJ5*, *ATP1A1*, *ATP2B3* and *CACNA1D* in adrenal glands.[22-25] *KCNJ5*, first described in APAs by *Choi et al*., encodes the inward rectifying potassium channel Kir3.4 that is present in the adrenal cortex and when mutated generates calcium influx of the adrenocortical cell, thus inducing activation of aldosterone synthesis.[22] In addition, *Choi et al*. hypothesize that these mutations in *KCNJ5* promote growth of

aldosterone secreting cells into APAs, since the mutation was present in 8 of 20 APAs studied. Other researchers confirmed the presence of the somatic *KCNJ5* mutations in about 20-40% of resected APAs.[8,23,25-31] The recently discovered *ATP1A1, ATP2B3* and *CACNA1D* mutations, accounting for about 7% of resected APAs, are also likely to increase intracellular calcium.[23,24]

Until now, most studies on these mutations have been performed on reportedly solitary APAs and lack data of the so often present hyperplasia.[26-30] One study that assessed additional hyperplasia in the resected adrenals found *KCNJ5* mutations in 40% of the samples classified as adenoma with associated hyperplasia.[8] However, this study lacks histopathological details of the glands that did or did not contain a *KCNJ5* mutation. A histopathological feature that has been reported is that adenoma tissue with the *KCNJ5* mutation resembles ZF-cells and that adenoma tissue with *ATP1A1* and *CACNA1D* mutations resembles ZG-cells.[25,27] This led us to systematically assess multinodularity and phenotypic and genotypic characteristics of adrenals removed because of suspicion of APA.

METHODS

SUBJECTS

We re-examined all retrievable adrenals (n=53) of patients with PA operated in the Radboud University Medical Center Nijmegen between 1997 and 2010 (n=65). All included patients had hypertension resistant to three or more drugs and/or hypertension accompanied by hypokalemia. The diagnosis of primary aldosteronism was confirmed by intravenous saline infusion test (SIT; n=45), oral salt loading test (n=3) or captopril suppression test (n=1) performed after cessation of medication and correction of hypokalemia in accordance to the current guidelines.[1] In four patients (nr.2, 5, 24 and 29, Table 3) no confirmation test was performed because of the potential risk of medication withdrawal that is necessary to create optimal conditions for a correct interpretation of the test results. In these patients the diagnosis was based on the triad of hypertension, hypokalemia and an increased aldosterone-to-renin ratio (ARR). The diagnosis of unilateral APA was based on adrenal venous sampling (n=36) or CT-scan (n=17), which was used because AVS was not yet available (before 2004, n=9), considered too hazardous because of the need for medication withdrawal (n=2) or

was unsuccessful (n=6). AVS was performed under continuous ACTH stimulation (5 µg/hr) using a selectivity index of ≥ 2.0 and a lateralization index of ≥ 4.0. For a lateralization index between 3.0 and 4.0 the decision to operate was reached by consensus, based on clinical details and CT-scan results (n=3). Post-operative follow-up information was available for three months in 7 and for at least one year in 46 patients. We defined outcome of surgery as either cured, improved or failed (Table 1).[32] The study was approved by the Medical Ethics Committee who waived the requirement for informed consent, absent in earlier cases, as use of anonymous or coded leftover material for scientific purposes is part of the standard treatment contract with patients in hospitals in the Netherlands. However, they set the condition that no genotyping of normal tissues (i.e. germline genotyping) was to be performed.

Table 1. Patient Characteristics. N=53

	Included (n=53)	Not included (n=12)	p
Gender: male/female	30/23	5/7	NS
Age	50±10	43±13	NS
BMI	27.4±5.0	26.4±4.6	NS
SBP (mmHg)	168±26	182±32	NS
DBP (mmHg)	99±13	104±18	NS
DDD*	4.0 (0.3-9.7)	2.3 (0.0-3.7)	< 0.01
Potassium (mmol/l)	3.2±0.6	3.4±0.5	NS
Aldosterone (nmol/l) *	0.78 (0.34 – 2.20)	0.85 (0.46-1.30)	NS

* median (range), DDD: defined daily dosages of antihypertensive medication (http://www.whocc.no/atc_ddd_index/)

HISTOPATHOLOGICAL PHENOTYPE

All adrenal glands resected were cut into 4-mm-thick slices after formaline fixation. These slices were assessed macroscopically, including the description of nodularity. Representative material was sampled for microscopical evaluation. These hematoxylin and eosin (HE) slides of all adrenal glands were assessed twice by an experienced pathologist (B.K.), who was blinded to patient characteristics and genotype results. Histopathological phenotyping of the glands consisted of assessment of zona glomerulosa thickening (continuous ZG and/or ZG thickness ≥200µm as measured by a micrometer), nodule diameter and the cellular

composition of the nodule(s) (Figure 1). The cellular composition of the lesions was determined to be ZG-like (predominantly compact cells), ZF-like (predominantly foamy or lipid-rich cells) or a combination of both. Additionally, nodules were assessed for the presence of atypical cells, showing enlargement, presence of nucleoli or hyperchromasia. In case of multiple nodules within one specimen, we assessed all nodules separately. Finally, we classified all adrenal glands as containing either 1) Adenoma: one well demarcated or encapsulated nodule, with the adjacent adrenal cortex resembling normal adrenal tissue without nodulation. 2) Nodular hyperplasia: presence of multiple nodules; Slight disturbances in the adrenal cortex were defined as a nodule in case they caused an increase in cortex thickness or caused distortion of the surrounding adrenal cortex.

IMMUNOHISTOCHEMISTRY

We performed immunohistochemistry on p450C18 and p450C11 expression in all glands to assess the functional differentiation of the adrenal cells for aldosterone and cortisol secretion, respectively. Immunohistochemistry was performed using the antibodies and protocols previously described by *Nishimoto et al*. [18] We defined the antigen expression areas as the percentage of the surface of the adrenal node expressing the antigen (Figure 1). Expression was qualified as weak or strong, in comparison to expression in pre-existent tissue to correct for background staining (Table 2). Additionally, all slides were screened for the presence of APCCs. APCCs were defined as cell clusters within the adrenal cortex that exhibits conventional cortex zonation (i.e. no nodulation with increase of cortex thickness or distortion of surrounding tissue) with marked p450C18, but no p450C11 expression (Figure 1). [18] Elongated p450C18 positive cell clusters (< 0.2mm) spreading over and merging with the zona glomerulosa were not classified as APCC, but were regarded to be part of the conventional adrenocortical zonation with sporadic expression of p450c18 in the zona glomerulosa.[18]

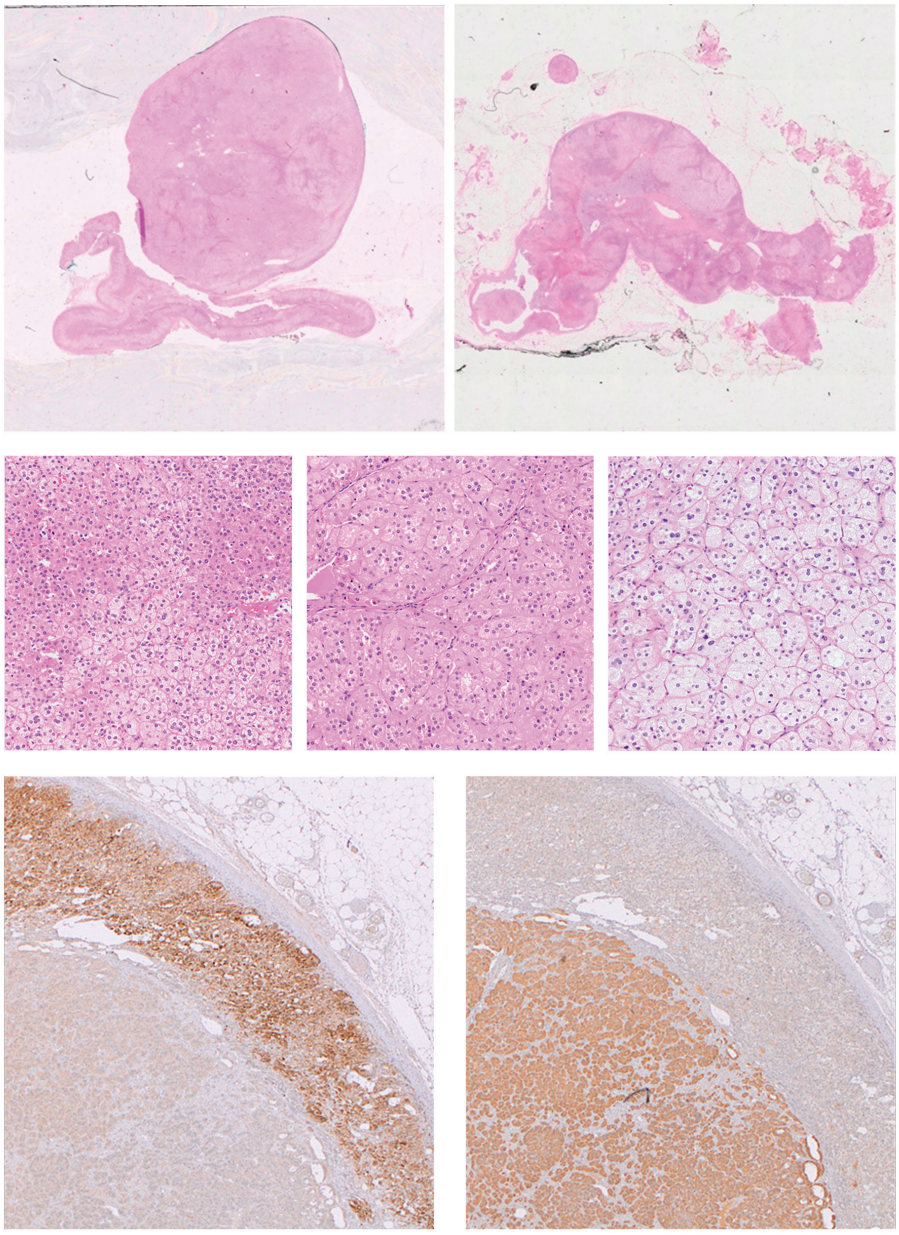


Figure 1. Examples of histopathological and immunohistochemical features of adrenal glands in primary aldosteronism.

Upper row, left panel, Solitary adenoma; right panel, multinodular hyperplasia (HE staining); middle row, left panel, ZG-like cells; middle panel, ZG-like + ZF-like cells; right panel, ZF-like cells (HE staining) lower row, immunohistochemistry; left panel, p450C11 staining, 0% of adenoma cell surface positive; right panel, p450C18, 100% of adenoma cell surface positive.

GENOTYPING

On each HE-slide all conspicuous nodules were demarcated by the pathologist (B.K.) by felt pen. Of each nodule demarcated three 20µm sections were manually micro-dissected. Genomic DNA was extracted from all separate nodules by overnight digestion with proteinase K and analyzed separately. For the mutation analysis, the crude extract was subsequently used to amplify the regions spanning the mutations. Primers used were for *KCNJ5* 5' TTGGCGACCAAGAGTGGATTCCTT3' and 5'CACCATGAAGGCATTGACGATGGA3', for *ATP1A1* exon4 5'CCACTACTCCTGAATGGATC3' and 5'TCCTCTTCTGTAGCAGCTTG3', for *ATP1A1* exon8 5'CTCTCATCCTTGAGTACACC3' and 5'TGCAAGCTGATCTGAGTCAG3', and for*ATP2B3* exon8 5'GATTGAGACGTTTGTCGTGG3' and 5'CCTTGACAGAGTAAGCTAAGG3' and analyzed by dideoxy sequencing. DNA samples of 50/53 patients were genotyped using custom TaqMan genotyping assays (Applied Biosystems) for the *CACNA1D* substitution mutations encoding c.T776A, c.G1207C, c.C2250G, and c.C4007G encoding p.Val259Asp, p.Gly403Arg, p.Ile1750Met, and p.Pro1336Arg, respectively. [25]

GENOTYPE-PHENOTYPE ANALYSIS

We compared *KCNJ5*, *ATP1A1*, *ATP2B3*, *CACNA1D* genotype results of adrenals classified as either adenoma or nodular hyperplasia to histopathological characteristics, immunohistochemistry, patient characteristics and treatment outcome.

STATISTICAL ANALYSIS

All data are presented as mean and standard deviation (sd) or, in case of skewed distributions, as median and range. To asses significance of difference between the histological classes, between glands with or without mutation, between demographic data, between histopathological characteristics and between treatment outcome we used the Chi-square test and Fisher's exact test for discrete data and one-way ANOVA (with Bonferroni correction) and Mann-Whitney U test (2 samples) or Kruskal-Wallis test (multiple samples) for continuous data with and without a normal distribution, respectively. Forward stepwise binary logistic regression analysis (LR method) was performed to determine the relation between patient characteristics and the presence of a mutation. Correlation between P450C11 and P450C11 expression was calculated using Spearman's rho. $P<0.05$ was considered significant. We used IBM SPSS statistics 20 and GraphPad Prism 5 for Windows for statistical analysis.

RESULTS

SUBJECTS AND ADRENAL GLANDS

The clinical features of the 53 patients whose adrenal glands were re-examined are shown in Table 2. Except for medication intake there were no significant differences in patient characteristics between those patients that could be included and those that could not (Table 2). The 53 glands studied contained 98 nodules (one to seven nodes per adrenal gland) which were all assessed separately (Table 3). Two adrenal glands that were severely damaged during adrenalectomy and consisted of tissue fragments only (nr 52 and 53) could not be classified as adenoma or nodular hyperplasia, and in one of these ZG characteristics could not be assessed. These two glands were counted as containing one nodule each. One nodule could not be assessed for immunohistochemistry because the immunostainer had not covered the entire specimen (nr. 47). DNA analysis was unsuccessful in four nodules (nr.15, 22, 31.1 and 51.2), because of DNA quality.

HISTOPATHOLOGICAL PHENOTYPE

The resected adrenal glands were classified as containing either a solitary adenoma or nodular hyperplasia in 23/51 and 28/51 of the cases, respectively (Table 4). Solitary adenomas were more often found in female patients and in younger patients. Adrenals of patients in whom the diagnosis of unilateral APA was based on CT-scan contained a solitary adenoma more frequently than adrenals of patients in whom adrenalectomy was based on AVS. The majority of the adrenals (43/52, 83%) demonstrated ZG hyperplasia, which did not differ significantly between glands with solitary adenoma and those with multiple nodules. Of the 98 individually assessed nodular structures 15 (15%) were composed of ZG-like cells, 38 (39%) of ZF-like cells and 45 (46%) of a combination of the two. The size of the largest nodule in each adrenal did not differ significantly between those classified as adenoma and those classified as nodular hyperplasia.

Table 2. Clinical, pathological, genetic and histochemical characteristics of 53 adrenal glands removed because of suspected unilateral disease in patients with primary aldosteronism

	Pre-operative								Pathology				Genetics		(Immuno)histology							Post-op
No	**M/F**	**Age**	**SBP**	**DBP**	**K+**	**Ald**	**ARR**	**CT/ AVS**	**Classification**	**ZG**	**ZG Ø (μm)**	**node**	**gene**	**mutation**	**size (mm)**	**Cell Comp.**	**atypic (% cells)**	**SB**	**% P450C18 (stain)**	**% p450C11 (stain)**	**APCC #**	**outcome**
KCNJ5 mutations																						
1	F	29	182	103	3.0	0.78	0.16	CT	adenoma	y	278	1	KCNJ5	c.503T>G (p.Leu168Arg)	23	ZF	5	n	25 (S)	33 (W)	0	cured
2	F	26	186	112	2.6	1.55	0.14	CT	adenoma	y	226	1	KCNJ5	c.503T>G (p.Leu168Arg)	23	ZF	25	n	80 (W)	30 (W)	2	impr
3	F	44	132	82	2.3	1.38	0.35	CT	adenoma	y	110	1	KCNJ5	c.503T>G(p. Leu168Arg)	15	ZG+ZF	10	n	70 (S)	60 (W)	0	cured
4	M	47	180	115	2.7	0.89	0.19	CT	adenoma	y	140	1	KCNJ5	c.503T>G (p.Leu168Arg)	14	ZG+ZF	30	y	33 (S)	100 (S/W)	0	impr
5	M	40	179	101	3.5	0.90	0.45	AVS	adenoma	y	230	1	KCNJ5	c.503T>G (p.Leu168Arg)	14	ZF	40	n	70 (S)	30 (S)	1	impr
6	F	30	142	97	3.5	0.40	0.20	AVS	adenoma	y	164	1	KCNJ5	c.503T>G (p.Leu168Arg)	13	ZF	25	n	40 (S)	0	1	cured
7	F	53	144	88	3.4	0.45	0.06	AVS	adenoma	y	255	1	KCNJ5	c.503T>G (p.Leu168Arg)	12	ZF	20	n	50 (S)	50 (W)	1	impr
8	F	51	162	92	3.8	1.74	0.24	CT	adenoma	y	174	1	KCNJ5	c.451G>C(p. Gly151Arg)	17	ZG+ZF	5	n	53 (S/W)	66 (W)	3	impr
9	F	44	166	106	2.8	0.87	0.09	AVS	adenoma	y	350	1	KCNJ5	c.451G>C(p. Gly151Arg)	12	ZF	5	n	50 (S/W)	20 (S/W)	2	impr
10	F	47	139	79	4.2	0.43	0.22	AVS	adenoma	y	182	1	KCNJ5	c.451G>C (p.Gly151Arg)	11	ZG+ZF	5	n	40 (S)	40 (W)	2	cured
11	F	51	220	120	3.3	1.10	0.12	CT	adenoma	n	164	1	KCNJ5	c.451G>A (p.Gly151Arg)	21	ZF	5	n	50 (S/W)	50 (W)	2	failed
12	F	31	188	110	2.2	0.79	0.15	AVS	adenoma	y	252	1	KCNJ5	c.451G>A (p.Gly151Arg)	12	ZF	5	n	40 (S)	25 (W)	1	failed
13	F	40	147	92	3.2	0.55	0.18	AVS	adenoma	y	237	1	KCNJ5	c.451G>A(p. Gly151Arg)	10	ZF	5	n	10 (S)	5 (S)	3	cured
14	F	55	184	98	3.2	0.59	0.08	AVS	Multinodular	y	200	1	KCNJ5	c.503T>G(p. Leu168Arg)	20	ZF	0	n	20 (W)	50 (W)	2	cured
												2	wt		6	ZG+ZF	5	n	0	40 (S)		
												3	wt		5	ZG+ZF	25	n	0	40 (S)		
15	F	53	149	79	2.8	0.85	0.43	AVS	Multinodular	y	260	1	wt		16	ZG+ZF	10	n	0	50 (S)	9	failed

											2	KCNJ5	c.503T>G (p.Leu168Arg)	15	ZG+ZF	30	n	45 (S/W)	0		
											3	wt		4	ZF	5	n	0	60 (S)		
16	F	42	164	98	3.3	0.76	0.04	CT	Multinodular	y	151	1	KCNJ5	c.503T>G(p. Leu168Arg)	13	ZF	20	n	25 (S)	40 (W)	1	impr
												2	wt		2	ZF	5	n	0	0		
												3	wt		1.5	ZF	5	n	0	0		
17	M	61	180	90	3.0	0.53	0.27	AVS	Multinodular	n	130	1	KCNJ5	c.503T>G (p.Leu168Arg)	11	ZG+ZF	30	n	40 (S/W)	40 (S)	0	failed
												2	wt		4	ZF	5	n	20 (S/W)	40 (S)		
												3	wt		6	ZG+ZF	10	n	0	40 (S)		
18	M	54	165	91	4.4	1.40	0.47	AVS	Multinodular	n	178	1	KCNJ5	c.451G>A (p.Gly151Arg)	19	ZG+ZF	20	n	70 (S/W)	25 (W)	3	impr
												2	KCNJ5	c.503T>G (p.Leu168Arg)	7	ZF	25	n	70 (S)	0		
												3	wt		4	ZG+ZF	5	n	0	40 (W)		
19	M	60	184	112	3.6	1.23	0.20	AVS	Multinodular	n	52	1	KCNJ5	c.451G>A(p. Gly151Arg)	19	ZF	25	n	15 (S)	50 (W)	0	impr
												2	wt		5	ZF	0	n	0	50 (W)		
20	F	71	145	99	4.7	1.66	0.49	CT	Multinodular	y	310	1	KCNJ5	c.451G>A (p.Gly151Arg)	17	ZG+ZF	25	n	30 (S/W)	80 (W)	0	failed
												2	wt		5	ZG+ZF	0	n	0	60 (W)		
												3	wt		5	ZG+ZF	0	n	0	70 (W)		
21	M	58	173	93	3.3	2.20	0.55	AVS	Multinodular	y	134	1	KCNJ5	c.451G>A (p.Gly151Arg)	15	ZF	25	n	25 (S)	10 (W)	0	cured
												2	wt		2.5	ZF	0	n	0	0		
22	M	54	197	114	3.9	0.68	0.08	AVS	Multinodular	y		1	KCNJ5	c.433G>C (p.Glu145Gln)	9	ZG+ZF	30	n	70 (W)	20 (S)	0	impr
												2	wt		9	ZF	5	n	0	40 (S)		
												3	wt		7	ZF	10	n	0	60 (S)		
												4	wt		7	ZF	10	n	50 (S)	50 (S)		
												5	wt		6	ZF	5	n	0	40 (S)		
												6	wt		5	ZG+ZF	5	n	0	40 (S)		
												7	wt		3	ZF	0	n	0	40 (S)		

>>

Table 2. Continued

	Pre-operative								Pathology				Genetics		(Immuno)histology							Post-op
No	**M/F**	**Age**	**SBP**	**DBP**	**K+**	**Ald**	**ARR**	**CT/ AVS**	**Classification**	**ZG**	**ZG Ø (μm)**	**node**	**gene**	**mutation**	**size (mm)**	**Cell Comp.**	**atypic (% cells)**	**SB**	**% P450C18 (stain)**	**% p450C11 (stain)**	**APCC #**	**outcome**
ATP2B3 mutations																						
23	M	56	147	88	3.2	1.39	0.25	AVS	adenoma	y	180	1	ATP2B3	c.1272_1277del (p.(Leu425_ Val426del))	9	ZG	0	n	100 (S)	0	0	failed
24	F	62	144	85	2.6	0.89	0.22	CT	adenoma	y	365	1	ATP2B3	c.1272_1277del (p.(Leu425_ Val426del))	9	ZG+ZF	0	n	80 (S)	10 (S)	0	cured
25	M	43	154	96	3.8	0.78	0.39	AVS	adenoma	y	184	1	ATP2B3	c.1272_1277del (p.(Leu425_ Val426del))	7	ZG	0	y	90 (S)	0	0	cured
26	M	49	222	111	2.4	0.72	0.24	AVS	adenoma	y	144	1	ATP2B3	c.1272_1277del (p.(Leu425_ Val426del))	8	ZG	0	n	60 (W)	20 (W)	0	impr
27	M	42	198	139	3.1	0.98	0.49	CT	adenoma	y	183	1	ATP2B3	c.1269_1275del (p.(Leu425_ Val426del))	8	ZG	0	y	90 (S)	0	1	impr
ATP1A1 mutations																						
28	M	48	186	87	3.0	1.16	0.17	AVS	Multinodular	n	179	1	ATP1A1	c.311T>G (p.Leu104Arg)	13	ZG	15	y	80 (S)	30 (W)	1	impr
												2	wt		10	ZF	10	n	0	30 (S)		
CACNA1D mutations																						
29	M	46	150	98	3.0	0.65	0.14	AVS	Multinodular	y	238	1	wt		6	ZG+ZF	5	n	0	40 (S)	3	failed
												2	CAC-NA1D	c.2250C>G (p.Ile1750Met) 1750Met)	5	ZG	0	y	100 (S)	0		
												3	wt		3	ZG	0	y	100 (S)	0		
No mutations																						
30	F	49	151	90	2.3	8.20	1.03	AVS	adenoma	n	155	1	wt		19	ZG+ZF	40	n	80 (S)	50 (W)	0	impr
31	F	24	138	98	3.1	0.61	0.20	AVS	adenoma	n	80	1	wt		10	ZG+ZF	0	n	80 (W)	20 (W)	0	cured
32	F	43	167	113	3.6	1.10	0.10	AVS	adenoma	y	176	1	wt		5	ZG	5	n	90 (S)	0	0	cured
33	F	69	181	94	2.9	0.54	0.18	CT	Multinodular	y	268	1	wt		23	ZG+ZF	5	n	0	66 (S/W)	4	failed

												2	wt.	4	ZG+ZF	0	n	0	0		
34	M	48	158	108	2.6	0.55	0.02	CT	Multinodular	y	233	1	wt	18	ZG	0	y	100 (S)	0	3	impr
												2	wt	5	ZG+ZF	0	n	0	40 (S)		
35	M	49	168	87	2.9	0.48	0.10	AVS	Multinodular	y	89	1	wt	10	ZF	0	n	100 (S)	10 (S)	3	impr
												2	wt	3	ZF	0	n	100 (S)	0		
36	M	49	122	82	2.6	0.53	0.27	AVS	Multinodular	y	98	1	wt	9	ZG+ZF	0	y	100 (S)	0	2	impr
												2	wt	2	ZF	5	n	0	50 (W)		
37	M	63	214	106	3.8	0.77	0.39	AVS	Multinodular	y	235	1	wt	8	ZG+ZF	10	n	100 (S)	10 (W)	0	failed
												2	wt	4	ZG+ZF	0	n	0	70 (S)		
38	F	62	212	98	3.4	1.10	0.22	AVS	Multinodular	y	138	1	wt	8	ZG	0	y	100 (S)	25 (W)	3	impr
												2	wt	4	ZF	0	n	0	70 (S)		
39	M	36	147	100	3.8	0.81	0.20	AVS	Multinodular	y	172	1	wt	7	ZF	0	n	80 (S)	0	0	failed
												2	wt	3	ZG+ZF	0	n	0	40 (S)		
40	M	62	211	112	2.6	0.78	0.09	AVS	Multinodular	y	250	1	wt	10	ZG+ZF	5	n	80 (S)	50 (W)	1	impr
												2	wt	5	ZF	5	n	0	50 (S)		
41	M	62	226	120	3.2	0.95	0.48	AVS	Multinodular	y	135	1	wt	9	ZG	0	y	80 (S)	40 (S/W)	1	impr
												2	wt	7	ZG	0	n	n.a.	n.a.		
42	M	56	160	88	2.8	0.34	0.03	AVS	Multinodular	y	80	1	wt	6	ZG+ZF	5	n	0	40 (S)	2	impr
												2	wt	4	ZG	5	n	0	40 (S)		
43	M	61	140	90	2.9	2.08	1.04	AVS	Multinodular	y	237	1	wt	6	ZF	5	n	0	66 (W)	2	failed
												2	wt	3	ZG+ZF	0	n	0	50 (S)		
44	M	53	179	125	3.7	0.44	0.10	AVS	Multinodular	y	300	1	wt	4	ZG+ZF	0	n	100 (S)	0	0	
												2	wt	3	ZG+ZF	0	n	0	40 (S)		
45	M	59	148	98	2.9	0.50	0.16	AVS	Multinodular	y	125	1	wt	10	ZG	5	n	70 (S)	0	2	impr
												2	wt	5	ZG+ZF	10	n	0	40 (S)		
												3	wt	4	ZG+ZF	5	n	0	40 (S)		
46	M	45	171	99	2.9	1.51	0.76	AVS	Multinodular	y	463	1	wt	15	ZF	20	n	0	50 (W)	0	failed
												2	wt	4	ZG+ZF	0	n	0	40 (S)		
												3	wt	4	ZG+ZF	0	n	0	40 (S)		
												4	wt	4	ZG+ZF	0	n	0	40 (S)		

>>

Table 2. Continued

	Pre-operative								Pathology				Genetics		(Immuno)histology							Post-op
No	**M/F**	**Age**	**SBP**	**DBP**	**K+**	**AId**	**ARR**	**CT/ AVS**	**Classification**	**ZG**	**ZG Ø (μm)**	**node**	**gene**	**mutation**	**size (mm)**	**Cell Comp.**	**atypic (% cells)**	**SB**	**% P450C18 (stain)**	**% p450C11 (stain)**	**APCC #**	**outcome**
47	M	55	132	76	4.4	0.40	0.02	AVS	Multinodular	y	250	1	wt		10	ZG+ZF	5	n	0	80 (S)	4	cured
												2	wt		4	ZG	0	n	0	80 (S)		
												3	wt		3	ZG+ZF	25	y	100 (S)	0		
												4	wt		2.5	ZF	5	n	0	20 (W)		
48	M	63	196	115	3.4	0.34	0.03	CT	Undefined §	y	124	1	wt		5	ZG+ZF	20	n	60(S)	40 (S)	1	impr
49	M	53	149	83	3.8	0.68	0.23	CT	Undefined§	n.a.	n.a.	1	wt		10	ZG+ZF	5	y	80 (S)	30 (S)	0	failed (rec.)
Mutation not assessable																						
50	F	44	200	120	3.5	0.81	0.06	CT	adenoma	y	265	1	n.a.		19	ZF	5	n	30 (S/W)	66 (S/W)	0	impr
51	F	48	126	82	4.0	1.10	0.52	CT	adenoma	n	73	1	n.a.		10	ZF	20	n	20 (W)	80 (S)	0	cured
52	M	58	169	94	3.3	0.53	0.05	CT	Multinodular	n	80	1	n.a.		16	ZF	10	n	10 (S)	60 (S)	-	impr
												2	wt		6	ZF	5	n	0	40 (S)		
53	M	48	151	105	3.1	0.45	0.09	AVS	Multinodular	y	274	1	wt		5	ZG+ZF	5	n	0	40 (S)	2	impr
												2	n.a.		4	ZG+ZF	0	n	0	50 (S)		

Table 3. Criteria for cure or improvement of PA at follow-up.

Definition	Criteria
Cure:	DBP < 90 mm Hg and SBP <140 mm Hg, no antihypertensive medications; Serum potassium ≥ 3.5 mmol/l Normal SIT (post-test aldosterone < 0.28nmol/l) or ARR < 0.09 nmol/mE
Improvement:	DBP <90 mm Hg and/or SBP <140 mm Hg on the same or reduced number of medications (or reduced number of defined daily doses as described by the World Health Organization) or a reduction in DBP by at least 15 mm Hg on the same or reduced number of medications. Serum potassium ≥ 3.5 mmol/l Normal SIT (post-test aldosterone < 0.28 nmol/l) or ARR < 0.09 nmol/mE
Failure:	No change or inability to meet above criteria for cure or improvement.

IMMUNOHISTOCHEMISTRY

Most adrenal glands (43/52 assessable glands) contained one single nodule positive for p450C18 expression (i.e. aldosterone production) with all other nodules in the same gland, when present, being negative. With the exception of two cases (nr 30, 46), this always concerned the largest nodule present. Four glands (nr. 26, 36, 40 and 44) contained an additional nodule positive for P450C18 staining, whereas five samples (nr. 24, 33, 48, 49, 50) showed P450C18 expression in none of the nodules studied. Mutated nodules always expressed P450C18. P450C11 expression (i.e. cortisol production) was present in most of the nodules and was inversely related to the P450C18 expression (r_s = -0.504, 95%CI -0.644 to – 0.330, p<0.0001). APCC's were found in 29 (55%) of the glands, ranging from 1 to 9 APCC's per gland, with cell cluster diameters ranging from 0.2mm to 1.2mm. We could not establish a relation between the presence of APCC's and patient characteristics, histopathology or immunohistochemistry of the adrenal gland.

Table 4. Differences in patient characteristics, histopathology, genotyping and treatment outcome in adrenal glands containing either adenoma or nodular hyperplasia.

	Adenoma (n=23, 23 nodules) ‡	Nodular hyperplasia (n=28, 73 nodules) ‡
Patient characteristics		
Gender: male	6/23 (20%)	22/28 (73%)§
Age	43.2±9.7	54.9±8.0§
Diagnostic strategy	11/23 (49%)	5/28 (18%)
CT-scan AVS	12/23 (52%)	23/28 (82%)
Histopathological characteristics		
ZG hypertrophy	19/23 (83%)	23/28 (82%)
Size (largest) nodule (mm)	12 (5-23)	10 (4-23)
Cell type		
ZF-like	11/23 (48%)	27/73 (37%)
ZG-like	5/23 (22%)	10/73 (14%)
ZG+ZF-like	7/23 (30%)	36/73 (49%)
Genotyping		
KCNJ5	13/23 (57%)	9/28 (32%)
ATP1A1	0/23 (0%)	1/28 (4%)
ATP2B3	5/23 (22%)	0/28 (0%)†
CACNA1D	0/23 (0%)	1/28 (4%)
Wild type	3/23 (13%)	15/28 (54%)†
Not assessable	2/23 (9%)	2/28 (7%)
Treatment outcome		
Cured	10/23 (43%)	3/28 (10%)†
Improved	10/23 (43%)	16/28 (53%)
Failed	3 /23 (14%)	11/28 (37%)

‡Two glands could not be classified as containing either adenoma or nodular hyperplasia due to severe tissue damage; §different from adenoma, significance level $p < 0.0001$; †different from adenoma; significance level $p < 0.05$; AVS = adrenal venous sampling; ZG = zona glomerulosa; ZF = zona fasciculata.

GENOTYPING: KCNJ5

KCNJ5 mutations were present in 13 (62%) and 9 (32%) of the assessable adrenals classified as a solitary adenoma and nodular hyperplasia, respectively, adding up to a total of 22 (42%) affected glands. The mutation was more frequently present in female patients compared to male patients (65% vs. 23%, $p < 0.01$). No relation between age, body mass index, blood

pressure, potassium or aldosterone levels and the presence of a *KCNJ5* mutation was found in univariate or multivariate analysis Nodules containing the *KCNJ5* mutation consisted of ZF-like cells more often compared to those wild-type for *KCNJ5* (61% vs. 28%, p = 0.04). *KCNJ5* mutations were never present in nodules consisting of only ZG-like cells. Nodules with the *KCNJ5* mutation showed more atypical cells than those without the mutation (median 20% (range 0-40%) vs. median: 5% (range 0-40%) p < 0.001).

GENOTYPING: ATP1A, ATP2B3 AND CACNA1D

ATP2B3 mutations were found in five nodules (9%) of adrenal glands all classified as solitary adenoma (nr 17,18,19,20 and 24). Four out of five patients were male and in these patients the mutated nodules consisted of ZG-like cells only. None of the nodules showed atypical cells. Nodules containing an *ATP2B3* mutation were significantly smaller than those containing a *KCNJ5* mutation (8.2mm vs. 14.9mm, p<0.001) and had a higher P450C18 expression (94% vs. 44%, p<0.001) and lower p450C11 expression (6% vs. 36%, p = 0.02). One *ATP1A1* and one *CACNA1D* mutation were both found in two male patients (nr 35 and nr 44, respectively) in a nodule of a multinodular gland, consisting of ZG-like cells only.

MULTINODULAR ADRENAL GLANDS

Regardless of the number of nodules in the 11 out of 28 multinodular glands that contained a somatic mutation of one of the four genes we studied, the mutation was present in only one of the nodules in each individual gland. All other nodules within the same adrenal did not contain one of these somatic mutations, except for gland nr. 26 that contained two P450C18 positive nodules, each containing a different *KCNJ5* mutation (Leu168Arg and Gly151Arg) (Figure 2). The first nodule consisted of both ZG-like and ZF-like cells, while the second consisted of only ZF-like cells. Both nodules expressed P450C18 in a relatively high percentage of the cell surface, and contained many atypical cells. A third nodule within the same adrenal gland was negative for both *KCNJ5* mutations and P450C18 expression.

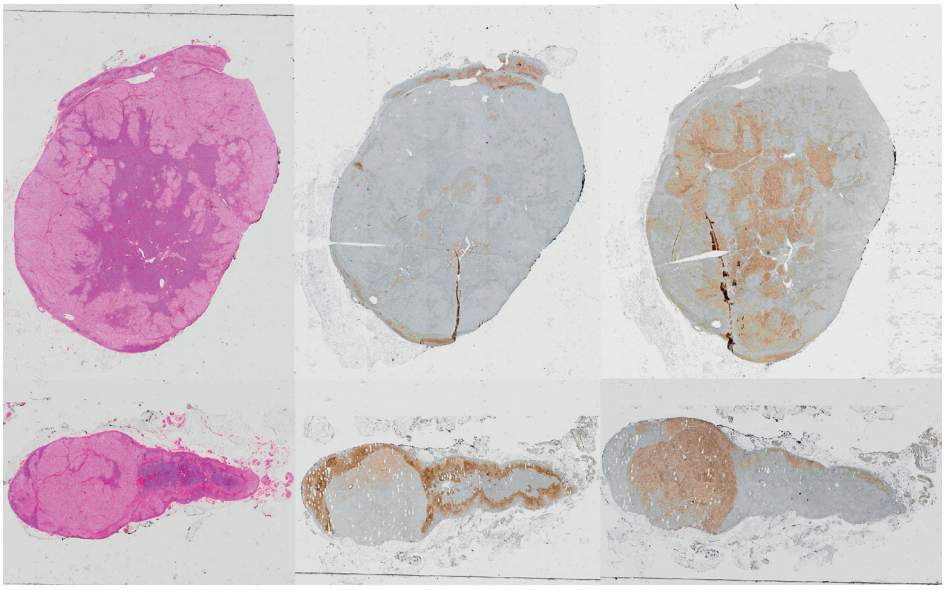


Figure 2. Two mutated nodules within one adrenal gland (number 26, Table 2).

From left to right, HE staining, p450C11 staining, and p450C18 staining. Upper panels, Node 1 with KCNJ5 (c.451G>A) mutation. Bottom panels, Node 2 with KCNJ5 (c.503T>G) mutation.

TREATMENT OUTCOME

At follow-up 13 patients (25%) were cured, 26 (49%) had improved and 14 (26%) had no improvement. One patient had recurrence of disease, because of incomplete adrenalectomy (nr. 45). Four patients did not undergo repeated biochemical testing because they were lost-to-follow-up (n=3) or deceased (n=1, melanoma). Patients whose adrenal contained a solitary adenoma were cured more often at follow-up compared to patients with an adrenal gland showing nodular hyperplasia (Table 4). Of the five cases that did not express p450C18 in any of the adrenal nodules, three had treatment failure. Two of these three had been diagnosed by AVS, and one by CT-scanning.

Of the 29 patients with a proven mutation, nine (31%) were cured, 13 (45%) improved, and seven (24%) had failure of treatment compared with 3 (15%), 10 (50%), and seven (35%) of the 20 patients without a proven mutation, respectively, which was not significantly different. Neither did we find a difference in the treatment outcome between the patients with different types of mutations.

DISCUSSION

The present study shows that a majority of adrenal glands removed because of suspicion of a unilateral aldosterone producing adenoma demonstrate hyperplasia instead of adenoma, similar to the observation recently reported by *Iacobone et al*.[7] *KCNJ5* mutations were present in 42% of the glands studied, which is in line with previous studies.[8,26,27] As in these studies, *KCNJ5* mutations in our cohort were more often present in nodules with a ZF-like cell type and were more frequently found in female patients.[8,27] The *ATP2B3*, *ATP1A1* and *CACNA1D* mutations, present in seven patients were predominantly present in ZG-like nodules of male patients. Mutations were found in adrenals with solitary adenomas and in adrenals with nodular hyperplasia. In a study that focused more on clinical details, Åkerström *et al*. described the presence of *KCNJ5* mutations in adrenals classified as adenoma with associated hyperplasia as well.[8]

Our study adds to previous reports that most of the removed glands, regardless of whether they are classified as adenoma or nodular hyperplasia, contain one nodule, usually the largest in multinodular glands, expressing p450C18 and that mutations were only found in these p450C18-positive nodules. It can be surmised that the remaining p450C18-positive nodules (like 39.1, 41.1 etc.) contain other, hitherto unidentified, mutations that cause aldosterone hypersecretion. This would lead to the hypothesis that in each (multinodular) gland the aldosterone hypersecretion can be attributed to one (or rarely two) mutated nodule(s). Whether the mutations found are causative in the development of the nodules remains to be proven, because if so, we would expect that within one gland each nodule contains a mutation, which was not the case in our study. A more plausible explanation is that the mutations are causative in aldosterone hypersecretion, but not in nodulation itself, which is also suggested by the functional effects of the mutations.[22,33] The overall hypothesis that we propose therefore is that some individuals for some reason develop multinodular adrenal cortices with ZG thickening and that only if a mutation occurs, for instance in *KCNJ5*, *ATP1A1*, *ATP2B3* or *CACNA1D*, but possibly also in other as yet unidentified genes, the clinical syndrome of PA develops. An intriguing question then is whether the contralateral adrenal gland is normal or that similar changes, perhaps to a lesser degree, are present. Since we clearly cannot obtain these contralateral glands we cannot answer this question. Recent case reports describe the development of aldosterone producing adenomas on the

contralateral or ipsilateral site in patients operated for APA,[34,35] which might be explained by newly arisen mutations in these glands. The questions, however, why a patient develops (unilateral or bilateral) multinodular cortices and why simultaneous ZG thickening occurs remains unanswered.

Our study showed some interesting associations between the histopathological phenotype, immunohistochemistry, and the genotype. For instance, KCNJ5 mutations were present in adrenal glands classified as either solitary adenoma or nodular hyperplasia, whereas ATP2B3 mutations were found only in solitary adenomas. ATP1A1 and CACNA1D were both found in a multinodular gland. KCNJ5-mutated nodules were rather large, often consisted of ZF-like cells and showed a relatively high number of atypical cells. On the contrary, all five ATP2B3-mutated tumours were less than 1 cm, consisted mainly of ZG-like cells and had no atypical cells, which was also the case in the tumour with the CACNA1D mutation. Concerning immunohistochemistry, most KCNJ5 mutations had strong staining for both P450C18 and P450C11. All ATP2B3 mutations had a strong staining for P450C18, whereas staining for P450C11 was absent or weak, suggesting predominant expression of aldosterone synthase. The number of mutations is, however, not large enough to determine whether these patients have higher aldosterone levels or higher BP, nor can we derive yet from the histological features with 100% certainty which mutations should be looked for in the first place.

In five patients no nodules positive for p450C18 expression were found. This can be explained by several mechanisms. First, aldosterone production can be attributed to APCC's. Four out of the five adrenals lacking a p450C18 positive nodule contained multiple APCCs. In their cohort *Nanba et al*. also found APCCs in adrenals containing a p450C18 negative nodule.[13] However, as APCCs are also present in normal adrenal tissues and its ontogeny is unknown, [18] it is unclear whether these cell clusters can be responsible for the aldosterone excess in PA. Second, the cross-section of the adrenal gland that was chosen by the pathologist may have missed the nodule responsible for aldosterone production in the multinodular adrenals. Especially for micronodular glands without evident nodules at macroscopy this might have been a problem. Third, it is possible that, despite thorough patient screening, the initial diagnosis of primary aldosteronism was not accurate, since specificity for ARR and saline infusion test may not be 100%.[36-38] Also, the diagnosis of unilateral APA established by AVS and/or CT-scan could have been inaccurate with the consequence that the patient was

falsely operated, as CT is known for its possible misclassification, just like AVS is susceptible to interpretative error.[25,39,40]

The clinical implication of our findings, in terms of prediction of treatment outcome, remains to be determined. We did not find a difference in treatment outcome between patients with different types of mutations. However, if it were possible to determine whether a mutation is present in an adrenal gland before adrenalectomy, this might be most helpful for the decision to proceed to adrenalectomy or not. As yet, there is no possibility to assess the presence of somatic mutations in one or both adrenal glands, but perhaps new forms of specific imaging or composition of adrenal venous blood might provide this information.

Our study had some limitations. Diagnostic work-up was not performed uniformly because our retrospective study spanned a long period of time in which diagnostic strategies changed from CT-scan to AVS. Although it has not been indisputably established, some clinicians and researchers regard CT-scan to be potentially misleading in PA diagnostic work-up.[25] As solitary adenomas were more easily diagnosed by CT-scan in our study, it is possible that the use of CT-scan has led to an inclusion bias towards patients with a solitary adenoma. However, as this study was not primarily designed to evaluate the prevalence of unilateral adrenal hyperplasia, this is of minor importance. Another limitation associated with the retrospective approach of the study is that the follow-up data of some patients were incomplete. However, essential information on outcome could be retrieved for all patients included. We could not assess the presence of germline mutations in our patients but it is unlikely that germline mutations were present, given that in all multinodular glands at least one of the nodules did not contain mutations in the four genes, although this does not exclude the possibility of mosaic mutations.

In conclusion, the concept that primary aldosteronism is caused by either a unilateral aldosterone producing adenoma (APA) or by bilateral adrenal hyperplasia (BAH) needs to be reconsidered. The majority of adrenal glands with supposedly unilateral aldosterone production displays multinodular pathology. In these cases the largest nodule is generally p450C18-positive and in more than half of all cases this nodule also contains a *KCNJ5*, *ATP1A1*, *ATP2B3* or *CACNA1D* mutation. These mutations probably occur after nodule formation because in multinodular samples only one of the nodules contains the mutation and because in one of our cases there were even two nodules that each contained a

different KCNJ5 mutation. These findings and the presence of ZG-hypertrophy need further investigation in order to understand the pathogenesis of PA. The relevance of these findings for clinical management remains to be determined.

ACKNOWLEDGEMENTS

We are grateful to Prof. Morris Brown who facilitated the genotyping for *CACNA1D*. This study was supported by a grant from ZonMW DoelmatigheidsOnderzoek 2010-2012 E&K (171002102) to JD.

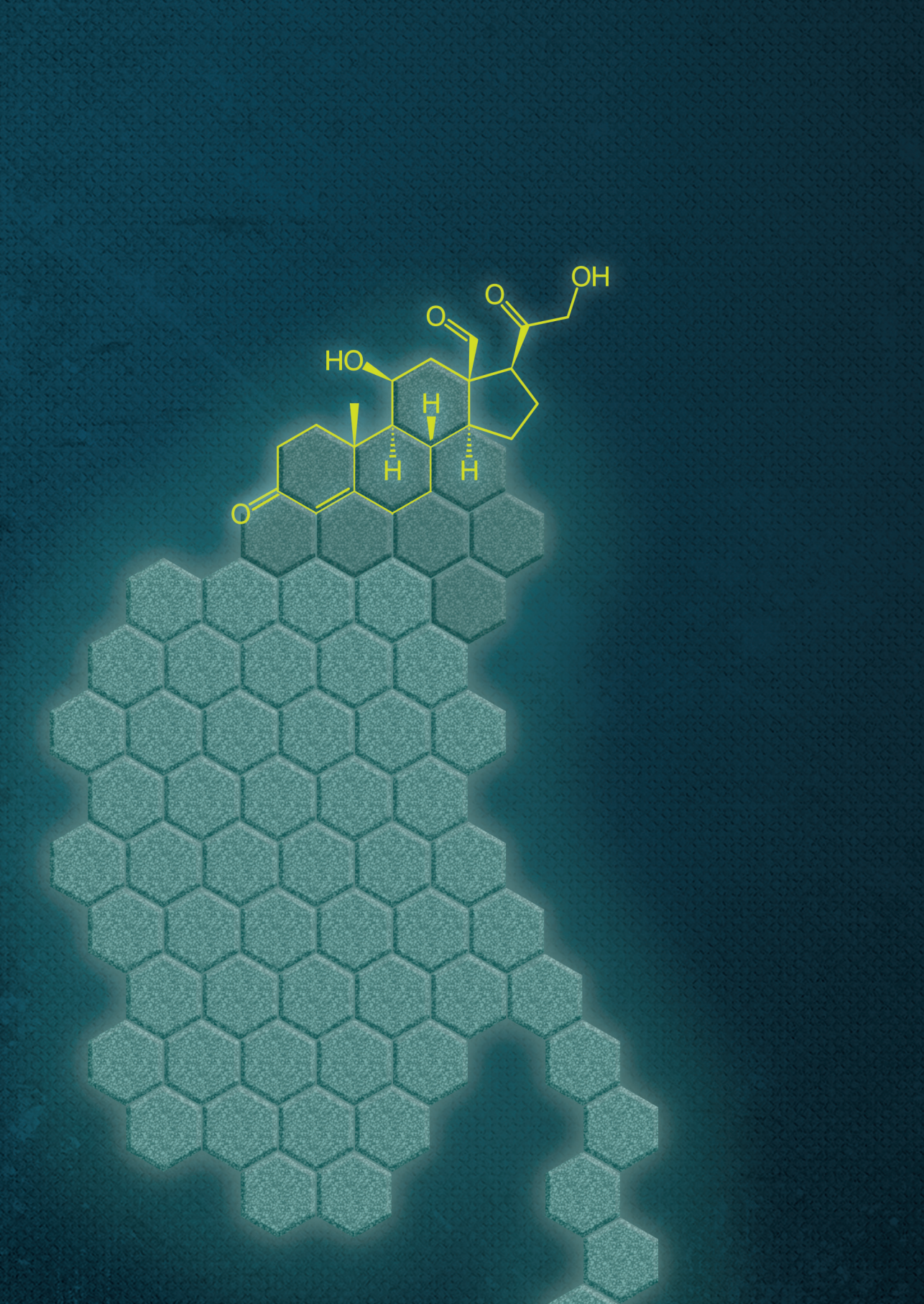
REFERENCES

1. Funder JW, Carey RM, Fardella C, et al. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. The Journal of clinical endocrinology and metabolism 2008;93:3266-81.
2. Mulatero P, Stowasser M, Loh KC, et al. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. The Journal of clinical endocrinology and metabolism 2004;89:1045-50.
3. Satoh F, Abe T, Tanemoto M, et al. Localization of aldosterone-producing adrenocortical adenomas: significance of adrenal venous sampling. Hypertension research : official journal of the Japanese Society of Hypertension 2007;30:1083-95.
4. Lo CY, Tam PC, Kung AW, Lam KS, Wong J. Primary aldosteronism. Results of surgical treatment. Annals of surgery 1996;224:125-30.
5. Fogari R, Preti P, Zoppi A, Rinaldi A, Fogari E, Mugellini A. Prevalence of primary aldosteronism among unselected hypertensive patients: a prospective study based on the use of an aldosterone/renin ratio above 25 as a screening test. Hypertension research : official journal of the Japanese Society of Hypertension 2007;30:111-7.
6. Enberg U, Volpe C, Hoog A, et al. Postoperative differentiation between unilateral adrenal adenoma and bilateral adrenal hyperplasia in primary aldosteronism by mRNA expression of the gene CYP11B2. European journal of endocrinology / European Federation of Endocrine Societies 2004;151:73-85.
7. Scholl UI, Nelson-Williams C, Yue P, et al. Hypertension with or without adrenal hyperplasia due to different inherited mutations in the potassium channel KCNJ5. Proceedings of the National Academy of Sciences of the United States of America 2012;109:2533-8.
8. Akerstrom T, Crona J, Delgado Verdugo A, et al. Comprehensive re-sequencing of adrenal aldosterone producing lesions reveal three somatic mutations near the KCNJ5 potassium channel selectivity filter. PloS one 2012;7:e41926.
9. Murashima M, Trerotola SO, Fraker DL, Han D, Townsend RR, Cohen DL. Adrenal venous sampling for primary aldosteronism and clinical outcomes after unilateral adrenalectomy: a single-center experience. Journal of clinical hypertension (Greenwich, Conn) 2009;11:316-23.
10. Proye CA, Mulliez EA, Carnaille BM, et al. Essential hypertension: first reason for persistent hypertension after unilateral adrenalectomy for primary aldosteronism? Surgery 1998;124:1128-33.

11. Tresallet C, Salepcioglu H, Godiris-Petit G, Hoang C, Girerd X, Menegaux F. Clinical outcome after laparoscopic adrenalectomy for primary hyperaldosteronism: the role of pathology. Surgery 2010;148:129-34.
12. Weisbrod AB, Webb RC, Mathur A, et al. Adrenal histologic findings show no difference in clinical presentation and outcome in primary hyperaldosteronism. Annals of surgical oncology 2013;20:753-8.
13. Nanba K, Tsuiki M, Sawai K, et al. Histopathological Diagnosis of Primary Aldosteronism Using CYP11B2 Immunohistochemistry. The Journal of clinical endocrinology and metabolism 2013 Apr;98(4):1567-74.
14. Iacobone M, Citton M, Viel G, et al. Unilateral adrenal hyperplasia: a novel cause of surgically correctable primary hyperaldosteronism. Surgery 2012;152:1248-55.
15. Sigurjonsdottir HA, Gronowitz M, Andersson O, et al. Unilateral adrenal hyperplasia is a usual cause of primary hyperaldosteronism. Results from a Swedish screening study. BMC endocrine disorders 2012;12:17.
16. Quillo AR, Grant CS, Thompson GB, Farley DR, Richards ML, Young WF. Primary aldosteronism: results of adrenalectomy for nonsingle adenoma. Journal of the American College of Surgeons 2011;213:106-12; discussion 12-3.
17. Ganguly A. Cellular origin of aldosteronomas. The Clinical investigator 1992;70:392-5.
18. Nishimoto K, Nakagawa K, Li D, et al. Adrenocortical zonation in humans under normal and pathological conditions. The Journal of clinical endocrinology and metabolism 2010;95:2296-305.
19. Shigematsu K, Yamaguchi N, Nakagaki T, Sakai H. A case of unilateral adrenal hyperplasia being difficult to distinguish from aldosterone-producing adenoma. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association 2009;117:124-8.
20. Fallo F, Pezzi V, Barzon L, et al. Quantitative assessment of CYP11B1 and CYP11B2 expression in aldosterone-producing adenomas. European journal of endocrinology / European Federation of Endocrine Societies 2002;147:795-802.
21. Boulkroun S, Samson-Couterie B, Dzib JF, et al. Adrenal cortex remodeling and functional zona glomerulosa hyperplasia in primary aldosteronism. Hypertension 2010;56:885-92.
22. Choi M, Scholl UI, Yue P, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. Science (New York, NY) 2011;331:768-72.

23. Beuschlein F, Boulkroun S, Osswald A, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. Nature genetics 2013 Apr;45(4):440-4, 444e1-2.
24. Azizan E, Poulsen H, Zhou J, et al. Mutations in the Na^+,K^+-ATPase a1 subunit identify a sub-group of adrenal aldosterone-producing adenomas. Nature genetics 2013;submitted.
25. Azizan EA, Poulsen H, Tuluc P, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. Nat Genet 2013 Sep;45(9):1055-60.
26. Azizan EA, Murthy M, Stowasser M, et al. Somatic mutations affecting the selectivity filter of KCNJ5 are frequent in 2 large unselected collections of adrenal aldosteronomas. Hypertension 2012;59:587-91.
27. Azizan EA, Lam BY, Newhouse SJ, et al. Microarray, qPCR, and KCNJ5 Sequencing of Aldosterone-Producing Adenomas Reveal Differences in Genotype and Phenotype between Zona Glomerulosa- and Zona Fasciculata-Like Tumors. The Journal of clinical endocrinology and metabolism 2012 May;97(5):E819-29.
28. Monticone S, Hattangady NG, Nishimoto K, et al. Effect of KCNJ5 mutations on gene expression in aldosterone-producing adenomas and adrenocortical cells. The Journal of clinical endocrinology and metabolism 2012;97:E1567-72.
29. Taguchi R, Yamada M, Nakajima Y, et al. Expression and mutations of KCNJ5 mRNA in Japanese patients with aldosterone-producing adenomas. The Journal of clinical endocrinology and metabolism 2012;97:1311-9.
30. Xekouki P, Hatch MM, Lin L, et al. KCNJ5 mutations in the National Institutes of Health cohort of patients with primary hyperaldosteronism: an infrequent genetic cause of Conn's syndrome. Endocr Relat Cancer 2012 May 3;19(3):255-60.
31. Seccia TM, Mantero F, Letizia C, et al. Somatic Mutations in the KCNJ5 Gene Raise the Lateralization Index: Implications for the Diagnosis of Primary Aldosteronism by Adrenal Vein Sampling. The Journal of clinical endocrinology and metabolism 2012 Dec;97(12):E2307-13.
32. Rundback JH, Sacks D, Kent KC, et al. Guidelines for the reporting of renal artery revascularization in clinical trials. American Heart Association. Circulation 2002;106:1572-85.
33. Azizan EA, Poulsen H, Tuluc P, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. Nature genetics 2013;45:1055-60.
34. Rizek P, Gorecki P, Lindenmayer A, Moktan S. Laparoscopic adrenalectomy for bilateral metachronous aldosteronomas. JSLS : Journal of the Society of Laparoendoscopic Surgeons / Society of Laparoendoscopic Surgeons 2011;15:100-4.

35. Calvo-Romero JM, Ramos-Salado JL. Recurrence of adrenal aldosterone-producing adenoma. Postgraduate medical journal 2000;76:160-1.
36. Schwartz GL, Chapman AB, Boerwinkle E, Kisabeth RM, Turner ST. Screening for primary aldosteronism: implications of an increased plasma aldosterone/renin ratio. Clinical chemistry 2002;48:1919-23.
37. Jansen PM, Boomsma F, van den Meiracker AH, Dutch AI. Aldosterone-to-renin ratio as a screening test for primary aldosteronism--the Dutch ARRAT Study. The Netherlands journal of medicine 2008;66:220-8.
38. Salva M, Cicala MV, Mantero F. Primary aldosteronism: the role of confirmatory tests. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2012;44:177-80.
39. Kempers MJ, Lenders JW, van Outheusden L, et al. Systematic review: diagnostic procedures to differentiate unilateral from bilateral adrenal abnormality in primary aldosteronism. Annals of internal medicine 2009;151:329-37.
40. Kline GA, Harvey A, Jones C, et al. Adrenal vein sampling may not be a gold-standard diagnostic test in primary aldosteronism: final diagnosis depends upon which interpretation rule is used. Variable interpretation of adrenal vein sampling. International urology and nephrology 2008;40:1035-43.


OH
O
O
HO
H
H
H
O

A pedunculated aldosterone-producing adenoma drained by an extra vein causing puzzling results of adrenal vein sampling.

Tanja Dekkers[1] | Marieke S. Velema[1] | Ad R.M.M. Hermus[1] | Henry J.L.M. Timmers[1]
Johannes F. Langenhuijsen[2] | Mark Arntz[3] | Benno Kusters[4] | Graeme Eisenhofer[5,6]
Jacques W.M. Lenders[1,6] | Jaap Deinum[1]

Velema and Dekkers equally contributed to this study.

[1] Department of Internal Medicine, Radboud University Medical Center, Nijmegen, the Netherlands.
[2] Department of Urology, Radboud University Medical Center, Nijmegen, the Netherlands.
[3] Department of Radiology, Radboud University Medical Center, Nijmegen, the Netherlands.
[4] Department of Pathology, Radboud University Medical Center, Nijmegen, the Netherlands.
[5] Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Germany.
[6] Department of Internal Medicine III, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Germany.

Clin Endocrinol (Oxf). Volume89, Issue2; August 2018; Pages 242-244

ABSTRACT

Context. Primary aldosteronism (PA), a common cause of endocrine hypertension, is mainly caused by autonomous aldosterone production in one or both adrenal glands. This distinction can be made by adrenal venous sampling (AVS).

Case. We assessed a 53-year-old male for primary aldosteronism. Computerized tomography (CT) showed normal adrenal glands. AVS was regarded to be non-selective at the right side based on cortisol levels. A second AVS was selective at both sides and indicated low aldosterone secretion on both sides discordant with peripheral aldosterone levels. Aldosterone, but not cortisol, turned out to be high, however, in the sample drawn from a presumed right adrenal vein obtained at the first AVS procedure, previously judged non-selective. Matching fluoroscopy images showed that these samples were derived from an extra vein draining into the inferior vena cava. Retrospective analysis of the CT disclosed a small tumour just superior to the right adrenal gland, previously interpreted as a protrusion of the liver. The extra vein drained this tumour, and was excised together with the right adrenal gland, resulting in remission of PA. Macroscopically, the adenoma was separated from the main adrenal gland. However, a very small adrenal tissue bridge was present on histological level. Immunohistochemical staining of the pedunculated adrenal adenoma was positive for both CYP11B1 and CYP11B2. Genetic analysis of this tumour revealed an ATP2B3 mutation. Steroid profiling of AVS samples showed co-production of mainly testosterone.

Conclusion. An aldosterone-producing tumour located just superior to the adrenal gland drained by an extra vein can result in confusing AVS and CT results. The apparently extra-adrenal adenoma turned out to be part of the adrenal histologically. Bilaterally low levels of aldosterone in AVS may indicate a second vein on one side.

INTRODUCTION

Primary aldosteronism (PA) is a common cause of endocrine hypertension in which two main subtypes are distinguished as follows: bilateral adrenal hyperplasia (BAH) and aldosterone-producing adenomas (APA).[1] Computed tomography (CT) and adrenal vein sampling (AVS) are used to differentiate between these subtypes. There are two basic conditions for AVS. First, aldosterone concentrations should be corrected for peripheral blood admixture by normalizing for cortisol concentration. Second, it is assumed that either adrenal is drained by one vein. Thus, aldosterone hypersecretion, indicated by a much higher aldosterone-to-cortisol ratio, is observed in one (in the case of APA) or both (for BAH) veins. In this report, we describe a patient with PA and initially bilaterally suppressed aldosterone secretion who turned out to have a pedunculated adrenal tumour secreting aldosterone through an accessory vein.

CASE

A 53-year-old man was referred for PA. Six years earlier, therapy-resistant hypertension and spontaneous hypokalemia occurred. The aldosterone-to-renin ratio was increased and a subsequent salt loading test confirmed the diagnosis of PA. Because of bilaterally normal adrenals on CT-scan, the patient had been treated with a mineralocorticoid receptor antagonist until referral.

We then performed sequential AVS with continuous ACTH infusion (50 µg/h) [2] to reassess whether the patient was eligible for adrenalectomy. A first AVS was unsuccessful, based on two attempts to catheterize the right adrenal vein revealing cortisol concentrations not significantly higher than those in a peripheral vein. A second AVS was successful with bilaterally selective cannulation. Remarkably, both aldosterone-to-cortisol ratios were significantly lower than in the peripheral vein (Table 1). We excluded concurrent autonomous cortisol secretion with a 1 mg dexamethasone suppression test. As use of metanephrine provides a useful alternative to assess selectivity,[3] we then measured concentrations of metanephrine and aldosterone in all blood samples from the first nonselective AVS. Metanephrine concentrations supported the nonselectivity at the right side of the first

AVS (Table 1). Surprisingly, in both right-sided blood samples, aldosterone concentrations were exceptionally high (Table 1). A repeated inspection of the AVS images showed that those samples had been derived from an extra vein entering the inferior vena cava dorsally (Figure 1A). In addition, on the CT images that vein was connected to an oval structure located just above the right adrenal gland, measuring 18 × 23 mm (Figure 1C). This structure had been interpreted as a protrusion of the liver.

We assumed that PA was caused by an extra-adrenal aldosterone-producing tumour. The patient underwent retroperitoneoscopic surgery and the tumour was visible craniomedially of the right adrenal gland with a fragile vein draining into the inferior vena cava. During surgery, the adrenal itself and the tumour were removed en bloc as they appeared to be connected. Microscopic pathological examination revealed a small tissue bridge between the tumour and the adrenal gland (Figure 2). The tumour had all characteristics of a benign adenoma. The adrenal itself showed no abnormalities. Immunohistochemical staining of the tumour was positive for both CYP11B1 and CYP11B2. Genetic analysis indicated a somatic ATP2B3 mutation (c.1269_1274del (p.(Leu425_Val426del))).

Postoperatively, the patient had a normal blood pressure with nifedipine SR 60 mg daily. He was in complete biochemical remission (normal potassium concentration and salt loading test one year after operation).

Steroid profiling of blood obtained during the second attempt of the first AVS showed in addition to the high aldosterone concentration a high testosterone concentration in the vein draining the pedunculated adenoma (Table 2).

Table 1. Results of cortisol, aldosterone and metanephrine concentrations as measured in blood during both AVS procedures

AVS	Location	Cortisol (µmol/L)	Aldo-sterone (nmol/L)	Meta-nephrine (pmol/L)	Cortisol CV/PVa	Aldo-sterone/ cortisol	Meta-nephrine CV/PVb
	LAV	8.7	34.2	9095	10.9	3.9	47.1
1.1	Right (extra vein) c	0.81	171	313	1.0	na	1.6
	PV	0.80	19.8	193	na	24.8	na
	LAV	15.7	36.7	14565	15.5	2.3	>86.7
1.2	Right (extra vein) c	1.26	212	541	1.24	na	3.22
	PV	1.01	24.3	<168	na	4.6	na
	LAV	15.9	35.3	13393	12.3	2.2	37.4
2	RAV	42.0	100.3	20348	32.6	2.4	56.8
	PV	1.29	18.6	358	na	14.4	na

1.1 = first AVS, first attempt; 1.2 = first AVS, second attempt; 2 = second AVS. AVS = adrenal vein sampling; CV = cannulated vein; LAV = left adrenal vein; RAV = right adrenal vein; PV = peripheral vein; na = not applicable. Conversion factors to metric units: cortisol µmol/L to µg/dL, 36.25; aldosterone nmol/L to ng/dL, 36.05; metanephrine pmol/L to pg/mL, 0.1972. a Cortisol CV/PV ≥ 3 indicates selective catheter positioning in adrenal vein. b Metanephrine CV/PV ≥ 12 indicates selective catheter positioning in adrenal vein. c Vein draining APA. Emphasis ital is the abnormal, extra vein, of which the results are most relevant.

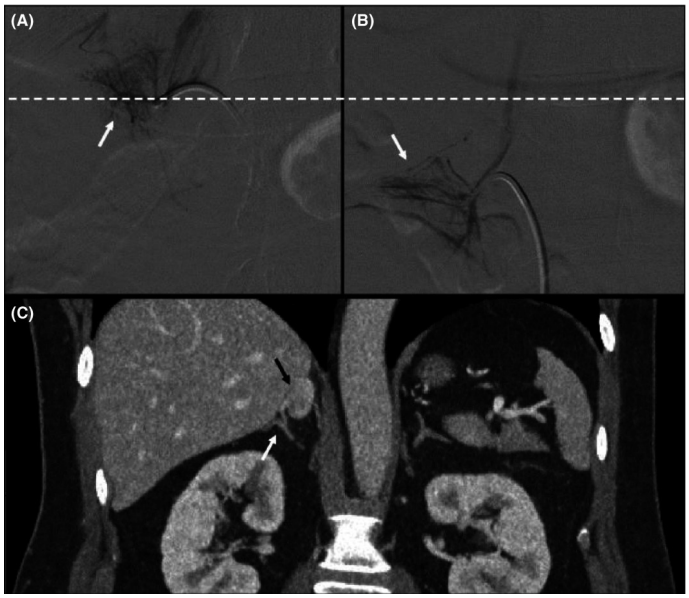


Figure 1. imaging of extra adrenal vein during AVS and on CT-scan.

A, First adrenal vein sampling (AVS) image showing contrast enhanced blood flow in right-sided aldosterone-producing tumour (white arrow). B, Second AVS procedure image showing contrast enhanced blood flow in right adrenal gland (white arrow). White dotted line = upper level of Th11. C, CT-scan (coronal plane) showing normal adrenal gland (white arrow) and aldosterone-producing tumour (black arrow)

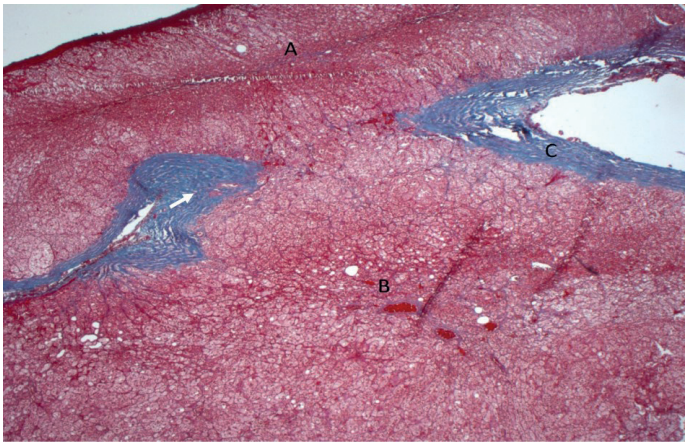


Figure 2. Microscopic pathological examination of the removed tumour.

Elastica van Masson staining showing tissue bridge (white arrow) between adrenal gland (A) and adenoma (B) surrounded by the capsule (C), original magnification x 50.

Table 2. Steroid profile first AVS procedure, second attempt.

Steroid	LAV (ng/ml)	Right (extra vein)* (ng/ml)	PV (ng/ml)	Right/LV	Right/PV
Aldosterone	9.38	30.45	7.46	3.246	4.082
Cortisol	4080	555	444	0.136	1.250
Androstendione	74.50	5.75	2.48	0.077	2.319
Corticosterone	1610.0	126.5	59.0	0.079	2.144
Cortisone	87.6	36.9	23.2	0.421	1.591
11-Deoxycorticosterone	51.50	22.10	4.89	0.429	4.519
11-Deoxycortisol	89.40	9.45	4.01	0.106	2.357
DHEA	272.00	18.50	6.58	0.068	2.812
DHEAS	1470	1430	1520	0.973	0.941
17-Hydroxyprogesterone	338.00	23.15	8.13	0.068	2.847
Progesterone	156.00	9.75	3.28	0.063	2.973
Testosterone	2.97	38.35	3.26	12.91	11.76
Pregnenolone	1580.00	87.00	40.30	0.055	2.159
21-Deoxycortisol	33.70	2.66	1.32	0.079	2.015
18-Hydroxyortisol	19.10	9.95	4.69	0.521	2.122
18-Oxocortisol	2.05	3.58	1.31	1.746	2.733

AVS= adrenal vein sampling, LAV= left adrenal vein, PV= peripheral vein. AVS was performed with continuous ACTH stimulation. * vein draining tumour

DISCUSSION

An extra-adrenal origin of PA is very rare (prevalence -0.5%).[4] Most published case reports describe aldosterone-producing ovarian tumours[5] or tumours originating from adrenal remnants located within the kidney.[4] One report describes an ectopic aldosteronoma located superior to the adrenal gland, but unlike in our patient, there appeared to be no connection with the adrenal.[6] In most cases, the tumours were considered as originating from

ectopic adrenocortical tissue that has been migrated with the gonads during embryological development, often consisting of adrenal cortex-like tissue only.[7]

In general, explanatory hypotheses of low aldosterone-to-cortisol ratios in adrenal veins in PA are fluctuating aldosterone secretion, accidental superselective cannulation of a tributary vein draining only normal adrenal tissue, an ectopic production of aldosterone, or anomalous anatomy of the adrenal veins.[8] An anatomical study showed the presence of duplicate right adrenal veins in 2 of 83 cases, of which one emptied into the vena cava and one joined an accessory hepatic vein.[9]

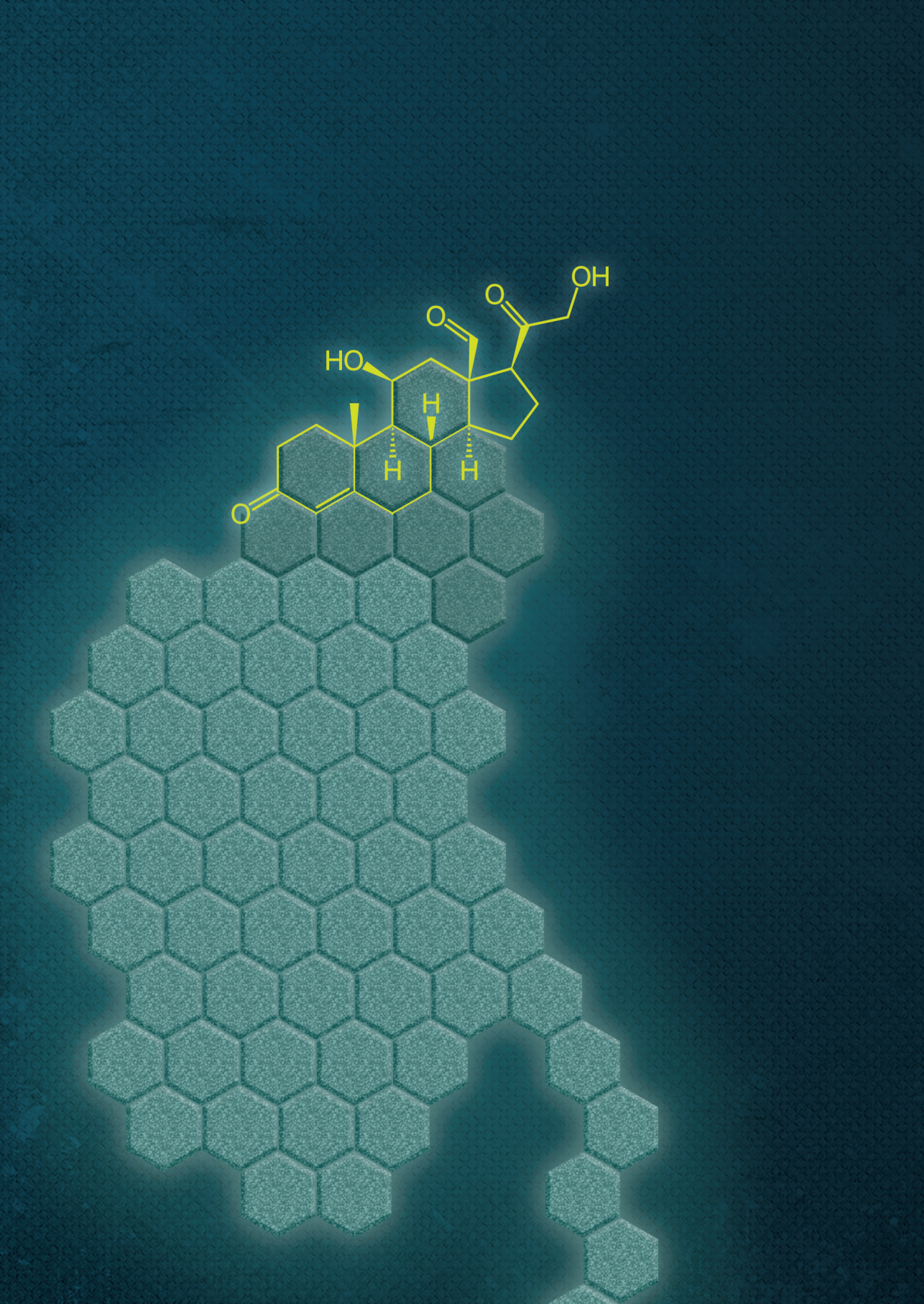
The adenoma contained a mutation in the ATP2B3 gene, which has been associated with PA.[10] It has been suggested that the steroid profile of the adrenal vein draining an APA-containing gland is specific for the presence of somatic mutations.[10] In such cases, nonadenomatous tissue also contributes to this profile. However, in this unique case, we have been able to specifically assess the steroids produced by an adenoma. We show that other steroid-generating enzymes, for example 17-hydroxysteroid-dehydrogenase, might be switched on as well, as exemplified by the increased production of testosterone. One other case of coproduction of testosterone by an APA has been reported. This tumour, in contrast to our case, also produced cortisol.[11]

Immunohistochemical investigation revealed that the adenoma stained positive not only for CYP11B2 but also for CYP11B1. This co-staining has been found in only 6% of patients with PA caused by ATPase or CACNA1D mutations compared to 49% and 45% in patients with a KCNJ5 mutant or wild-type tumour, respectively.[12] Although positive staining of CYP11B1 in APAs is associated with higher concentrations of cortisol after 1 mg dexamethasone overnight, only a minority of these patients have (subclinical) Cushing's syndrome.[13] In our patient, the results of the 1 mg dexamethasone test and the low cortisol concentrations in the vein draining the adenoma indicate the absence of significant cortisol production by the adenoma.

In conclusion, this case demonstrates that the combination of a CT-scan and AVS may not immediately succeed in localizing the source of aldosterone excess. Bilaterally low concentrations of aldosterone relative to cortisol in AVS may indicate a missed unilateral accessory vein on one side. Awareness of the possibility of abnormal anatomy is essential for correct interpretation of AVS results.

REFERENCES

1. Funder JW, Carey RM, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. The Journal of clinical endocrinology and metabolism 2016;101:1889-916.
2. Rossi GP, Auchus RJ, Brown M, et al. An expert consensus statement on use of adrenal vein sampling for the subtyping of primary aldosteronism. Hypertension 2014;63:151-60.
3. Dekkers T, Deinum J, Schultzekool LJ, et al. Plasma metanephrine for assessing the selectivity of adrenal venous sampling. Hypertension 2013;62:1152-7.
4. Abdelhamid S, Muller-Lobeck H, Pahl S, et al. Prevalence of adrenal and extra-adrenal Conn syndrome in hypertensive patients. Archives of internal medicine 1996;156:1190-5.
5. Kulkarni JN, Mistry RC, Kamat MR, Chinoy R, Lotlikar RG. Autonomous aldosterone-secreting ovarian tumor. Gynecologic oncology 1990;37:284-9.
6. Arnold J, Mitchell A. Conn's syndrome due to an ectopic adrenal adenoma. Postgraduate medical journal 1989;65:847-8.
7. Falls JL. Accessory adrenal cortex in the broad ligament: incidence and functional significance. Cancer 1955;8:143-50.
8. Shibayama Y, Wada N, Umakoshi H, et al. Bilateral aldosterone suppression and its resolution in adrenal vein sampling of patients with primary aldosteronism: analysis of data from the WAVES-J study. Clinical endocrinology 2016;85:696-702.
9. MacGillivray DC, Khwaja K, Shickman SJ. Confluence of the right adrenal vein with the accessory right hepatic veins. A potential hazard in laparoscopic right adrenalectomy. Surg Endosc 1996;10:1095-6.
10. Williams TA, Peitzsch M, Dietz AS, et al. Genotype-Specific Steroid Profiles Associated With Aldosterone-Producing Adenomas. Hypertension 2016;67:139-45.
11. Markou A, Tsigou K, Papadogias D, et al. A unique case of a benign adrenocortical tumor with triple secretion of cortisol, androgens, and aldosterone: development of multiple sclerosis after surgical removal of the tumor. Hormones (Athens, Greece) 2005;4:226-30.
12. Monticone S, Castellano I, Versace K, et al. Immunohistochemical, genetic and clinical characterization of sporadic aldosterone-producing adenomas. Mol Cell Endocrinol 2015;411:146-54.
13. Nanba K, Tsuiki M, Sawai K, et al. Histopathological diagnosis of primary aldosteronism using CYP11B2 immunohistochemistry. The Journal of clinical endocrinology and metabolism 2013;98:1567-74.


OH
O
O
HO
H
H
H
O

Discussion and perspectives

DISCUSSION AND PERSPECTIVES

This thesis discusses three aspects of primary aldosteronism (PA): (1) Prevalence; (2) Subtyping by AVS versus CT-scan; (3) Histopathology: the dichotomy of aldosterone producing adenoma (APA) and bilateral adrenal hyperplasia (BAH). We elaborate on the issues raised in the introduction in the light of the findings in this thesis.

1. PREVALENCE

Based on autopsy and laboratory data Dr. Conn estimated a PA prevalence of 10-20% of the hypertensive population.[1,2] Later, this was contested by subsequent studies with contradictory results.[3,4] Nevertheless in recent years most papers on PA introduce the disease as being the most frequent form of secondary hypertension, with percentages of anywhere between 5 and 20%.[5-9] Those who believe in a relatively high prevalence of PA in the hypertensive population base that notion on the studies showing a prevalence of 10-20%.[10] Opponents claim that the high prevalence is only the reflection of selection and referral bias and use of the disputed ARR to screen for PA.[11] The debate is appropriate because the true prevalence of PA determines the clinical relevance of screening all hypertensive patients for PA.[12] In that context, clarity on PA prevalence might have important consequences for policy decisions in the organization of health care.

STUDY HETEROGENEITY

In our systematic review, described in chapter 2, we tried to establish the prevalence of PA performing a systematic review. Thirty-six studies using confirmation testing to establish the diagnosis of PA showed a PA prevalence ranging from 1% to almost 30%. This wide range was mainly attributable to the gross heterogeneity in study design and date of the included studies. Previous reviews on PA prevalence faced the same problem.[9,13] In their review *Jansen et al.* acknowledged the heterogeneity in study design, screening tests, cut-off levels of the used tests and study population of the included studies.[13] Focusing on the aldosterone-to-renin ratio (ARR), they concluded that test conditions and medication use during ARR measurement can have important consequences for the test results and thus for the prevalence of PA diagnosis. In 2012 *Hannemann et al.* updated the review of *Jansen et al.* and confirmed that PA prevalence is highly dependent on study population, kind of assays, cut-offs for ARR and test conditions.[9]

In our study we found a strong heterogeneity in study design of PA prevalence studies too. Therefore, we chose to report prevalence ranges as we could not establish a weighted mean. In addition to the previous reviews we analyzed the factors underlying the wide ranges and we reported studies carried out in primary care and in referral centres separately. For referral centres our model showed the highest prevalence when (1) studies were performed after 2000; (2) studies were performed in Australia; (3) the study was retrospective; (4) the study objective was to assess the prevalence of secondary hypertension; (5) patient inclusion was consecutive; and (6) when no PA screening test was performed. Higher prevalences found after 2000 can be explained by the growing awareness in clinicians on the importance to detect PA. High PA prevalence in Australian studies might reflect the retrospective study methodology with inclusion of self-selected patients, although a higher prevalence in the Australian population cannot be excluded. The high prevalence in those studies relying only on PA confirmation testing without prior screening might reflect limited reliability of the screening test in other studies (false negative) or a limited reliability of the confirmation test (false positive).[14-16]

FUTURE RESEARCH

Study heterogeneity and methodological challenges hampered providing reliable estimates of PA prevalence. Ideally, a prospective, multi-continental, population based study should be conducted, including consecutive patients with newly diagnosed hypertension, using standardized and accepted screening and confirmation tests applied to all patients. A recent study that was conducted in the Netherlands met at least some of these criteria by prospectively including newly diagnosed patients with hypertension in primary care centres. The researchers found a PA prevalence of 2.6% (95% CI: 1.4-4.9). However, only a low proportion of the patients (9.2%) was screened for PA, reflecting the difficulty of including consecutive patients in primary care settings, resulting in an increased risk of selection bias. [17] So, before the 'ideal prevalence study' could be designed and implemented, standardization and validation of diagnostic protocols is of the utmost importance. Yet, the discussion on the optimal diagnostic protocols is as old as the one regarding PA prevalence and has not been settled yet.

2. SUBTYPING: AVS VERSUS CT-SCAN

As PA patients have an increased risk of cardiovascular complications, proper treatment is of key importance.[18-25] This treatment consists of either adrenalectomy or medical treatment with mineralocorticoid receptor antagonists (MRA). As described in the introduction it is important to select those patients that might benefit from surgery, i.e. patients with unilateral PA. CT-scan and AVS are used for this purpose but in many centres where AVS is not available, the selection of patients is done by CT scan only. However, a review of a large number of case series showed a limited concordance of 62.2% between CT and AVS regarding the localisation of excess aldosterone production.[26] The key question is whether any of these techniques is superior over the other.

SPARTACUS TRIAL

The proper way to solve this question is to perform a randomized trial instead of relying on retrospective, observational studies. With the Spartacus trial, described in chapter 3, we conducted such a prospective, randomized trial. Special about the Spartacus trial was the outcome-based and pragmatic character of the study design. The trial led to unexpected results when compared with previous retrospective or observational studies. We were unable to establish a clear difference in treatment outcome between AVS-guided or CT-guided treatment of PA patients. This can be attributed to several factors. Given that we observed a 50% discordance between CT and AVS derived conclusions, the presence of identical rates of adrenalectomy and similar treatment outcomes in both groups suggests that both methods may be fallible for different reasons. Here we discuss the factors that may explain our findings.

FLAWS IN STUDY DESIGN

First we should consider the possibility that study outcome was influenced by methodological issues in the Spartacus trial. A primary aspect to consider is study blinding. Although it was a randomized trial it was not blinded and both patients and treating physicians were aware of diagnostic allocation and treatment strategy. The question is whether this caused bias in office blood pressure measurements and patient compliance. As our primary outcome was the amount of medication needed to achieve target blood pressure this could be of significant importance. However, 24-hour blood pressure measurement is in fact a blinded way of assessing blood pressure. In addition, the fact that there was no difference in 24-hour blood pressure levels at the end of the study between both diagnostic groups refutes a relevant

bias in blood pressure measurements. Another aspect to be discussed is compliance to the use of medication. Although patient compliance is often compromised and no compliance monitoring was performed in our trial, it is not to be expected that this has been of influence on our trial results, as it is not likely that the allocated diagnostic strategy would be of influence on patient compliance. Non-compliance is expected to be distributed evenly.

Secondly, the selection of our primary endpoint, i.e. the intensity of antihypertensive treatment, can be questioned. A primary endpoint has to be applicable to the entire study population and not only to a subcohort, in this case only the operated patients. Therefore we chose the intensity of antihypertensive treatment which concerns the entire PA population, both operated patients and those treated with MRA. This contrasts with many previous observational studies that focused on adrenalectomised patients only. Also blood pressure itself would not have been a satisfactory primary endpoint, as it is not ethically justified to leave severe hypertension untreated for a prolonged period if it would persist after adrenalectomy or maximal MRA treatment. Therefore, the endpoint of intensity of antihypertensive treatment to achieve a target blood pressure was chosen, expressed in Defined Daily Dose (DDD). The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults, and has been recommended by the World Health Organisation (https://www.whocc.no). For example, 5 mg of amlodipin has a DDD of 1, as does 10 mg of lisinopril. If both drugs are taken together, the DDD is 2. However, in the case of PA the DDD of its main antihypertensive drugs, spironolactone and eplerenone, differs greatly. A dose of spironolactone 75 mg once daily is therapeutically equivalent to eplerenone 75 mg twice daily, but in terms of DDD correspond to 1DDD and 3DDD respectively. This could have interfered with our results and conclusions in case of unequal distribution of eplerenone use between the diagnostic groups. However, no unequal distribution was found in post-hoc analyses and confounding of our conclusions is therefore unlikely. Recently, an international consensus statement was published on the clinical and biochemical outcome measure to establish successful treatment in unilateral PA.[27] However, since the criteria were only published after our study was finished and are only applicable for patients treated with adrenalectomy, they could not be used in the Spartacus trial.

A third aspect that can be challenged is the power of the study. As our study addressed the clinical problem of how to select patients for adrenalectomy from the whole group of PA patients the power calculation was based on the entire PA cohort. When a clinician is confronted with a PA patient, he/she does not know the result of subtyping beforehand and hence, does not know whether an adrenalectomy or MRA is the appropriate treatment.

Therefore it is scientifically preferable to base the power analysis and outcome assessment on all patients with PA who are subjected to either of the two diagnostic strategies. However, because of this, the power for the subgroup analyses in the adrenalectomised patients might have been insufficient. We found a non-significant trend in favour of AVS in our secondary biochemical endpoint of resolved PA. This trend in favour of AVS over CT was also present for the patients who reached normotension without medication. In a larger cohort, these differences might become statistically significant. However, the question is whether it would be clinically relevant, because the magnitude of the difference was very small. A recent, large retrospective study in PA patients with a CT-based or AVS-based adrenalectomy did find a significant difference in complete biochemical remission in favour of the AVS-group.[28] It should be noted, however, that this was a retrospective study with a substantial risk of reporting bias. In addition, despite the difference in biochemical remission rate, no significant difference in clinical outcome was observed. This is in agreement with the Spartacus trial, since in both studies post-operative blood pressure and medication use was comparable between the CT-based and AVS-based treated patients. We would suggest that the difference in biochemical remission between the treatment groups is of clinical importance only if this results in meaningful differences in future cardiovascular complications or in health related quality of life. Although this may be expected on the basis of previous research, this could not be assessed in this study.[18,21-25,29,30] Also in case of the Spartacus trial, sample size and follow-up period precluded assessment of differences between the two study arms in terms of cardiovascular complications.

A fourth aspect that should be discussed is the skewed gender distribution in our study population, with more than three-quarter of the included subjects being male. Many previous studies on PA show a nearly equal gender distribution[14,17,28], although there are other studies that report skewed gender distributions, with inclusion of more male patients, as well.[31,32] This may be partly explained by a generally lower participation rate of female patients in clinical research. [33,34] However, in our study, we also found a gender difference in the initial 275 patients screened for inclusion, of whom 73% was male. This could be attributed to a different PA detection rates in male and female patients. Recent research shows that PA is more often diagnosed in male patients compared to female patients (60% vs. 40% respectively), which might be caused by differences in clinical presentation of the disease.[35] In our study, this skewed distribution has been of influence on treatment strategy, as more female patients were treated by adrenalectomy than male patients (65% versus 46%). However, as randomization between CT-scan and AVS was minimized for gender,

gender distribution between the diagnostic strategies was equal and is therefore unlikely to have influenced our trial conclusions.

Finally, the AVS and CT protocols adopted in the trial might have been of impact on the results. There is considerable debate on what constitutes optimal work-up in these patients. For example, there is no consensus on AVS cut-offs, the use of cosyntropin and the performance of consecutive or simultaneous bilateral sampling. In addition, there are no standardized protocols for CT assessment. Different protocols may therefore lead to different conclusions.[36-38] Some of these aspects regarding CT-scan and AVS will be addressed in the paragraphs below. Nonetheless, all protocols applied in our trial were in line with clinical guidelines and current clinical practice in many medical centres.

Now that the possible methodological issues of the Spartacus trial have been discussed, we should focus on the aspects that potentially compromise the accuracy of AVS or CT-scan.

CAUSES OF CT SCAN MISCLASSIFICATION

CT-scan has the potential flaw that it has a restricted detection limit, resolution and specificity, while considerable interobserver variation for the detection of adrenal adenomas is observed. Also, the physiological size difference between the left and right adrenal gland could confound conclusions. Recent studies have shown adrenal gland size differences due to patient's age, sex and weight.[39,40] *Degenhart et al.* showed a physiological difference in size and volume between the left and right adrenal gland, with a larger left adrenal gland.[41] This might lead to false-negative results in the right adrenal gland, or false-positive results in the left adrenal gland. In the Spartacus trial we did not account for such a physiological difference and a cut-off of 7mm was applied to define adrenal enlargement in both left and right adrenal. At this cut-off level, the CT-group showed solitary left-sided anomalies in 40% of the patients and solitary right-sided anomalies in only 12% of the patients. At follow-up those operated on a right-sided adenoma had a better outcome than those operated on a left-sided adenoma. This might be attributed to false positive results in CT-scans that showed solitary left-sided enlargement, which would be in line with the findings of *Degenhart et al*. When assessing the concordance between CT and AVS it is also striking that discordance was more often seen in case of left-sided CT anomalies (22 out of 42) compared to right-sided CT anomalies (2 out of 7) (Figure S1 supplemental data Spartacus trial). The physiological size difference between the left and right adrenal gland might have led to misclassification of a substantial number of patients in the CT-group. If so, it is intriguing why this has not led to a difference between the AVS and CT group in treatment outcome. Nevertheless, future

research of adrenal CT scanning should account for this by using age, sex, weight and gland localisation adjusted cut-offs. Also the use of adrenal volume instead of diameter might improve CT accuracy.[40]

CAUSES FOR AVS MISCLASSIFICATION

Misclassification by AVS might be contributed to several factors of which most are related to the AVS protocol used. The first question is whether cortisol is the right comparator to verify selective sampling and to adjust for venous non-adrenal blood mixture. *Arlt et al.* showed that patients with PA have a relatively high excretion of cortisol and other glucocorticoids in 24h urine samples.[42] In the Spartacus trial we did not systematically perform a dexamethasone suppression test to exclude autonomous cortisol excess. However, according to the study of *Arlt et al.*, performing dexamethasone suppression tests would not have helped in preventing AVS misclassification as almost all PA patients in their study had a normal overnight dexamethasone test despite relatively high 24h urine cortisol secretion. The reason for this remains unclear.[42]

Asymmetric co-secretion of cortisol in PA patients could explain AVS misclassification as AVS relies on the assumption that cortisol is equally secreted by both adrenal glands (Figure 1A). This assumption might be false and cortisol secretion might also be increased in the affected gland. This would result in an underestimation of the lateralization ratio in the adrenal vein of the affected gland (Figure 1B). The use of cortisol has other disadvantages. Because of the long circulating half life of cortisol (100 minutes), increases in adrenal vein concentrations above levels of peripheral venous concentrations are relatively mild under physiological conditions. Furthermore, due to physiological corticotropin fluctuations, cortisol secretion is fluctuating. Fluctuating cortisol levels can interfere with the interpretation of AVS selectivity.[43-45] In chapter 5 we show that metanephrine may be a better marker for selectivity. Although no prospective studies have been performed on the value of metanephrine to determine lateralisation, a recent case report shows successful AVS-based treatment outcome using metanephrine in calculations of both selectivity and lateralisation of AVS.[46] Finally, other steroids such as 11-deoxycortisol, DHEA and androstenedione, might be better options than cortisol as indicator of selectivity as well as normaliser for lateralisation, having superior plasma ratios between peripheral and adrenal blood.[47-51]

Second, the use of cosyntropin might have had an influence on sampling outcome. Cosyntropin is used to stimulate and to stabilize cortisol secretion, thus facilitating determination of sampling selectivity. The assumption is that in this way the problems of the relatively

low adrenal to peripheral cortisol gradient and the fluctuating cortisol secretion can be overcome. However, several studies suggest that cosyntropin may modify the lateralization index and might influence sampling conclusions, for example by increasing aldosterone secretion in the non-affected gland (Figure 1C).[52-55] A recent study of *El Ghorahyeb et al.* showed discordance between basal (non-stimulated) lateralization ratios and cosyntropin-stimulated lateralization ratios in 28% of the cases.[52] However, this study was performed using a large bolus of cosyntropin and not the continuous low dose cosyntropin infusion used in the Spartacus trial, and the significance of this difference is unknown.

Third, variations in adrenal anatomy could lead to erroneous conclusions from AVS results (Figure 1D). Upon examination of 546 laparoscopic adrenalectomies *Scholten et al.* found 70 adrenals (13%) with a deviant adrenal vein anatomy.[56] The following variants found were: one main adrenal vein with additional small veins (n=11), two draining adrenal veins (n=20), more than two adrenal veins (n=14), no main adrenal vein identifiable (n=18), and variants of the adrenal vein drainage to the inferior vena cava, hepatic vein or inferior phrenic vein (n=7). In the first three options described (8% of the patients), it could be that only one of the veins actually drains the aldosterone producing adenoma, while the others drain normal adrenal tissue. During an AVS procedure it is possible that only the vein that drains the normal adrenal tissue (and not the adenoma) is sampled, which would result in a selective sampling without lateralization, despite the presence of an adenoma (Figure 1D). This is also described in our case-report, chapter 7.

IMPACT OF SOMATIC MUTATIONS ON AVS

Besides the factors described above, theoretically, specific somatic adrenal mutations may determine sampling outcome. Several studies have been performed on the influence of somatic mutations on lateralization indices. *Seccia et al.* and *Williams et al.* found a higher lateralization index in adrenal glands harbouring a KCNJ5 mutation.[57,58] However, these findings were not supported by *Osswald et al.*[59] If specific somatic mutations influence the amount of aldosterone produced, this could affect AVS conclusions, especially in case of bilateral adenomas harbouring different mutations (Figure 2A). Besides the influence of a somatic mutation on aldosterone production itself, it could also influence the aldosterone response to cosyntropin stimulation. A recent case report shows that germline KCNJ5 mutations in hypertensive patients without PA can increase the adrenal aldosterone response to cosyntropin stimulation.[60] The question is whether that might also be the case in PA patients with a somatic KCNJ5 mutation (Figure 2B). In theory, specific mutations could also

have a different influence on cortisol production or the adrenal cortisol response on ACTH. As AVS is based on the assumption that cortisol is secreted equally by both adrenal glands, this could seriously affect sampling results (Figure 2C and 2D).

PROSPECTS

The Spartacus trial , despite its pragmatic character, can be considered as a proof-of-concept study and it shows that the concept of AVS superiority over CT scan in general may be questioned. These outcomes have caused a stir in the scientific PA community, splitting it into those who are in favor and those who are opposed to the use of AVS (in its present form).[61-68]

The key question is, however: how to proceed from here? We discuss three options: improve AVS, replace AVS with another diagnostic option, or abandon surgery.

The option to improve AVS is appealing since AVS is conceptually sound and insight into its strengths and weaknesses is increasing. If we would choose to improve AVS, we need further prospective randomized studies to shed light on several aspects of this technique as mentioned in the previous paraghaphs. Also, better understanding of the pathobiology of adrenal adenomas and hyperplasia may lead to better modalities of how AVS should be performed. Knowledge on the co-secretion of cortisol and other steroids, for instance 11-deoxycortisol, DHEA and androstenedione, can improve the AVS procedure and interpretation by taking into account pathophysiological processes as described above and in Figure 1. Also a better understanding of the histopathology and aetiology of adrenal nodules and hyperplasia might change our view on AVS.

We could also choose to abandon AVS and focus on other techniques. Non-invasive functional imaging is advancing fast. New techniques as PET-methomidate, or PET/MRI with CYP11B2 specific imaging or use of other ligands might make CT and AVS redundant.[69-74] Another rapidly advancing option is steroid profiling in peripheral blood. In this technique different steroid concentrations and their ratios predict the presence of a unilateral adenoma or bilateral hyperplasia.[47,58,75-77] However, the diagnostic protocols of these new techniques have not yet been validated. Prospective, outcome-based, randomized studies with a similar design as the Spartacus trial would be needed to prove their superiority.

A third option could be to find another treatment strategy for PA, which would render surgery redundant. When there is no need for surgery it is no longer important to select those that would benefit from surgery. As discussed in the introduction, conventional MRAs cannot replace surgery as they might give less protection against (subclinical) organ damage, impair quality of life, and are less cost-effective.[24,25,78-83] However, new drugs that might overcome

these problems are in the pipeline: tissue-specific aldosterone antagonists, non-steroidal MRAs or aldosterone synthase inhibitors. Especially the last category is very interesting as it does not block the effect of aldosterone but blocks its production. Aldosterone synthase (encoded by the *CYP11B2* gene) catalyzes the last three steps in the biosynthesis of aldosterone. By inhibiting these steps plasma levels of aldosterone will decrease. However, the high homology of aldosterone synthase with steroid β-hydroxylase (*CYP11B1*) poses an important problem as higher doses cause both aldosterone and cortisol suppression making patients prone for an Addison crisis. Hence, it is interesting to see that currently, more selective drugs are being developed.[84]

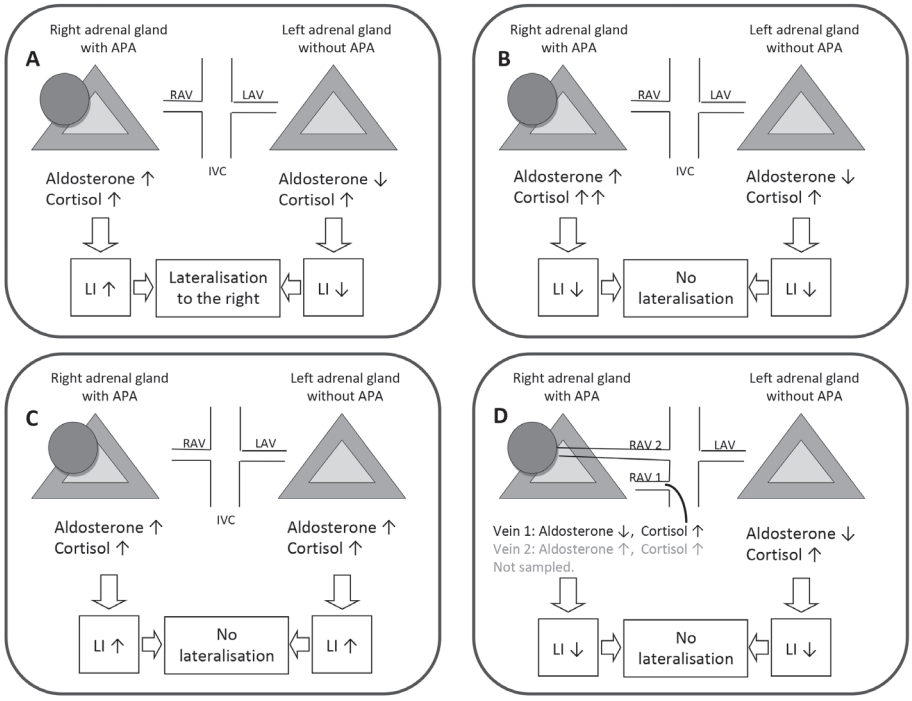


Figure 1. Potential causes of AVS "failure" in case of unilateral aldosterone producing adenoma in cosyntropin stimulated procedures.

A. Normal situation of cosyntropin-stimulated AVS in case of normal adrenal vein anatomy. Assumption: cosyntropin stimulates similar bilateral cortisol secretion and has no influence on the aldosterone production. B. Aldosterone producing adenoma with co-secretion of cortisol. C. cosyntropin stimulation causing aldosterone hypersecretion from the non-affected gland. D. aberrant adrenal vein anatomy with two veins of which only one drains the aldosterone producing adenoma. LI = lateralisation index. See introduction for interpretation of these indices.

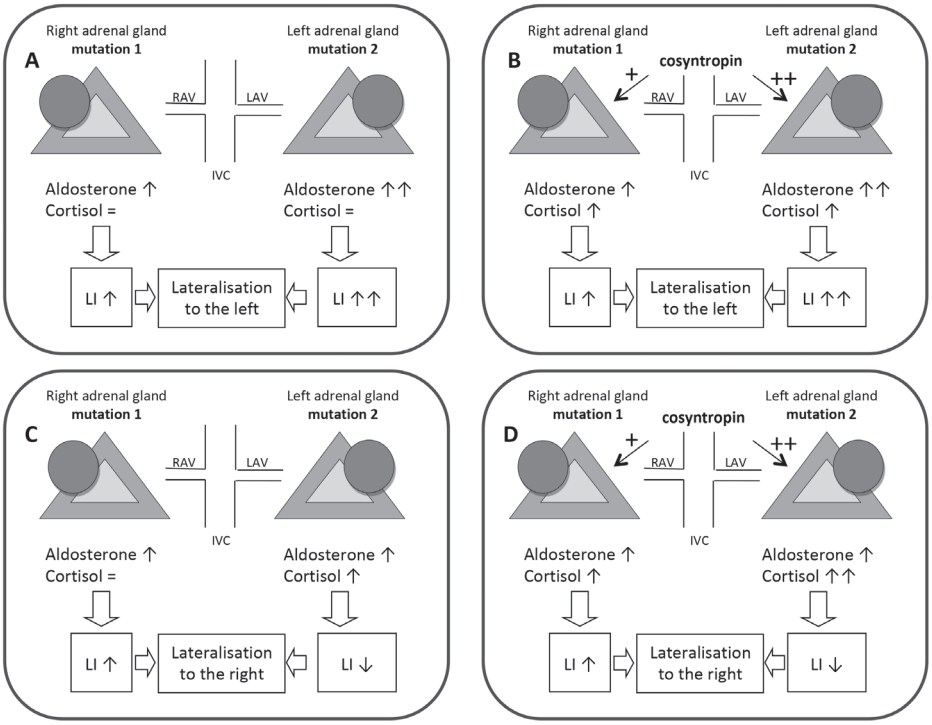


Figure 2. Theoretical flaws in adrenal vein sampling in case of bilateral aldosterone production due to somatic mutations.

A. a specific somatic mutation in the left adrenal causes a higher increase in aldosterone production than another mutation present in the right gland. Despite bilateral adenomas, there will be a lateralization to the left. B. a somatic mutation in the left adrenal gland causes further increased aldosterone production upon cosyntropin stimulation. Another mutation in the right adrenal gland does not. Despite bilateral adenomas, there will be a lateralization to the left. C. a specific mutation in the left adrenal gland causes both aldosterone and cortisol overproduction, another mutation in the right adrenal gland causes only aldosterone overproduction. Despite bilateral adenomas, there will be a lateralization to the right. D. a somatic mutation in the left adrenal gland causes increased cortisol production upon cosyntropin stimulation. Another mutation in the right adrenal gland does not. Despite bilateral adenomas, there will be a lateralization to the right. RAV = right adrenal vein; LAV = left adrenal vein; IVC = inferior vena cava; LI = lateralisation index.

3. PA HISTOPATHOLOGY: THE DICHOTOMY OF ALDOSTERONE PRODUCING ADENOMA AND BILATERAL ADRENAL HYPERPLASIA

As described in the introduction, PA is considered to be caused by either a unilateral adenoma or bilateral hyperplasia. Already in 1956 differences between the two were described.[85] Since then, the entire clinical work-up of PA is based upon this principle of dichotomy, and also the treatment strategies depend on it. Many previous studies, showing

single adenomas in all excised glands, have lent support for this dichotomy.[6,86-88] However, the assumption that all adrenal glands excised because of a presumed unilateral adenoma, harbour only one nodule is questionable. Evidence that contradicts this is accumulating.[89-93]

CORTICAL NODULATION AND SOMATIC MUTATIONS

In line with previous investigations[89-93] we show in chapter 6, that many adrenals with a presumed single adenoma demonstrate various patterns of macronodular or micronodular hyperplasia. Our study also suggests that not the entire adrenal gland, but only one (or two) of the nodules in a multinodular gland is actually responsible for aldosterone excess. In our study most removed glands, regardless of whether the gland was classified as an adenoma or nodular hyperplasia, contained only one nodule with *p450C18* (CYP11B2) expression, and with that the capability of aldosterone production. Other nodules in that same multinodular gland were negative for aldosterone production based on the absence of aldosterone synthase activity. We found only four glands harbouring more than one *p450C18* (CYP11B2) expressing nodule. Later studies seem to have found adrenals with multiple *p450C18* positive nodules more frequently.[94,95]

In our study we investigated the presence of somatic mutations (KCNJ5, ATP1A1, ATP2B3, CACNA1D) in the adrenal nodules, both with and without *p450C18* (CYP11B2) expression. Strikingly, not all mutations seemed to originate from the same adrenal cortex cell type. KCNJ5 mutations were more often present in zona fasciculate-like cells (foamy and lipid-rich adrenal cortical cells) and were more frequently found in female patients. In male patients we found more ATP1A1, ATP2B3 and CACNA1D mutations. These genotype-phenotype related findings are consistent with other studies.[96-101] In our study, somatic mutations were only present in those nodules staining positive for aldosterone synthase. In one of the four adrenals harbouring multiple *p450C18* (CYP11B2) positive nodules we found a different mutation in each nodule. Some other studies have also reported the presence of multiple mutations within one gland.[94,102,103] Although we did not find a mutation in every nodule with aldosterone synthase activity, it is plausible that the remaining aldosterone synthase expressing nodules contain other aldosterone-stimulating mutations that have not been discovered yet.

There are several hypotheses linking the somatic mutations to nodulation and aldosterone excess. *Choi et al.* hypothesized that the somatic mutations promote growth of aldosterone secreting cells, thus causing the formation of aldosterone producing adenomas. In chapter 6 we hypothesize that the mutations are causative for the aldosterone hypersecretion but not

necessarily for the nodulation itself as a mutation is not present in all nodules. The hypothesis we propose is that some individuals for some reason develop multinodular adrenal cortices and that only if a mutation occurs aldosterone hypersecretion occurs. This is in line with the hypothesis of *Zennaro et al.*, suggesting a two-hit mechanism with one hit causing adrenocortical cell proliferation and a second hit causing hormone hypersecretion.[104]

The discovery of the so-called aldosterone producing cell clusters (APCCs) could also help developing new hypotheses regarding PA pathophysiology. APCCs are small (0.2-1.0 mm diameter) aldosterone synthase expressing islands in the adrenal cortex. They seem to develop in the adrenal during lifetime and do not necessarily cause PA.[105] The real function of these APCCs is still unknown.[106,107] In our own study, chapter 6, all adrenals were screened for the presence of APCCs, and they were detected in more than half of the glands. Unfortunately, we could not establish a relation between the presence of APCCs and patient characteristics, histopathology or immunohistochemistry. We did not assess the presence of somatic mutations in APCCs. In a study by *Nishimoto et al.*, somatic mutations known to cause excess aldosterone production were identified in APCCs in adrenals of both healthy individuals and PA patients.[106,108] Although it is still unclear if a mutated APCC can develop in an aldosterone producing adenoma, a recent case-report on APCC-to-APA transitional lesions suggests that this may indeed be the case.[109] The fact that APCCs harbour mutations without nodule formation might be one of the hits required for the two-hit mechanism suggested in the previous paragraph.

ALTERNATIVE PATHOGENESIS

All these recent pathological findings challenge the commonly assumed dichotomy of unilateral versus bilateral disease in PA. The extensive variation in histopathological adrenal characteristics and the presence of APCCs raise the possibility that many cases of presumed unilateral aldosterone hypersecretion may in fact represent bilateral asymmetric nodular hyperplasia as a result of somatic mutations.[110] PA may be a disease of the adrenal glands where a patient may be anywhere on the line between unilateral and bilateral hypersecretion.[110] However, this hypothesis would imply that the contralateral adrenal gland is always involved to some extent. The question is whether this should influence treatment decisions, as this hypothesis suggests that adrenalectomy cannot be curative for a lifetime. However, in clinical practice most patients with a long-term post-adrenalectomy follow-up never show signs of recurrent PA.[111] The reason for this could be threefold. 1. Inadequate follow-up. In clinical practice, patients are often referred back to their general

physician when blood-pressure normalizes or is treated successfully within the first year after adrenalectomy. There are few studies with a follow-up of more than five years and none of them repeat saline infusion tests or even ARR regularly at follow-up.[111]; 2. The process causing hyperaldosteronism is initially stopped by removal of the affected adrenal gland. This process may commence (or continue) in the contralateral gland, but it takes more than the remaining patient's lifetime for the contralateral gland to cause true hyperaldosteronism again. In that case the presence of bilateral disease would have no clinical consequences, or; 3. The hypothesis stated above is incorrect and there is indeed real unilateral PA. In that case the real question is: is there a different aetiology for unilateral and bilateral disease?
To shed more light on this subject further research is needed. Studies linking adrenal vein steroid profiles with imaging studies, histopathological characteristics and somatic mutations could provide further insight in the presence of bilateral involvement. In the long-term, post-adrenalectomy follow-up studies with lifetime annual or quinquennial biochemical testing and imaging would be very interesting. Also autopsy studies on PA patients could be of great value to determine bilateral involvement. Even more promising could be the results of functional imaging studies, which would be able to show adrenal functional activity of the non-excised adrenal gland during long-time follow-up.

CONCLUDING REMARKS

I would like to end this thesis with the patient case presented in the introduction: a 48 years old, male patient with a blood pressure of 150/94 mmHg and a decreased plasma potassium level of 3.1 mmol/l. Upon further examination he is diagnosed with PA and he is willing to undergo adrenal surgery if evidence for a unilateral adenoma is found. His treating specialist in a peripheral hospital, without AVS facilities, performs a CT-scan which shows a unilateral lesion in the left adrenal gland. Now the question is: Should he refer the patient to a surgeon for a unilateral adrenalectomy or should he refer him to an AVS-performing centre for further evaluation?
Currently, the results of the SPARTACUS trial seem to suggest that AVS is unlikely to improve the prospects of the patient. However, a single trial is rarely considered to provide conclusive evidence. Hence, further studies would be needed to either challenge or support the recommendation by the Endocrine Society guideline that all patients should undergo AVS. Based on the Spartacus trial results we might conclude that this patient has the same chance of good clinical outcome when treatment is based on CT-scan results compared to AVS results. However, what this trial taught us too is that both techniques are imperfect.

As researchers and clinicians we cannot be satisfied with the diagnostic options that are currently available and we have to expand our knowledge. Also for that reason, this patient should be referred to an AVS-performing centre. Not to perform an AVS in the context of standard of care, but to participate in further well organized, multicentre and international diagnostic trials to improve AVS or explore new diagnostic options. The only valid argument to continue AVS systematically in all patients is to improve this technique. In case referral is considered not feasible, treatment can be safely based on CT-scan result only.

As discussed above, there are many aspects of AVS possibly causing misclassification which could be further investigated in these new diagnostic trials. One of these aspects could be the use of metanephrine or steroid profiles instead of cortisol to determine AVS selectivity and to correct for non-adrenal venous mixture in the assessment of lateralisation. In a diagnostic trial a PA patient would be randomized between cortisol-based AVS and metanephrine-based AVS with treatment outcome as a reference standard. The advantage in methodology, compared to the Spartacus trial, would be that both cortisol and metanephrine can be determined in all patients with randomization determining which one to use for determination of treatment strategy. In this way the study could also be blinded for both patient and physician. In this study we should ensure to obtain a thorough histopathological assessment of the resected adrenals, including a somatic mutation analysis. Ideally, it would be followed by an extended study period assessing annual or, in the long-term, quinquennial biochemical outcome and adrenal imaging.

The study proposed above is just one of the many possible studies to optimize PA diagnostic work-up. Many other aspects of the diagnostic work-up might be improved. However, whatever aspect we would choose to address we should no longer focus on retrospective studies but focus on diagnostic, outcome-based randomized trials. The Spartacus trial has shown that this is feasible.

REFERENCES

1. Conn JW. The evolution of primary aldosteronism: 1954-1967. Harvey lectures 1966;62:257-91.
2. Schteingart DE. The 50th anniversary of the identification of primary aldosteronism: a retrospective of the work of Jerome W. Conn. The Journal of laboratory and clinical medicine 2005;145:12-6.
3. Berglund G, Andersson O, Wilhelmsen L. Prevalence of primary and secondary hypertension: studies in a random population sample. British medical journal 1976;2:554-6.
4. Swales JD. Primary aldosteronism: how hard should we look? British medical journal (Clinical research ed) 1983;287:702-3.
5. Rossi GP, Bernini G, Caliumi C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. Journal of the American College of Cardiology 2006;48:2293-300.
6. Mulatero P, Stowasser M, Loh KC, et al. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. The Journal of clinical endocrinology and metabolism 2004;89:1045-50.
7. Gordon RD, Stowasser M, Tunny TJ, Klemm SA, Rutherford JC. High incidence of primary aldosteronism in 199 patients referred with hypertension. Clinical and experimental pharmacology & physiology 1994;21:315-8.
8. Funder JW, Carey RM, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. The Journal of clinical endocrinology and metabolism 2016;101:1889-916.
9. Hannemann A, Wallaschofski H. Prevalence of primary aldosteronism in patient's cohorts and in population-based studies--a review of the current literature. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2012;44:157-62.
10. Calhoun DA. Is there an unrecognized epidemic of primary aldosteronism? Pro. Hypertension 2007;50:447-53; discussion 447-53.
11. Kaplan NM. Is there an unrecognized epidemic of primary aldosteronism? Con. Hypertension 2007;50:454-8; discussion 454-8.
12. Buffolo F, Monticone S, Burrello J, et al. Is Primary Aldosteronism Still Largely Unrecognized? Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2017;49:908-14.
13. Jansen PM, Boomsma F, van den Meiracker AH. Aldosterone-to-renin ratio as a screening test for primary aldosteronism--the Dutch ARRAT Study. The Netherlands journal of medicine 2008;66:220-8.

14. Jansen PM, van den Born BJ, Frenkel WJ, et al. Test characteristics of the aldosterone-to-renin ratio as a screening test for primary aldosteronism. Journal of hypertension 2014;32:115-26.
15. Willenberg HS, Vonend O, Schott M, et al. Comparison of the saline infusion test and the fludrocortisone suppression test for the diagnosis of primary aldosteronism. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2012;44:527-32.
16. Mulatero P, Milan A, Fallo F, et al. Comparison of confirmatory tests for the diagnosis of primary aldosteronism. The Journal of clinical endocrinology and metabolism 2006;91:2618-23.
17. Kayser SC, Deinum J, de Grauw WJ, et al. Prevalence of primary aldosteronism in primary care: a cross-sectional study. The British journal of general practice : the journal of the Royal College of General Practitioners 2018;68:e114-e22.
18. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. Journal of the American College of Cardiology 2005;45:1243-8.
19. Monticone S, Burrello J, Tizzani D, et al. Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice. Journal of the American College of Cardiology 2017;69:1811-20.
20. Monticone S, D'Ascenzo F, Moretti C, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. The lancet Diabetes & endocrinology 2018;6:41-50.
21. Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular complications associated with primary aldosteronism: a controlled cross-sectional study. Hypertension 2013;62:331-6.
22. Born-Frontsberg E, Reincke M, Rump LC, et al. Cardiovascular and cerebrovascular comorbidities of hypokalemic and normokalemic primary aldosteronism: results of the German Conn's Registry. The Journal of clinical endocrinology and metabolism 2009;94:1125-30.
23. Catena C, Colussi G, Nadalini E, et al. Cardiovascular outcomes in patients with primary aldosteronism after treatment. Archives of internal medicine 2008;168:80-5.
24. Mulatero P, Monticone S, Bertello C, et al. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. The Journal of clinical endocrinology and metabolism 2013;98:4826-33.
25. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. The lancet Diabetes & endocrinology 2018;6:51-9.
26. Kempers MJ, Lenders JW, van Outheusden L, et al. Systematic review: diagnostic procedures to differentiate unilateral from bilateral adrenal abnormality in primary aldosteronism. Annals of internal medicine 2009;151:329-37.

27. Williams TA, Lenders JWM, Mulatero P, et al. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. The lancet Diabetes & endocrinology 2017;5:689-99.
28. Williams TA, Burrello J, Sechi LA, et al. Computed Tomography and Adrenal Venous Sampling in the Diagnosis of Unilateral Primary Aldosteronism. Hypertension 2018 Sep;72(3):641-649.
29. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Incidence of Atrial Fibrillation and Mineralocorticoid Receptor Activity in Patients With Medically and Surgically Treated Primary Aldosteronism. JAMA cardiology 2018;3:768-74.
30. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Renal Outcomes in Medically and Surgically Treated Primary Aldosteronism. Hypertension 2018;72:658-66.
31. Young WF, Stanson AW, Thompson GB, Grant CS, Farley DR, van Heerden JA. Role for adrenal venous sampling in primary aldosteronism. Surgery 2004;136:1227-35.
32. Nwariaku FE, Miller BS, Auchus R, et al. Primary hyperaldosteronism: effect of adrenal vein sampling on surgical outcome. Archives of surgery (Chicago, Ill : 1960) 2006;141:497-502; discussion 502-3
33. Martin SS, Ou FS, Newby LK, et al. Patient- and trial-specific barriers to participation in cardiovascular randomized clinical trials. Journal of the American College of Cardiology 2013;61:762-9.
34. Ding EL, Powe NR, Manson JE, Sherber NS, Braunstein JB. Sex differences in perceived risks, distrust, and willingness to participate in clinical trials: a randomized study of cardiovascular prevention trials. Archives of internal medicine 2007;167:905-12.
35. Heinrich DA, Adolf C, Rump LC, et al. Primary aldosteronism: key characteristics at diagnosis: a trend toward milder forms. European journal of endocrinology / European Federation of Endocrine Societies 2018;178:605-11.
36. Kline GA, Harvey A, Jones C, et al. Adrenal vein sampling may not be a gold-standard diagnostic test in primary aldosteronism: final diagnosis depends upon which interpretation rule is used. Variable interpretation of adrenal vein sampling. International urology and nephrology 2008;40:1035-43.
37. Lethielleux G, Amar L, Raynaud A, Plouin PF, Steichen O. Influence of diagnostic criteria on the interpretation of adrenal vein sampling. Hypertension 2015;65:849-54.
38. Mulatero P, Bertello C, Sukor N, et al. Impact of different diagnostic criteria during adrenal vein sampling on reproducibility of subtype diagnosis in patients with primary aldosteronism. Hypertension 2010;55:667-73.
39. Carsin-Vu A, Oubaya N, Mule S, et al. MDCT Linear and Volumetric Analysis of Adrenal Glands: Normative Data and Multiparametric Assessment. European radiology 2016;26:2494-501.

40. Schneller J, Reiser M, Beuschlein F, et al. Linear and volumetric evaluation of the adrenal gland--MDCT-based measurements of the adrenals. Academic radiology 2014;21:1465-74.
41. Degenhart C, Schneller J, Osswald A, et al. Volumetric and densitometric evaluation of the adrenal glands in patients with primary aldosteronism. Clinical endocrinology 2017;86:325-31.
42. Arlt W, Lang K, Sitch AJ, et al. Steroid metabolome analysis reveals prevalent glucocorticoid excess in primary aldosteronism. JCI insight 2017 Apr 20;2(8).
43. Seccia TM, Miotto D, Battistel M, et al. A stress reaction affects assessment of selectivity of adrenal venous sampling and of lateralization of aldosterone excess in primary aldosteronism. European journal of endocrinology / European Federation of Endocrine Societies 2012;166:869-75.
44. Spark RF, Kettyle WR, Eisenberg H. Cortisol dynamics in the adrenal venous effluent. The Journal of clinical endocrinology and metabolism 1974;39:305-10.
45. Tanemoto M, Suzuki T, Abe M, Abe T, Ito S. Physiologic variance of corticotropin affects diagnosis in adrenal vein sampling. European journal of endocrinology / European Federation of Endocrine Societies 2009;160:459-63.
46. Goupil R, Wolley M, Ungerer J, et al. Use of plasma metanephrine to aid adrenal venous sampling in combined aldosterone and cortisol over-secretion. Endocrinology, diabetes & metabolism case reports 2015;2015:150075.
47. Eisenhofer G, Dekkers T, Peitzsch M, et al. Mass Spectrometry-Based Adrenal and Peripheral Venous Steroid Profiling for Subtyping Primary Aldosteronism. Clinical chemistry 2016;62:514-24.
48. Peitzsch M, Dekkers T, Haase M, et al. An LC-MS/MS method for steroid profiling during adrenal venous sampling for investigation of primary aldosteronism. The Journal of steroid biochemistry and molecular biology 2015;145:75-84.
49. Ceolotto G, Antonelli G, Maiolino G, et al. Androstenedione and 17-alpha-Hydroxyprogesterone Are Better Indicators of Adrenal Vein Sampling Selectivity Than Cortisol. Hypertension 2017;70:342-6.
50. Li H, Zhang X, Shen S, et al. Adrenal androgen measurement for assessing the selectivity of adrenal venous sampling in primary aldosteronism. Steroids 2018;134:16-21.
51. Nilubol N, Soldin SJ, Patel D, et al. 11-Deoxycortisol may be superior to cortisol in confirming a successful adrenal vein catheterization without cosyntropin: a pilot study. International journal of endocrine oncology 2017;4:75-83.
52. El Ghorayeb N, Mazzuco TL, Bourdeau I, et al. Basal and Post-ACTH Aldosterone and Its Ratios Are Useful During Adrenal Vein Sampling in Primary Aldosteronism. The Journal of clinical endocrinology and metabolism 2016;101:1826-35.

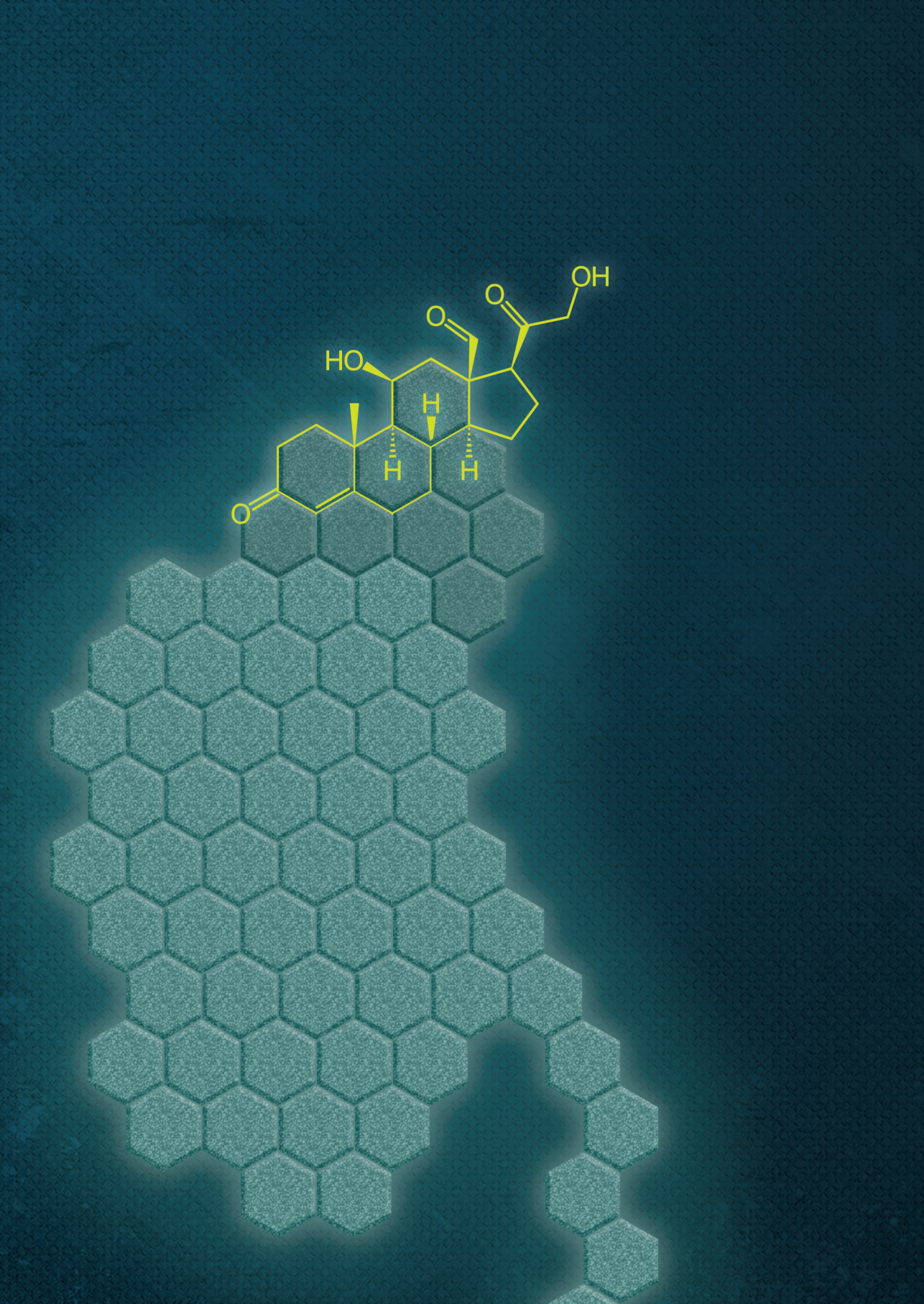
53. Rossitto G, Maiolino G, Lenzini L, et al. Subtyping of primary aldosteronism with adrenal vein sampling: Hormone- and side-specific effects of cosyntropin and metoclopramide. Surgery 2017.
54. Rossi GP, Pitter G, Bernante P, Motta R, Feltrin G, Miotto D. Adrenal vein sampling for primary aldosteronism: the assessment of selectivity and lateralization of aldosterone excess baseline and after adrenocorticotropic hormone (ACTH) stimulation. Journal of hypertension 2008;26:989-97.
55. Seccia TM, Miotto D, De Toni R, et al. Adrenocorticotropic hormone stimulation during adrenal vein sampling for identifying surgically curable subtypes of primary aldosteronism: comparison of 3 different protocols. Hypertension 2009;53:761-6.
56. Scholten A, Cisco RM, Vriens MR, Shen WT, Duh QY. Variant adrenal venous anatomy in 546 laparoscopic adrenalectomies. JAMA surgery 2013;148:378-83.
57. Seccia TM, Mantero F, Letizia C, et al. Somatic mutations in the KCNJ5 gene raise the lateralization index: implications for the diagnosis of primary aldosteronism by adrenal vein sampling. The Journal of clinical endocrinology and metabolism 2012;97:E2307-13.
58. Williams TA, Peitzsch M, Dietz AS, et al. Genotype-Specific Steroid Profiles Associated With Aldosterone-Producing Adenomas. Hypertension 2016;67:139-45.
59. Osswald A, Fischer E, Degenhart C, et al. Lack of influence of somatic mutations on steroid gradients during adrenal vein sampling in aldosterone-producing adenoma patients. European journal of endocrinology / European Federation of Endocrine Societies 2013;169:657-63.
60. Sertedaki A, Markou A, Vlachakis D, et al. Functional characterization of two novel germline mutations of the KCNJ5 gene in hypertensive patients without primary aldosteronism but with ACTH-dependent aldosterone hypersecretion. Clinical endocrinology 2016;85:845-51.
61. Williams TA, Reincke M. MANAGEMENT OF ENDOCRINE DISEASE: Diagnosis and management of primary aldosteronism: the Endocrine Society guideline 2016 revisited. European journal of endocrinology / European Federation of Endocrine Societies 2018;179:R19-r29.
62. Funder JW, Rossi GP. Adrenal vein sampling versus CT scanning in primary aldosteronism. The lancet Diabetes & endocrinology 2016;4:886.
63. van der Wilt GJ, Dekkers T, Lenders JW, Deinum J. Adrenal vein sampling versus CT scanning in primary aldosteronism - Authors' reply. The lancet Diabetes & endocrinology 2016;4:886-7.
64. Beuschlein F, Mulatero P, Asbach E, et al. The SPARTACUS Trial: Controversies and Unresolved Issues. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2017;49:936-42.
65. Deinum J, Prejbisz A, Lenders JWM, van der Wilt GJ. Adrenal Vein Sampling Is the Preferred Method to Select Patients With Primary Aldosteronism for Adrenalectomy: Con Side of the Argument. Hypertension 2018;71:10-4.

66. Rossi GP, Funder JW. Adrenal Vein Sampling Is the Preferred Method to Select Patients With Primary Aldosteronism for Adrenalectomy: Pro Side of the Argument. Hypertension 2018;71:5-9.
67. Rossi GP, Funder JW. Adrenal Venous Sampling Versus Computed Tomographic Scan to Determine Treatment in Primary Aldosteronism (The SPARTACUS Trial): A Critique. Hypertension 2017;69:396-7.
68. Rossi GP, Mulatero P, Satoh F. 10 good reasons why adrenal vein sampling is the preferred method for referring primary aldosteronism patients for adrenalectomy. Journal of hypertension 2019;37:603-11.
69. Mendichovszky IA, Powlson AS, Manavaki R, et al. Targeted Molecular Imaging in Adrenal Disease-An Emerging Role for Metomidate PET-CT. Diagnostics (Basel, Switzerland) 2016 Nov 18;6(4).
70. Powlson AS, Gurnell M, Brown MJ. Nuclear imaging in the diagnosis of primary aldosteronism. Current opinion in endocrinology, diabetes, and obesity 2015;22:150-6.
71. Burton TJ, Mackenzie IS, Balan K, et al. Evaluation of the sensitivity and specificity of (11) C-metomidate positron emission tomography (PET)-CT for lateralizing aldosterone secretion by Conn's adenomas. The Journal of clinical endocrinology and metabolism 2012;97:100-9.
72. Abe T, Naruse M, Young WF, Jr., et al. A Novel CYP11B2-Specific Imaging Agent for Detection of Unilateral Subtypes of Primary Aldosteronism. The Journal of clinical endocrinology and metabolism 2016;101:1008-15.
73. Naruse M, Umakoshi H, Tsuiki M, et al. The Latest Developments of Functional Molecular Imaging in the Diagnosis of Primary Aldosteronism. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2017;49:929-35.
74. Heinze B, Fuss CT, Mulatero P, et al. Targeting CXCR4 (CXC Chemokine Receptor Type 4) for Molecular Imaging of Aldosterone-Producing Adenoma. Hypertension 2018;71:317-25.
75. Yang Y, Burrello J, Burrello A, et al. Classification of microadenomas in patients with primary aldosteronism by steroid profiling. The Journal of steroid biochemistry and molecular biology 2019 May;189:274-282.
76. Meyer LS, Wang X, Susnik E, et al. Immunohistopathology and Steroid Profiles Associated With Biochemical Outcomes After Adrenalectomy for Unilateral Primary Aldosteronism. Hypertension 2018;72:650-7.
77. Buffolo F, Monticone S, Williams TA, et al. Subtype Diagnosis of Primary Aldosteronism: Is Adrenal Vein Sampling Always Necessary? International journal of molecular sciences 2017 Apr 17;18(4).
78. Reincke M, Fischer E, Gerum S, et al. Observational study mortality in treated primary aldosteronism: the German Conn's registry. Hypertension 2012;60:618-24.

79. Ahmed AH, Gordon RD, Sukor N, Pimenta E, Stowasser M. Quality of life in patients with bilateral primary aldosteronism before and during treatment with spironolactone and/or amiloride, including a comparison with our previously published results in those with unilateral disease treated surgically. The Journal of clinical endocrinology and metabolism 2011;96:2904-11.
80. Apostolopoulou K, Kunzel HE, Gerum S, et al. Gender differences in anxiety and depressive symptoms in patients with primary hyperaldosteronism: a cross-sectional study. The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry 2014;15:26-35.
81. Kunzel HE, Apostolopoulou K, Pallauf A, et al. Quality of life in patients with primary aldosteronism: gender differences in untreated and long-term treated patients and associations with treatment and aldosterone. Journal of psychiatric research 2012;46:1650-4.
82. Kline GA, Pasieka JL, Harvey A, So B, Dias VC. Medical or surgical therapy for primary aldosteronism: post-treatment follow-up as a surrogate measure of comparative outcomes. Annals of surgical oncology 2013;20:2274-8.
83. van den Berg TN, Rongen GA, Frohlich GM, Deinum J, Hausenloy DJ, Riksen NP. The cardioprotective effects of mineralocorticoid receptor antagonists. Pharmacology & therapeutics 2014;142:72-87.
84. Sakakibara R, Sasaki W, Onda Y, et al. Discovery of Novel Pyrazole-Based Selective Aldosterone Synthase (CYP11B2) Inhibitors: A New Template to Coordinate the Heme-Iron Motif of CYP11B2. Journal of medicinal chemistry 2018;61:5594-608.
85. Doorenbos H, Elings HS, Van Buchem FS. Primary aldosteronism due to adrenocortical hyperplasia. Lancet (London, England) 1956;271:335-7.
86. Satoh F, Abe T, Tanemoto M, et al. Localization of aldosterone-producing adrenocortical adenomas: significance of adrenal venous sampling. Hypertension research : official journal of the Japanese Society of Hypertension 2007;30:1083-95.
87. Lo CY, Tam PC, Kung AW, Lam KS, Wong J. Primary aldosteronism. Results of surgical treatment. Annals of surgery 1996;224:125-30.
88. Fogari R, Preti P, Zoppi A, Rinaldi A, Fogari E, Mugellini A. Prevalence of primary aldosteronism among unselected hypertensive patients: a prospective study based on the use of an aldosterone/renin ratio above 25 as a screening test. Hypertension research : official journal of the Japanese Society of Hypertension 2007;30:111-7.
89. Enberg U, Volpe C, Hoog A, et al. Postoperative differentiation between unilateral adrenal adenoma and bilateral adrenal hyperplasia in primary aldosteronism by mRNA expression of the gene CYP11B2. European journal of endocrinology / European Federation of Endocrine Societies 2004;151:73-85.

90. Murashima M, Trerotola SO, Fraker DL, Han D, Townsend RR, Cohen DL. Adrenal venous sampling for primary aldosteronism and clinical outcomes after unilateral adrenalectomy: a single-center experience. Journal of clinical hypertension (Greenwich, Conn) 2009;11:316-23.
91. Weisbrod AB, Webb RC, Mathur A, et al. Adrenal histologic findings show no difference in clinical presentation and outcome in primary hyperaldosteronism. Annals of surgical oncology 2013;20:753-8.
92. Tresallet C, Salepcioglu H, Godiris-Petit G, Hoang C, Girerd X, Menegaux F. Clinical outcome after laparoscopic adrenalectomy for primary hyperaldosteronism: the role of pathology. Surgery 2010;148:129-34.
93. Nanba K, Tsuiki M, Sawai K, et al. Histopathological diagnosis of primary aldosteronism using CYP11B2 immunohistochemistry. The Journal of clinical endocrinology and metabolism 2013;98:1567-74.
94. Nanba K, Chen AX, Omata K, et al. Molecular Heterogeneity in Aldosterone-Producing Adenomas. The Journal of clinical endocrinology and metabolism 2016;101:999-1007.
95. Yamazaki Y, Nakamura Y, Omata K, et al. Histopathological Classification of Cross-Sectional Image-Negative Hyperaldosteronism. The Journal of clinical endocrinology and metabolism 2017;102:1182-92.
96. Scholl UI, Healy JM, Thiel A, et al. Novel somatic mutations in primary hyperaldosteronism are related to the clinical, radiological and pathological phenotype. Clinical endocrinology 2015;83:779-89.
97. Akerstrom T, Crona J, Delgado Verdugo A, et al. Comprehensive re-sequencing of adrenal aldosterone producing lesions reveal three somatic mutations near the KCNJ5 potassium channel selectivity filter. PloS one 2012;7:e41926.
98. Azizan EA, Murthy M, Stowasser M, et al. Somatic mutations affecting the selectivity filter of KCNJ5 are frequent in 2 large unselected collections of adrenal aldosteronomas. Hypertension 2012;59:587-91.
99. Azizan EA, Lam BY, Newhouse SJ, et al. Microarray, qPCR, and KCNJ5 sequencing of aldosterone-producing adenomas reveal differences in genotype and phenotype between zona glomerulosa- and zona fasciculata-like tumors. The Journal of clinical endocrinology and metabolism 2012;97:E819-29.
100. Azizan EA, Poulsen H, Tuluc P, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. Nature genetics 2013;45:1055-60.

101. Lenzini L, Rossitto G, Maiolino G, Letizia C, Funder JW, Rossi GP. A Meta-Analysis of Somatic KCNJ5 K(+) Channel Mutations In 1636 Patients With an Aldosterone-Producing Adenoma. The Journal of clinical endocrinology and metabolism 2015;100:E1089-95.
102. Omata K, Yamazaki Y, Nakamura Y, et al. Genetic and Histopathologic Intertumor Heterogeneity in Primary Aldosteronism. The Journal of clinical endocrinology and metabolism 2017;102:1792-6.
103. Fernandes-Rosa FL, Giscos-Douriez I, Amar L, et al. Different Somatic Mutations in Multinodular Adrenals With Aldosterone-Producing Adenoma. Hypertension 2015;66:1014-22.
104. Zennaro MC, Boulkroun S, Fernandes-Rosa F. Genetic Causes of Functional Adrenocortical Adenomas. Endocrine reviews 2017;38:516-37.
105. Nishimoto K, Seki T, Hayashi Y, et al. Human Adrenocortical Remodeling Leading to Aldosterone-Producing Cell Cluster Generation. International journal of endocrinology 2016;2016:7834356.
106. Nishimoto K, Nakagawa K, Li D, et al. Adrenocortical zonation in humans under normal and pathological conditions. The Journal of clinical endocrinology and metabolism 2010;95:2296-305.
107. Nanba K, Tsuiki M, Sawai K, et al. Histopathological Diagnosis of Primary Aldosteronism Using CYP11B2 Immunohistochemistry. The Journal of clinical endocrinology and metabolism 2013 Apr;98(4):1567-74.
108. Nishimoto K, Tomlins SA, Kuick R, et al. Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands. Proceedings of the National Academy of Sciences of the United States of America 2015;112:E4591-9.
109. Nishimoto K, Seki T, Kurihara I, et al. Case Report: Nodule Development From Subcapsular Aldosterone-Producing Cell Clusters Causes Hyperaldosteronism. The Journal of clinical endocrinology and metabolism 2016;101:6-9.
110. Gomez-Sanchez CE, Kuppusamy M, Reincke M, Williams TA. Disordered CYP11B2 Expression in Primary Aldosteronism. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2017;49:957-62.
111. Citton M, Viel G, Rossi GP, Mantero F, Nitti D, Iacobone M. Outcome of surgical treatment of primary aldosteronism. Langenbeck's archives of surgery 2015;400:325-31.


OH
O
O
HO
H
H
H
O

Summary

SUMMARY OF THIS THESIS

Aldosterone is produced in the adrenal cortex as one of the major end products of the renin-angiotensin-aldosterone system. This system is one of the major blood pressure regulating systems in the human body. In primary aldosteronism (PA) the physiological regulation of the renin-angiotensin-aldosterone system is overruled by excessive autonomous aldosterone secretion by one or both diseased adrenal glands. PA is an important cause of secondary hypertension, affecting 5–15% of the hypertensive population.[1] Early diagnosis and treatment are important because patients have a higher cardiovascular morbidity and mortality than blood-pressure-matched controls with primary hypertension.[2-11] In most cases, PA is caused by either a unilateral aldosterone-producing adenoma (APA) or by bilateral hyperplasia (BAH).[1] Proper distinction between the two is crucial, because the former is treated by adrenalectomy (ADX), and the latter by mineralocorticoid receptor antagonists (MRA).[1]

Chapter 1 gives a general introduction on PA. It summarizes the current knowledge on PA and highlights the most important developments in the field over the past years. We also introduce three debatable aspects of PA that are addressed in this thesis: the prevalence, the subtyping of PA by CT-scan or adrenal vein sampling (AVS) and the assumed dichotomy between APA and BAH in the histopathology of PA.

The first aspect, the prevalence of PA, is discussed in **chapter 2**. The actual prevalence of PA is a matter of continuing debate as prevalence rates reported in literature are highly variable. For health care planning and allocation of resources, realistic estimation of the prevalence of PA is necessary. In a systematic review we assessed the prevalence of PA in primary and secondary care and we evaluated the factors determining the wide variety of prevalences found in recently performed studies. Thirty-nine studies provided data on 42 510 patients. Prevalence estimates varied from 3.2% to 12.7% in primary care and from 1% to 29.8% in referral centres. Heterogeneity was too high to establish point estimates. Meta-regression analysis showed higher prevalences in studies: 1) published after 2000, 2) from Australia, 3) aimed at assessing prevalence of secondary hypertension, 4) that were retrospective, 5) that selected consecutive patients, and 6) not using a screening test. Higher prevalences found after 2000 can be explained by the growing awareness in clinicians on the importance to detect PA. High PA prevalence in Australian studies might reflect the retrospective study

methodology with inclusion of self-selected patients, although a true higher prevalence in the Australian population cannot be excluded. The high prevalence in those studies relying only on PA confirmation testing without prior screening might reflect limited reliability of the screening test in other studies (false negative) or a limited reliability of the confirmation test (false positive).[12-14] This study demonstrates that it is pointless to claim low or high prevalence of PA based on published reports. Because of the significant impact of a diagnosis of PA on health care resources and the necessary facilities, our findings urge for a prevalence study whose design takes into account the factors identified in the meta-regression analysis.

The second aspect addressed in this thesis in **chapter 3** is the use of CT-scan or AVS for subtyping of PA. As described above most cases of PA are caused by either a unilateral APA, which is best treated with ADX, or by BAH, which is best with MRA.[1] Because of these different treatment modalities distinction between the two is crucial. Whether CT or AVS represents the best test for diagnosis was controversial. Therefore, we compared the outcome of CT-based management with AVS-based management for patients with PA, using the design of a diagnostic, randomized, controlled trial. We randomly assigned 200 patients with PA to undergo either adrenal CT or AVS to determine the presence of APA (with subsequent ADX treatment) or BAH (with subsequent MRA treatment). In this outcome-based trial, no differences were found at 1 year follow up in treatment outcome, expressed as the intensity of antihypertensive medication required to control blood pressure. No statistically significant differences were observed in secondary endpoints either, including biochemical outcome, health-related quality of life, and adverse events. This finding challenges the current recommendation to perform AVS in all patients with PA.[1]

As discussed in chapter 3 and chapter 8, these findings may be interpreted in a number of ways. First, methodological issues in the Spartacus trial should be considered. However, as described in chapter 8 it is unlikely that these issues have compromised our study outcome. Instead, we should seriously consider the possibility that both CT and AVS are imperfect tests to identify patients who might benefit from ADX. CT may fail for obvious reasons such as restricted detection limit, resolution and specificity, and substantial interobserver variation. However, also a physiological size difference between the left and right adrenal glands (in favour of the left gland) might be of influence of CT accuracy.[15] Challenges in interpreting results from AVS include multiple vein drainage, selective cannulation of contributory veins not draining an APA, or asymmetrical cortisol secretion.[16,17] Besides these

factors, theoretically, specific somatic adrenal mutations may determine sampling outcome. Additionally, several other AVS procedure-related factors, such as use of cosyntropin,[18,19] sequential or simultaneous sampling of adrenal veins,[20] or varying criteria for selectivity and lateralisation[21,22] can affect AVS conclusions.[23,24]

When questioning the accuracy of AVS we have several options on how to procede: improve AVS, replace AVS with another diagnostic option or abandon ADX, rendering AVS redundant. In chapter 8 we discuss the current techniques under development that could make us relinquish ADX (such as aldosterone synthase inhibitors), or that could make us replace AVS (such as by functional imaging or steroid profiling). However, also the option to improve AVS is appealing as AVS is conceptually sound and insight into its strengths and weaknesses is increasing. In chapter 4 and chapter 5 we discuss two aspects that could improve the efficiency and accuracy of AVS.

Chapter 4 comprises a small study regarding AVS cost minimalisation by the use of single instead of duplicate blood samples per sampling location during the AVS procedure. Ninety-six AVS procedures with duplicate measurements performed in our university medical centre between 2005 and 2010 were evaluated retrospectively. We compared the conclusions regarding selectivity and lateralization based on the first sample taken (A) to the conclusions based on the average of duplicate samples (AB). The concordance in AVS conclusions between samples A and AB was 98–100%, depending on the criteria used for selectivity and lateralization. With permissive and strict criteria the number needed to be sampled in duplicate were infinite and 48, respectively. Based on these results we conclude that the incremental benefit of duplicate sampling compared to single sampling is low. Therefore conclusions can also be reliably drawn from a single blood sample.

Chapter 5 addresses the use of another metabolite, metanephrine instead of cortisol, to determine selectivity (i.e. correct catheter position) in AVS. The use of cortisol to determine selectivity might not be ideal due its relative low ratio between adrenal and peripheral blood, its fluctuating secretion and the fact that a recent study indicates increased cortisol secretion in PA patients.[25-28] Plasma metanephrine represents an alternative parameter. In our study we aimed to determine whether plasma metanephrine concentrations can be used to establish correct catheter positioning during AVS. We included 52 cosyntropin-stimulated and 34 nonstimulated sequential procedures. Among procedures assessed as selective using

cortisol, the adrenal to peripheral vein ratio of metanephrine was 6-fold higher than that of cortisol. Concordance in sampling success rates determined by cortisol and metanephrine was substantially higher in cosyntropin-stimulated than in nonstimulated samplings. For the latter procedures, sampling success rates determined by metanephrine were higher than those determined by cortisol. Based on this we can conclude that metanephrine provides a superior analyte compared with cortisol in assessing the selectivity of adrenal vein sampling without cosyntropin stimulation. Since then, studies have shown that also other hormones (e.g. 11-deoxycortisol, androstenedione, DHEA and 17-α-hydroxyprogesterone) can be successfully used to replace cortisol to determine selectivity in the AVS procedure. [29-31]

Chapter 6 discusses the third aspect of this thesis, the presumed dichotomy between APA and BAH. Classically, PA is considered to be caused by either an APA or BAH. The entire clinical work-up and treatment of PA is based upon this principle of dichotomy. However, the assumption that all adrenal glands excised because of a presumed APA, harbour only one nodule is questionable.[32-36] In chapter 6 we assessed the adrenals of 53 PA patients, removed because of the suspicion of unilateral APA, for multinodularity and phenotypic and genotypic characteristics. Glands contained a solitary adenoma in 43% and nodular hyperplasia in 53% of the cases. Most (multinodular) glands contained only one nodule positive for P450C18 expression, with all other nodules negative. Somatic mutations (*KCNJ5, ATP1A1, ATP2B3, CACNA1D*) were not limited to APAs but were also found in the multinodular adrenals. Mutations were always located in the P450C18-positive nodule. In one gland two nodules containing two different KCNJ5 mutations were present. Based on these findings we hypothesize that the mutations are causative for the aldosterone hypersecretion but not necessarily for the nodulation itself as a mutation is not present in all nodules. The hypothesis we propose is that some individuals for some reason develop multinodular adrenal cortices and that only if a mutation occurs aldosterone hypersecretion occurs. This suggests a two-hit mechanism with one hit causing adrenocortical cell proliferation and a second hit causing hormone hypersecretion.[37]

These findings challenge the commonly assumed dichotomy of unilateral versus bilateral disease in PA. This raises the possibility that many cases of presumed unilateral aldosterone hypersecretion may in fact represent bilateral asymmetric nodular hyperplasia as a result of somatic mutations.[38] PA may be a disease of the adrenal glands where a patient may be anywhere on the line between unilateral and bilateral hypersecretion.[38]

Chapter 7 is a case-report that is illustrative for several aspects discussed in this thesis. In this case-report we describe a 53-year-old male with PA in whom there were no adrenal anomalies on CT-scan and a bilateral suppression of adrenal aldosterone production on AVS. Upon more thorough examination he turned out to have a pedunculated APA drained by an extra vein. This case shows us some of the pitfalls of CT-scan and AVS as both techniques initially misclassified the patient. However, it also gives us some insight in the pathophysiology of PA as the steroid profile of solely adenomatous tissues, without adrenal venous blood mixture from nonadenomatous tissue, could be assessed.

Finally, a general discussion of the studies described in this thesis and prospects of future research is presented in **chapter 8**.

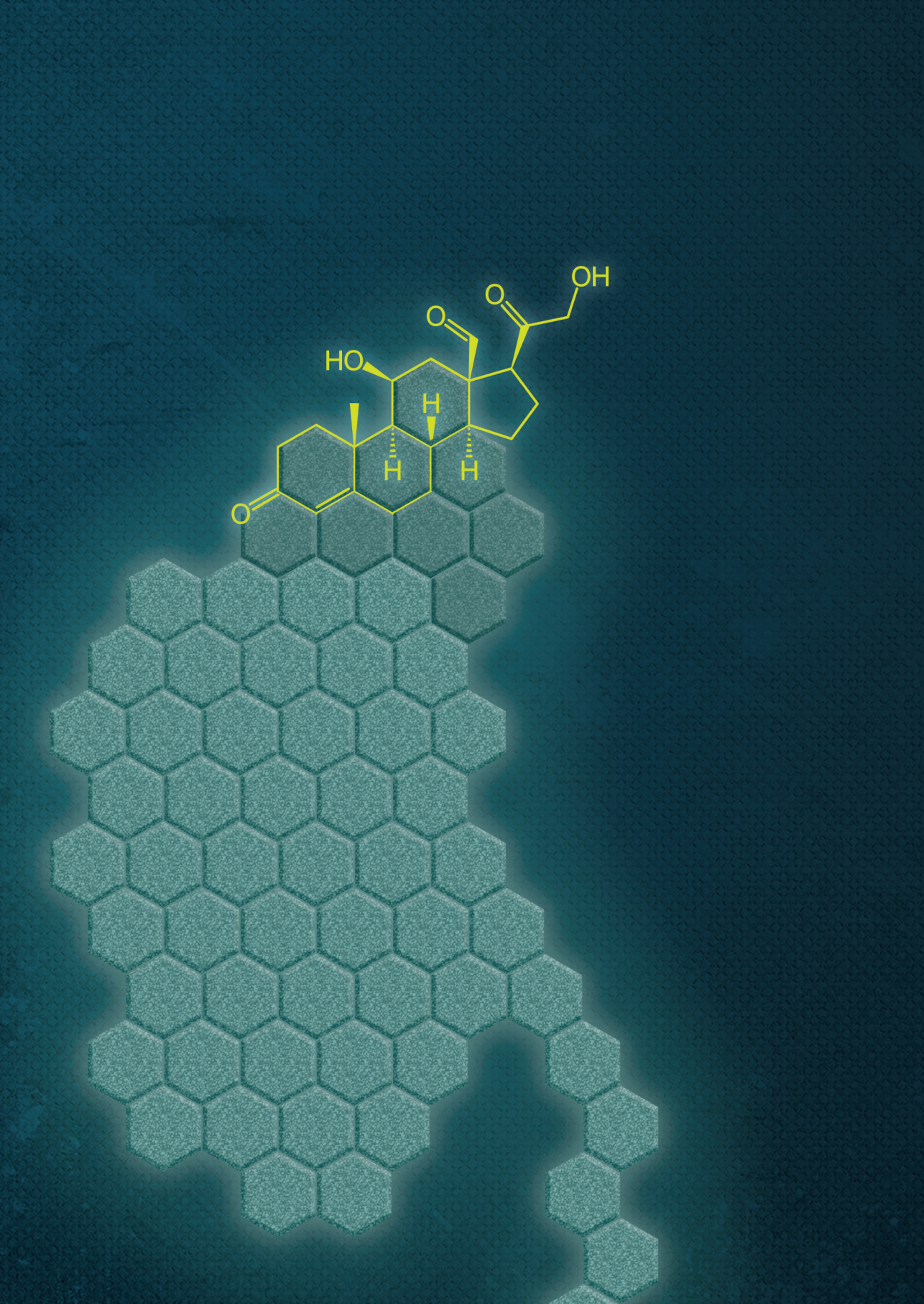
REFERENCES

1. Funder JW, Carey RM, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. The Journal of clinical endocrinology and metabolism 2016;101:1889-916.
2. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. Journal of the American College of Cardiology 2005;45:1243-8.
3. Born-Frontsberg E, Reincke M, Rump LC, et al. Cardiovascular and cerebrovascular comorbidities of hypokalemic and normokalemic primary aldosteronism: results of the German Conn's Registry. The Journal of clinical endocrinology and metabolism 2009;94:1125-30.
4. Catena C, Colussi G, Lapenna R, et al. Long-term cardiac effects of adrenalectomy or mineralocorticoid antagonists in patients with primary aldosteronism. Hypertension 2007;50:911-8.
5. Catena C, Colussi G, Nadalini E, et al. Cardiovascular outcomes in patients with primary aldosteronism after treatment. Archives of internal medicine 2008;168:80-5.
6. Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular complications associated with primary aldosteronism: a controlled cross-sectional study. Hypertension 2013;62:331-6.
7. Mulatero P, Monticone S, Bertello C, et al. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. The Journal of clinical endocrinology and metabolism 2013;98:4826-33.
8. Monticone S, D'Ascenzo F, Moretti C, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. The lancet Diabetes & endocrinology 2018;6:41-50.
9. Monticone S, Burrello J, Tizzani D, et al. Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice. Journal of the American College of Cardiology 2017;69:1811-20.
10. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Renal Outcomes in Medically and Surgically Treated Primary Aldosteronism. Hypertension 2018;72:658-66.
11. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Incidence of Atrial Fibrillation and Mineralocorticoid Receptor Activity in Patients With Medically and Surgically Treated Primary Aldosteronism. JAMA cardiology 2018;3:768-74.

12. Jansen PM, van den Born BJ, Frenkel WJ, et al. Test characteristics of the aldosterone-to-renin ratio as a screening test for primary aldosteronism. Journal of hypertension 2014;32:115-26.
13. Willenberg HS, Vonend O, Schott M, et al. Comparison of the saline infusion test and the fludrocortisone suppression test for the diagnosis of primary aldosteronism. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2012;44:527-32.
14. Mulatero P, Milan A, Fallo F, et al. Comparison of confirmatory tests for the diagnosis of primary aldosteronism. The Journal of clinical endocrinology and metabolism 2006;91:2618-23.
15. Schneller J, Reiser M, Beuschlein F, et al. Linear and volumetric evaluation of the adrenal gland--MDCT-based measurements of the adrenals. Academic radiology 2014;21:1465-74.
16. Satoh F, Morimoto R, Ono Y, et al. 8D.04: CLINICAL BENEFITS OF ADMINISTERING SUPER-SELECTIVE SEGMENTAL ADRENAL VENOUS SAMPLING AND PERFORMING ADRENAL SPARING SURGERY IN THE PATIENTS WITH PRIMARY ALDOSTERONISM. Journal of hypertension 2015;33 Suppl 1:e114.
17. Kline GA, Dias VC, So B, Harvey A, Pasieka JL. Despite limited specificity, computed tomography predicts lateralization and clinical outcome in primary aldosteronism. World journal of surgery 2014;38:2855-62.
18. Kline GA, So B, Dias VC, Harvey A, Pasieka JL. Catheterization during adrenal vein sampling for primary aldosteronism: failure to use (1-24) ACTH may increase apparent failure rate. Journal of clinical hypertension (Greenwich, Conn) 2013;15:480-4.
19. Seccia TM, Miotto D, De Toni R, et al. Adrenocorticotropic hormone stimulation during adrenal vein sampling for identifying surgically curable subtypes of primary aldosteronism: comparison of 3 different protocols. Hypertension 2009;53:761-6.
20. Carr CE, Cope C, Cohen DL, Fraker DL, Trerotola SO. Comparison of sequential versus simultaneous methods of adrenal venous sampling. Journal of vascular and interventional radiology : JVIR 2004;15:1245-50.
21. Lethielleux G, Amar L, Raynaud A, Plouin PF, Steichen O. Influence of diagnostic criteria on the interpretation of adrenal vein sampling. Hypertension 2015;65:849-54.
22. Mulatero P, Bertello C, Sukor N, et al. Impact of different diagnostic criteria during adrenal vein sampling on reproducibility of subtype diagnosis in patients with primary aldosteronism. Hypertension 2010;55:667-73.
23. Stewart PM, Allolio B. Adrenal vein sampling for Primary Aldosteronism: time for a reality check. Clinical endocrinology 2010;72:146-8.
24. Auchus RJ, Wians FH, Jr., Anderson ME, et al. What we still do not know about adrenal vein sampling for primary aldosteronism. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2010;42:411-5.

25. Spark RF, Kettyle WR, Eisenberg H. Cortisol dynamics in the adrenal venous effluent. The Journal of clinical endocrinology and metabolism 1974;39:305-10.
26. Seccia TM, Miotto D, Battistel M, et al. A stress reaction affects assessment of selectivity of adrenal venous sampling and of lateralization of aldosterone excess in primary aldosteronism. European journal of endocrinology / European Federation of Endocrine Societies 2012;166:869-75.
27. Tanemoto M, Suzuki T, Abe M, Abe T, Ito S. Physiologic variance of corticotropin affects diagnosis in adrenal vein sampling. European journal of endocrinology / European Federation of Endocrine Societies 2009;160:459-63.
28. Arlt W, Lang K, Sitch AJ, et al. Steroid metabolome analysis reveals prevalent glucocorticoid excess in primary aldosteronism. JCI insight 2017 Apr 20;2(8).
29. Nilubol N, Soldin SJ, Patel D, et al. 11-Deoxycortisol may be superior to cortisol in confirming a successful adrenal vein catheterization without cosyntropin: a pilot study. International journal of endocrine oncology 2017;4:75-83.
30. Ceolotto G, Antonelli G, Maiolino G, et al. Androstenedione and 17-alpha-Hydroxyprogesterone Are Better Indicators of Adrenal Vein Sampling Selectivity Than Cortisol. Hypertension 2017;70:342-6.
31. Li H, Zhang X, Shen S, et al. Adrenal androgen measurement for assessing the selectivity of adrenal venous sampling in primary aldosteronism. Steroids 2018;134:16-21.
32. Enberg U, Volpe C, Hoog A, et al. Postoperative differentiation between unilateral adrenal adenoma and bilateral adrenal hyperplasia in primary aldosteronism by mRNA expression of the gene CYP11B2. European journal of endocrinology / European Federation of Endocrine Societies 2004;151:73-85.
33. Murashima M, Trerotola SO, Fraker DL, Han D, Townsend RR, Cohen DL. Adrenal venous sampling for primary aldosteronism and clinical outcomes after unilateral adrenalectomy: a single-center experience. Journal of clinical hypertension (Greenwich, Conn) 2009;11:316-23.
34. Weisbrod AB, Webb RC, Mathur A, et al. Adrenal histologic findings show no difference in clinical presentation and outcome in primary hyperaldosteronism. Annals of surgical oncology 2013;20:753-8.
35. Tresallet C, Salepcioglu H, Godiris-Petit G, Hoang C, Girerd X, Menegaux F. Clinical outcome after laparoscopic adrenalectomy for primary hyperaldosteronism: the role of pathology. Surgery 2010;148:129-34.
36. Nanba K, Tsuiki M, Sawai K, et al. Histopathological diagnosis of primary aldosteronism using CYP11B2 immunohistochemistry. The Journal of clinical endocrinology and metabolism 2013;98:1567-74.

37. Zennaro MC, Boulkroun S, Fernandes-Rosa F. Genetic Causes of Functional Adrenocortical Adenomas. Endocrine reviews 2017;38:516-37.
38. Gomez-Sanchez CE, Kuppusamy M, Reincke M, Williams TA. Disordered CYP11B2 Expression in Primary Aldosteronism. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2017;49:957-62.


OH
O
O
HO
H
H
H
O

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Voor mijn vrienden, familie en iedereen die zich de voorgaande 60 000 woorden wil besparen, volgt nu een korte Nederlandse samenvatting van dit proefschrift. Eerst zal ik het ziektebeeld primair hyperaldosteronisme toelichten, om vervolgens in te gaan op de verschillende aspecten van mijn proefschrift.

PRIMAIR HYPERALDOSTERONISME

DE EERSTE PATIËNT

Het is 1955 wanneer een jonge vrouw de spreekkamer van Dr. Jerome Conn binnen komt lopen. Ze heeft klachten van spierzwakte, spiertrekkingen en kramp in de handen. Bij onderzoek blijkt zij een hoge bloeddruk en een laag kaliumgehalte in het bloed te hebben. Dr. Conn (Figuur 1) is een Amerikaanse arts, die zich tijdens de Tweede Wereldoorlog vooral heeft toegelegd op onderzoek naar zoutverlies via het zweet van soldaten.[1] Hij ontdekte dat een hormoon uit de bijnier betrokken is bij het vasthouden van zout en vocht in het lichaam. Later werd door Simpson en Tait vastgesteld dat het hier om het hormoon aldosteron gaat.[2] Op grond van zijn ervaringen dacht Dr. Conn dat dit aldosteron een rol kon spelen in het ziektebeeld van zijn patiënte. Deze vrouw bleek in haar bloed inderdaad veel te veel aldosteron te hebben. Ze werd uiteindelijk genezen door het verwijderen van een gezwel in de bijnier. Dit nieuwe ziektebeeld werd bestempeld als “het syndroom van Conn”, tegenwoordig bekend als “primair hyperaldosteronisme”.[3]

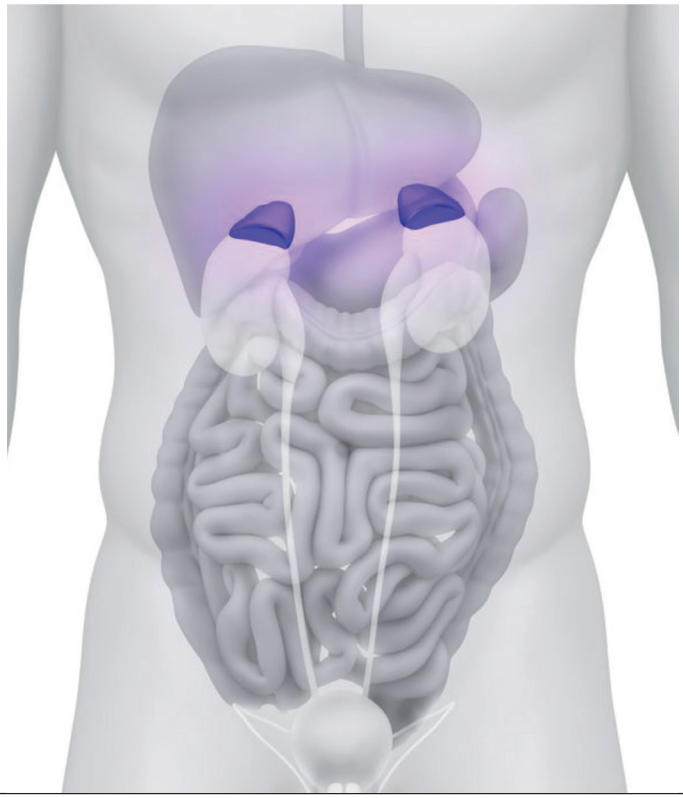
Figuur 1. Jerome Conn (1907-1994)[1]

ALDOSTERON

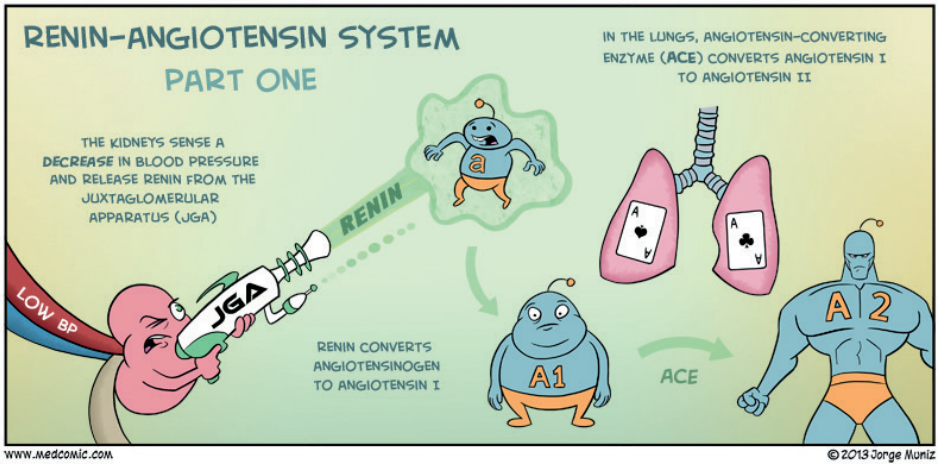
Bij de ziekte primair hyperaldosteronisme wordt er in het lichaam te veel aldosteron aangemaakt. Aldosteron is een hormoon dat

wordt gemaakt in de bijnieren, twee kleine orgaantjes die boven de nieren liggen (Figuur 2). Het is één van de eindproducten van het Renine-angiotensine-aldosteron-systeem (ofwel RAAS). Dit systeem is een zeer belangrijk regelmechanisme voor de bloeddruk (Figuur 3A en 3B). Door een verhoogd aldosterongehalte in het bloed houden de nieren meer water en zout vast en scheiden meer kalium uit. Hierdoor krijgen patiënten een hoge bloeddruk en vaak een laag kaliumgehalte in het bloed. Van dit lage kaliumgehalte kunnen zij klachten als spierkramp krijgen. Ook kunnen patiënten psychische klachten krijgen zoals depressie of angststoornissen.[4] Het is niet duidelijk wat de oorzaak hiervan is, maar meest waarschijnlijk heeft dit te maken met een direct effect van aldosteron op de hersenen.

Waarom een patiënt primair hyperaldosteronisme krijgt, weten we nog niet. De laatste jaren zijn wel een aantal DNA-mutaties gevonden in de bijnieren van patiënten met primair hyperaldosteronisme die de hoge aldosteronproductie lijken te veroorzaken. Hoe patiënten aan deze mutaties komen is nog onduidelijk.

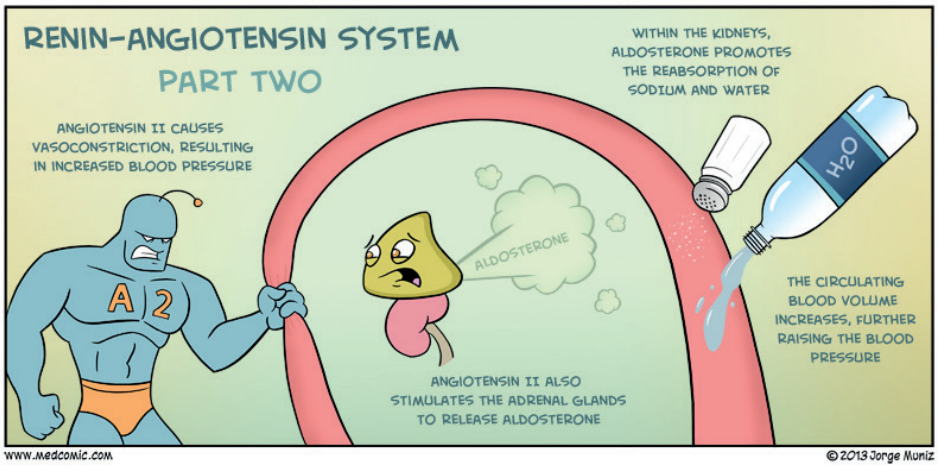


Figuur 2: De bijnieren (paars) gelegen boven de nieren. Bron: 123RF



Figuur 3A. Renine-Angiotensine-Aldosteron-Systeem (deel 1).

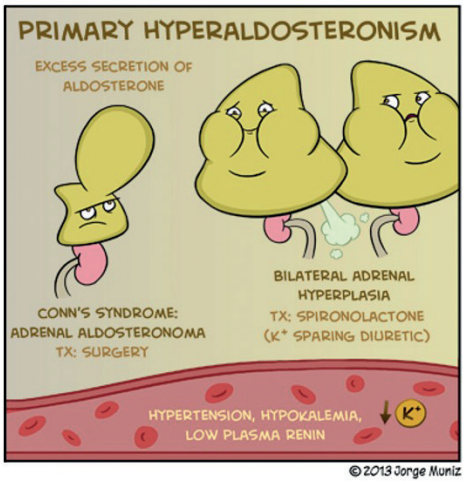
Als de bloeddruk (BP) te laag wordt, wordt dit door de nier opgemerkt via het zogenaamde juxtoglomerulaire apparaat (JGA). Hierop geeft de nier "renine" af. Renine zorgt ervoor dat angiotensinogeen (a) wordt omgezet in angiotensine 1 (A1). Angiotensine 1 is eigenlijk maar een tussenproduct en heeft zelf niet heel veel effect in het lichaam. Pas als het wordt omgezet in angiotensine 2 (A2) wordt het effectief. Deze omzetting gebeurt door het "angiotensine converting enzyme" (ook wel ACE) dat onder andere afkomstig is uit de longen. Gepubliceerd met de toestemming van www.medcomic.com



Figuur 3B. Renine-Angiotensine-Aldosteron-Systeem (deel 2).

Angiotensine 2 (A2) zorgt ervoor dat de bloedvaten samenknijpen en stimuleert de afgifte van aldosteron door de bijnieren. Aldosteron zorgt ervoor dat de nieren water en zout vasthouden. Door deze extra hoeveelheid vocht gaat de bloeddruk omhoog. Daarnaast gaat de nier meer kalium uitscheiden, waardoor het kaliumgehalte in het lichaam daalt. Gepubliceerd met de toestemming van www.medcomic.com

ZELDZAME ZIEKTE?



Figuur 4. Eenzijdige aldosteron producerend adenoom (links) versus bilaterale bijnierhyperplasie (rechts).

Gepubliceerd met de toestemming van www.medcomic.com

Toen primair hyperaldosteronisme in de jaren vijftig ontdekt werd, ging men ervan uit dat het een veel voorkomende oorzaak van hoge bloeddruk was.[5] Deze gedachte liet men op basis van andere onderzoeken echter snel varen en lange tijd werd primair hyperaldosteronisme beschouwd als een zeer zeldzaam fenomeen.[6,7] In de laatste 15 jaar komt men daar echter weer van terug. Volgens verschillende onderzoeken heeft ongeveer 5 tot 10 procent van de mensen met hoge bloeddruk, en zelfs 10-20 procent van de mensen met moeilijk te behandelen hoge bloeddruk, primair hyperaldosteronisme.[8] Ervan uitgaande dat in Nederland zo'n 30% van de mensen hoge bloeddruk heeft, zouden er in Nederland 250 000 patiënten met primair hyperaldosteronisme zijn. Helaas wordt de ziekte maar zelden vastgesteld. Dat kan ernstige gevolgen hebben voor de patiënt. Vaak blijft deze doorlopen met een verhoogde bloeddruk, met als gevolg schade aan hart en bloedvaten zoals hartfalen, ritmestoornissen, hartinfarcten en beroerten. Het blijkt dat deze schade bij primair hyperaldosteronisme patiënten ook nog eens groter is dan bij patiënten met "gewone hoge bloeddruk". [9] Dit kan voorkomen worden door primair hyperaldosteronisme tijdig vast te stellen en te behandelen. [9]

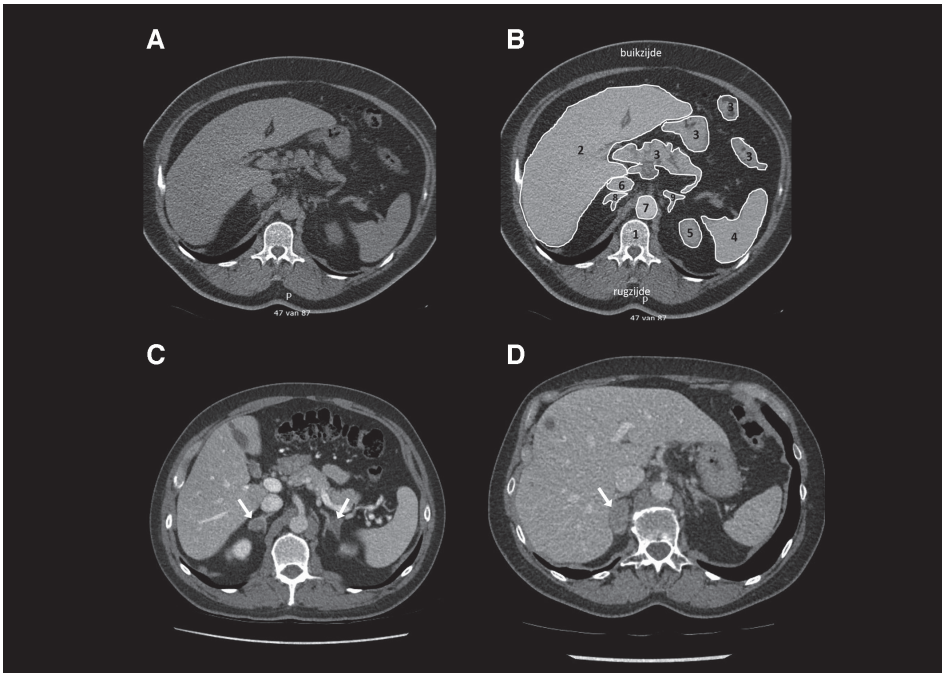
EENZIJDIG OF DUBBELZIJDIG BIJNIERPROBLEEM

Bij primair hyperaldosteronisme wordt de hoge aldosteronproductie veroorzaakt door een afwijking in één of in beide bijnieren (Figuur 4).[8] Bij ongeveer de helft van de patiënten is er sprake van een goedaardig gezwel in één van de bijnieren (aldosteron producerend adenoom) en is de andere bijnier gezond. Deze patiënten kunnen het beste van hun primair hyperaldosteronisme worden genezen door de zieke bijnier operatief te verwijderen.[8] In de andere helft van de patiënten zijn beide bijnieren ziek (bilaterale bijnierhyperplasie). In dit geval is primair hyperaldosteronisme helaas niet te genezen, omdat operatief verwijderen

van beide bijnieren geen optie is. Daarom worden patiënten bij wie beide bijnieren aangedaan zijn, behandeld met speciale medicijnen: mineralocorticoidreceptorantagonisten (spironolacton of eplerenon). Het nadeel is dat deze medicijnen levenslang gebruikt moeten worden en soms vervelende bijwerkingen hebben.

CT-SCAN OF BIJNIERVENESAMPLING

Om te kijken of één of beide bijnieren ziek zijn, zijn er verschillende technieken bedacht. De belangrijkste technieken zijn de CT-scan en de bijniervenesampling. Bij de CT-scan (Figuur 5) worden er door middel van röntgenstraling afbeeldingen van de bijnieren gemaakt. Hierop kan de radioloog beoordelen of slechts één bijnier vergroot is (en de patiënt dus operatief behandeld kan worden) of dat beide bijnieren vergroot zijn (en de patiënt dus medicamenteus behandeld moet worden). Soms zien de bijnieren er op de CT-scan helemaal normaal uit. Ook dan kiezen we ervoor om met medicijnen te behandelen.[8]

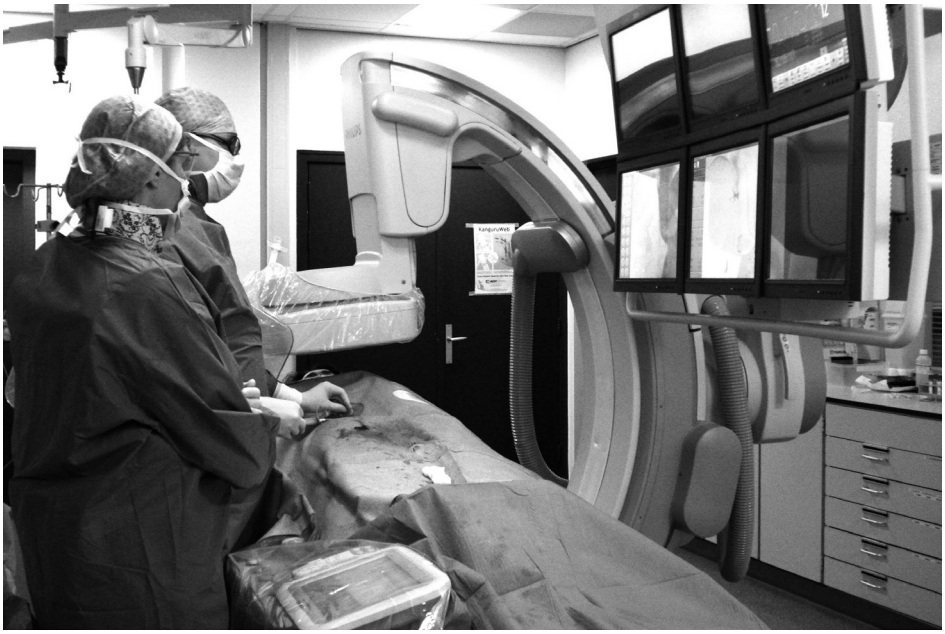


Figuur 5. CT-scan.

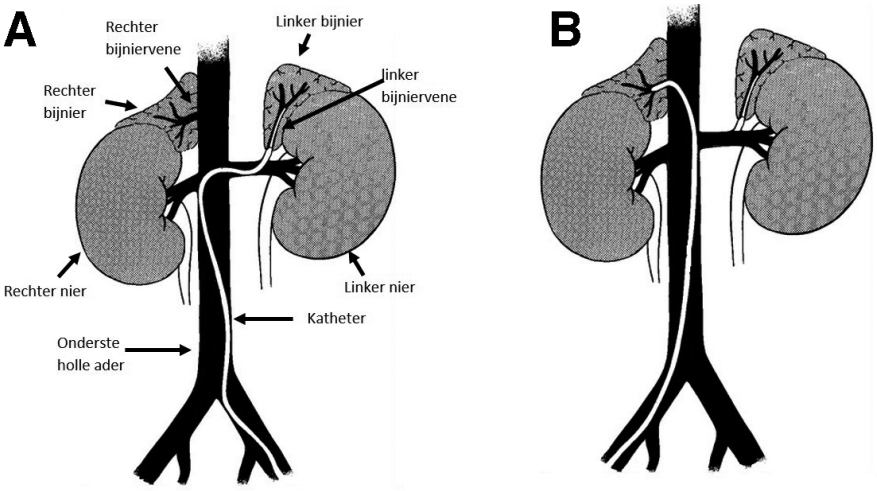
Bij de CT-scan worden er door middel van röntgenstralen dwarsdoorsneden van het lichaam afgebeeld. A/B. CT-scan van de buik met normale bijnieren. Buikorganen: 1. Ruggenwervels; 2: lever; 3: darmen; 4: milt; 5: bovenpool linker nier; 6: vena cava inferior (onderste holle lichaamsader); 7: aorta (grote lichaamsslagader); 8: rechter bijnier; 9: linker bijnier. C. CT-scan van een patiënt met bilaterale bijnierhyperplasie (zie pijlen). D: CT-scan van een patiënt met een eenzijdig adenoom (zie pijl).

Bij een bijniervenesampling (Figuur 6 en 7) prikt een interventieradioloog de ader (vene) in de lies aan en schuift vanuit hier een katheter op tot in de aders die het bloed afvoeren vanuit de bijnieren (bijniervenen).[10] De interventieradioloog lokaliseert deze bijniervenen met behulp van doorlichting met röntgenstraling en gebruik van contrastmiddel. Vervolgens neemt hij/zij bloedmonsters af uit deze bijniervenen, waarin het aldosteron wordt bepaald. Als de ene bijnier verhoudingsgewijs veel meer aldosteron afgeeft dan de andere bijnier, wordt deze bijnier als oorzaak van het primair hyperaldosteronisme beschouwd. Deze bijnier kan dan operatief verwijderd worden. Indien beide bijnieren ongeveer evenveel aldosteron afgeven, wordt de ziekte als dubbelzijdig beschouwd en wordt een patiënt met medicijnen behandeld.[8]

Het voordeel van de CT-scan is dat deze eenvoudig uitvoerbaar, veilig, goedkoop en weinig belastend voor de patiënt is. Ook is de CT-scan in ieder ziekenhuis in Nederland beschikbaar. Er zijn echter artsen en onderzoekers die van mening zijn dat de CT-scan niet goed genoeg is. Aan de ene kant kun je de diagnose van een adenoom missen als de aldosteron-producerende afwijkingen heel klein zijn. Aan de andere kant kun je de diagnose van een adenoom ten onrechte stellen in geval van grote afwijkingen die geen overmaat aan aldosteron produceren. Daarom zijn sommigen van mening dat eigenlijk alle patiënten met hyperaldosteronisme een bijniervenesampling zouden moeten ondergaan. Deze sampling is echter technisch moeilijk uitvoerbaar, duur en belastend voor de patiënt. Tevens moeten hiervoor in een ziekenhuis speciale faciliteiten aanwezig zijn en moeten interventieradiologen zijn opgeleid. Om deze reden kan dit onderzoek maar in een paar ziekenhuizen in Nederland worden uitgevoerd.



Figuur 6. Interventieradioloog voert bijniervenesampling uit.



Figuur 7. Bijniervenesampling. Ligging van de katheter (witte slangetje) in de linker bijnier (A) en rechter bijnier (B).

bron: The New England Journal of Medicine 1967; 277:1050-6, overgenomen met toestemming van de uitgever.

BELANGRIJKE ASPECTEN VAN DIT PROEFSCHRIFT

Ondanks dat we ondertussen steeds meer weten van primair hyperaldosteronisme zijn er nog aspecten die tot discussie leiden. Sommige van de algemeen geaccepteerde opvattingen over primair hyperaldosteronisme zijn niet gebaseerd op overtuigend wetenschappelijk bewijs. In dit proefschrift heb ik drie van deze aspecten belicht 1. Hoe vaak komt primair hyperaldosteronisme nu eigenlijk voor? 2. Kunnen we het beste de CT-scan of bijniervenesampling gebruiken voor de diagnostiek?, en 3. Klopt de aanname wel, dat er sprake is van een eenzijdig adenoom of een dubbelzijdige hyperplasie van de bijnier?

HOE VAAK KOMT PRIMAIR HYPERALDOSTERONISME VOOR?

Het eerste punt dat ik in dit proefschrift heb belicht is de prevalentie van primair hyperaldosteronisme. Met andere woorden: hoe vaak komt primair hyperaldosteronisme nu daadwerkelijk voor? Zoals hierboven beschreven schatten we dat primair hyperaldosteronisme voorkomt bij ongeveer 5 tot 10 procent van de mensen met hoge bloeddruk, en zelfs bij 10 tot 20 procent van de mensen met moeilijk te behandelen hoge bloeddruk.[8] Er worden echter heel uiteenlopende percentages gerapporteerd in de literatuur. In **hoofdstuk 2** hebben we de literatuur die hierover verschenen is op een rijtje gezet door middel van een *systematic review* (systematische zoekstrategie naar en beoordeling van eerder uitgevoerde onderzoeken en verschenen artikelen). Na het screenen van 1679 wetenschappelijke artikelen hebben we uiteindelijk 36 studies over de prevalentie van primair hyperaldosteronisme opgenomen in ons review. Deze studies beschreven een prevalentie van primair hyperaldosteronisme van 1% tot bijna 30% bij mensen met hoge bloeddruk. In de eerstelijns zorg (huisartsenpraktijk) was dit 3.2% tot 12.7% en in de tweedelijns zorg (ziekenhuizen) 1% tot 29.8%. Echter, uit onze analyse bleek dat de studies zodanig in opzet van elkaar verschilden, dat de gevonden prevalenties eigenlijk niet goed met elkaar te vergelijken zijn. We hebben daardoor geen gemiddelde schatting kunnen maken van de werkelijke prevalentie van primair hyperaldosteronisme.

Wel hebben we bekeken welke factoren nu bepalen of de onderzoeker een hoog of laag aantal patiënten met primair hyperaldosteronisme vond. Dit blijkt vooral samen te hangen met de setting waar het onderzoek werd uitgevoerd (huisartsenpraktijk versus ziekenhuis), hoe recent de studie was (recentere studies vonden hogere percentages dan oudere studies), het land waar de studie werd uitgevoerd en de manier waarop de studie was opgezet. Op grond van deze inzichten zouden we een nieuwe studieopzet kunnen maken. Binnen deze studie zouden we bij voorkeur *wereldwijd*, *opeenvolgende* patiënten met *nieuw*

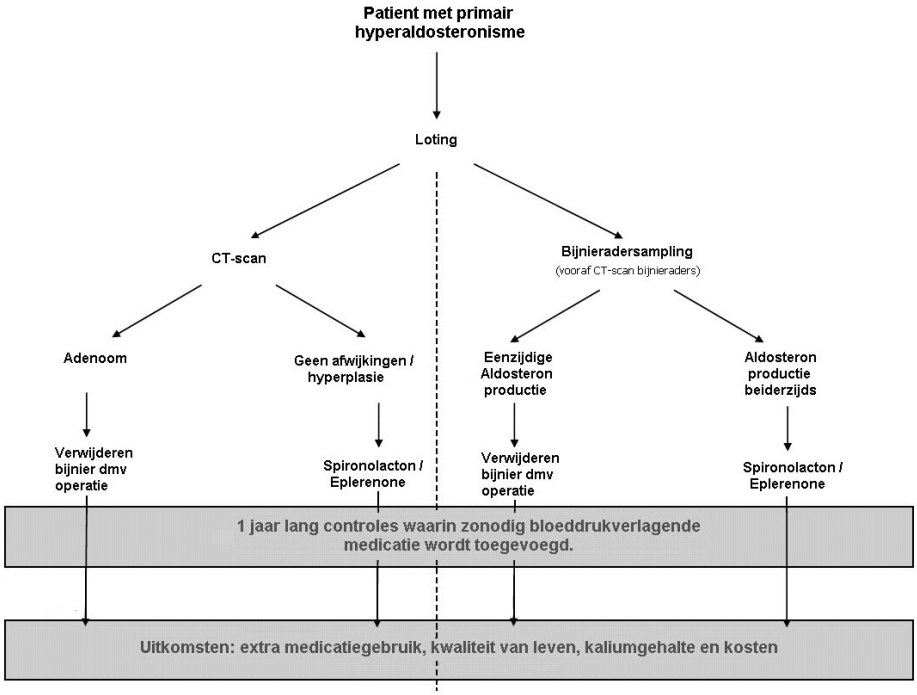
gediagnostiseerde hoge bloeddruk moeten screenen op primair hyperaldosteronisme. Een cruciaal probleem is echter dat er wereldwijd veel verschillende protocollen gebruikt worden voor de diagnostiek van primair hyperaldosteronisme. Voordat een grote studie naar de werkelijke prevalentie van primair hyperaldosteronisme opgezet kan worden, dient er eerst tussen centra/landen overeenstemming te komen wat het optimale protocol voor zo'n studie is. Het is niet te verwachten dat de animo hiervoor groot is en daarom is een dergelijke studie niet haalbaar.

CT-SCAN OF BIJNIERVENESAMPLING?

Het tweede aspect dat ik in dit proefschrift heb onderzocht is het gebruik van de CT-scan of de bijniervenesampling om te bepalen of iemand een eenzijdige of dubbelzijdige bijnierafwijking heeft. In de internationale richtlijnen stelt men dat de CT-scan niet goed genoeg is en dat alle patiënten een bijniervenesampling moeten krijgen.[8] Er was tot op heden echter geen gedegen onderzoek om dit te onderbouwen. Om te kijken wat nu het beste is, hebben wij de Spartacus studie opgezet (**hoofdstuk 3**). Spartacus staat voor: **S**ubtyping **P**rimary **A**ldosteronism: a **R**andomised **T**rial comparing **A**drenal vein sampling and **C**omp**U**ted tomography **S**can. De belangrijkste vraag van deze studie was: Is er een verschil in *behandeluitkomst* tussen patiënten bij wie de behandelkeuze is gebaseerd op CT-scan en patiënten bij wie die keuze is gebaseerd op bijniervenesampling? Met andere woorden: welke patiënten doen het na behandeling het beste?

In deze studie werd geloot of een patiënt met bewezen primair hyperaldosteronisme een CT-scan of een bijniervenesampling kreeg om de verdere behandeling te bepalen: een bijnieroperatie bij een eenzijdige bijnierafwijking/aldosteron overproductie of medicatie bij een dubbelzijdige bijnierafwijking/aldosteron overproductie (Figuur 8). Een jaar na de operatie of na de start van de medicijnen werd gekeken met welke groep patiënten het het beste ging: de CT-groep of de bijniervenesampling-groep. Dit werd vooral afgemeten aan de hoeveelheid medicijnen die een patiënt nog nodig had om een normale bloeddruk te krijgen. Daarnaast werd gekeken naar de kwaliteit van leven van de patiënten, de kalium-

en aldosteronwaarden bij de patiënten die geopereerd waren en de kosten van behandeling en diagnostiek. Hierbij werd ervan uitgegaan dat bij patiënten die goed op een behandeling reageerden, de juiste diagnose was gesteld met hetzij CT-scan, hetzij bijniervenesampling. De resultaten van de Spartacus studie waren opmerkelijk: we konden geen verschil aantonen tussen de patiënten die op basis van CT-scan waren behandeld en de patiënten die op basis van bijniervenesampling waren behandeld. Patiënten hadden een vergelijkbare bloeddruk en gebruikten een vergelijkbare hoeveelheid medicijnen. Ook was er geen duidelijk verschil tussen het aantal patiënten dat genezen was van het primair hyperaldosteronisme na een operatie, al was er wel een lichte trend in het voordeel van de bijniervenesampling.



Figuur 8. Opzet van de Spartacus studie.

Bij de patiëntengroep die de bijniervenesampling had geloot, was voorafgaand aan de sampling ook een CT-scan gemaakt. Deze CT-scan werd niet gebruikt om de behandeling te bepalen, maar was alleen bedoeld voor de interventieradioloog om vooraf de ligging van de bijniervenen te kunnen beoordelen. Achteraf hebben we op deze scans gekeken of er

een eenzijdige of tweezijdige afwijking te zien was. In de helft van de gevallen kwam de conclusie van de CT-scan niet overeen met de uitkomst van de bijniervenesampling. Dit is opvallend, zeker omdat we geen verschil in behandeluitkomst tussen de groepen hebben gevonden. Kennelijk heeft bij sommige patiënten de CT-scan, en bij andere patiënten de bijniervenesampling het bij het rechte eind. Met andere woorden, beide testen hebben hun eigen beperkingen als we willen bepalen wie het beste geopereerd kan worden en wie het beste met medicijnen behandeld kan worden.

De grote vraag is, wat is er dan mis met de CT-scan en met de bijniervenesampling? Ten eerste kan de CT-scan hele kleine afwijkingen in een bijnier missen. Ook zien we vaak dat er verschillen zijn in de beoordeling van een CT-scan tussen verschillende radiologen. Daarnaast is bij de CT-scan het probleem dat je nooit zeker weet of een bijnier die op de CT-scan vergroot is ook daadwerkelijk te veel aldosteron afgeeft. Op oudere leeftijd hebben mensen kans om goedaardige gezwellen in de bijnieren te ontwikkelen zonder dat deze gezwellen aldosteron produceren. Ten slotte zijn er aanwijzingen dat ook bij gezonde mensen de bijnieren niet precies even groot zijn. De linker bijnier lijkt bij de meeste mensen iets groter te zijn dan de rechter bijnier. Het zou kunnen zijn dat we ons op basis hiervan soms vergissen als we op basis van de CT-scan beslissen welke bijnier het grootste is.[11] Bij de patiënten die in onze studie behandeld werden op basis van de CT-scan werd ook vaker de linker bijnier verwijderd (40% van de patiënten) dan de rechter bijnier (12% van de patiënten).

Ook bij de bijniervenesampling zijn een aantal redenen te bedenken waarom deze techniek er soms naast zit. Zo kan het zijn dat de manier waarop we de sampling uitvoeren niet goed is of dat de gebruikte afkapwaarden om te bepalen of er een eenzijdig of dubbelzijdige overproductie van aldosteron is niet goed zijn. In hoofdstuk 4 en hoofdstuk 5 hebben we beschreven hoe we de bijniervenesampling zouden kunnen verbeteren of versimpelen. In **hoofdstuk 4** wordt een studie beschreven waarin werd onderzocht of het zin heeft om tijdens de bijniervenesampling dubbele in plaats van enkele bloedmonsters af te nemen. We hebben in 96 bijniervenesamplings met terugwerkende kracht gekeken of de conclusie van de sampling veranderde als je deze baseerde op het aldosterongehalte in één bloedbuisje per samplinglocatie of op het gemiddelde van twee bloedbuisjes die net na elkaar zijn afgenomen. Dit bleek géén verschil te maken. De conclusie van een bijniervenesampling kan dus veilig op basis van enkelvoudige bloedmonsters gesteld kan worden. Dit is belangrijk

omdat de katheter bij de sampling makkelijk uit de bijniervene schiet waardoor er soms maar één betrouwbaar bloedmonster beschikbaar is.
Een manier om de bijniervenesampling eventueel te verbeteren is door andere hormonen te meten tijdens de bijniervenesampling. Normaal gesproken gebruiken we cortisolmetingen om te bepalen of de katheter daadwerkelijk in de bijnierader zit (en niet per ongeluk in een ander bloedvat dat niet uit de bijnier komt) op het moment dat we bloed afnemen. Het gebruik van cortisol heeft echter een aantal nadelen. Zo is bijvoorbeeld de cortisolconcentratie in het bijnierbloed maar minimaal verhoogd ten opzichte van het bloed in de rest van het lichaam. In **hoofdstuk 5** kijken we of *metanefrine* een beter alternatief is. Om te kijken of we beter metanefrinewaarden kunnen gebruiken dan cortisolwaarden, hebben we in 86 bijniervenesamplings zowel cortisol als metanefrine gemeten. Hieruit blijkt dat metanefrine voor dit doel beter geschikt is dan cortisol. Sindsdien zijn er ook enkele studies verschenen die andere stoffen die in de bijnieren worden gemaakt (zoals 11-deoxycortisol, androstenedione, DHEA and 17-α-hydroxyprogesterone) hebben getest voor dit doel. Ook deze lijken een goed alternatief voor het gebruik van cortisol.
In **Hoofdstuk 7** beschrijven we de ziektegeschiedenis van een patiënt bij wie zowel de CT-scan als de bijniervenesampling ernaast zat, niet vanwege technisch falen, maar vanwege een opmerkelijke bijnierafwijking bij de patiënt. Bij de patiënt die we beschrijven was er namelijk geen sprake van een adenoom ín de bijnier, maar van een adenoom buiten de bijnier met een ander afvoerend bloedvat. Dit was maar met een heel klein stukje weefsel aan de bijnier verbonden. Deze casus laat zien dat ook anatomische variaties bij de patiënt ons in het diagnostisch proces voor de gek kunnen houden.

Op basis van al deze onderzoeken is de vraag hoe we in de toekomst nu verder moeten met de bijniervenesampling. Aangezien de bijniervenesampling veel duurder en omslachtiger is dan de CT-scan moeten we goed overwegen of we dit onderzoek bij alle patiënten moeten uitvoeren zoals in de richtlijn wordt aangeraden. Wat betreft de toekomst van de bijniervenesampling hebben we mijns inziens drie opties: de sampling verbeteren, de sampling vervangen door andere onderzoeken of een alternatieve behandeling vinden voor de bijnieroperatie zodat de sampling overbodig wordt. Voorbeelden voor het verbeteren van de sampling worden hierboven reeds gegeven. Ook wordt er momenteel veel onderzoek gedaan naar alternatieve technieken om het onderscheid tussen een eenzijdige en dubbelzijdige bijnierafwijking kunnen maken, zoals een speciale PET-scan of uitgebreid

bloedonderzoek (steroïdprofiel) van monsters die door normale bloedafname in de arm verkregen kunnen worden.[12,13] Als vervanging van de bijnieroperatie wordt momenteel veel onderzoek gedaan naar medicijnen die de aldosteronproductie remmen.[14] Helaas is het optimale middel nog niet gevonden. Tot het zo ver is, zullen we in de kliniek verder moeten met de diagnostiek en behandeling van patiënten met primair hyperaldosteronisme. Moeten we alle patiënten nog steeds bijniervenesamplings laten ondergaan? Voordat we daadwerkelijk stellen dat de bijniervenesampling geen meerwaarde heeft ten opzichte van de CT-scan, zullen de uitkomsten van de Spartacus studie eerst door nieuwe studies bevestigd moeten worden. Ik denk dus dat we samplings uit moeten blijven voeren onder de voorwaarde dat deze worden uitgevoerd in onderzoeksverband. Ook nieuwe alternatieve technieken zullen eerst in prospectieve, goed opgezette, diagnostische studies onderzocht moeten worden. De Spartacus studie heeft laten zien dat dergelijk onderzoek goed haalbaar is.

EENZIJDIG ADENOOM OF DUBBELZIJDIGE HYPERPLASIE VAN DE BIJNIER?

Het derde aspect dat in dit proefschrift aan de orde is gekomen, is de veronderstelde tweedeling tussen een eenzijdig adenoom en dubbelzijdige hyperplasie van de bijnier. Bij de diagnostiek en behandeling van primair hyperaldosteronisme gaan we uit van deze tweedeling: of er is sprake van één goedaardig gezwel in één van de bijnieren (aldosteron producerend adenoom), of van algehele zwelling of meerdere gezwellen in beide bijnieren (bilaterale bijnierhyperplasie).[8] Maar is deze scheiding wel zo zwart wit?

In **hoofdstuk 6** hebben we een onderzoek beschreven waarin we bijnieren van 53 patiënten hebben onderzocht die verwijderd zijn vanwege de verdenking op een eenzijdig bijniergezwel (adenoom) dat aldosteron produceerde. Volgens de huidige opvattingen zou een dergelijk bijnier dan ook maar één aldosteron producerende gezwel (nodus) bevatten. Bij beoordeling onder de microscoop viel echter op dat in meer dan de helft van de gevallen er sprake is van meerdere gezwellen (nodi) binnen dezelfde bijnier (nodulaire hyperplasie). Met speciale kleurstoffen hebben we aangetoond welke van deze nodi aldosteron kan produceren. Hierbij is het opvallend dat in de meeste gevallen in iedere bijnier maar één van de nodi aldosteron leek te kunnen produceren. Er waren echter ook een paar bijnieren waarin twee van de nodi aldosteron konden produceren. Vervolgens hebben we gekeken naar de aanwezigheid van mutaties in het DNA van de nodi in alle bijnieren. Hierbij hebben we gezocht naar DNA-mutaties waarvan bekend is dat ze primair

hyperaldosteronisme veroorzaken (KCNJ5, ATP1A1, ATP2B3, CACNA1D mutaties). Deze mutaties werden gevonden in zowel bijnieren met maar één nodus, als in bijnieren met nodulaire hyperplasie. Binnen al deze bijnieren werden alleen mutaties gevonden in nodi die ook aldosteron konden produceren. In nodi die geen aldosteron konden produceren vonden we geen mutaties. Op basis hiervan hebben we de mogelijkheid geopperd dat de mutaties overproductie van aldosteron veroorzaken, maar niet de vorming van de nodi in de bijnier zelf. We denken dat de patiënt om nog onbekende redenen één of meerdere nodi in de bijnier ontwikkelt en dat alleen als er een mutatie optreedt in een dergelijke nodus ook daadwerkelijk primair hyperaldosteronisme ontstaat.

Deze bevindingen trekken de klassieke tweedeling "eenzijdig adenoom" versus "dubbelzijde hyperplasie" in twijfel. Zou het niet zo kunnen zijn dat er meer sprake is van een soort van continu spectrum tussen deze twee uitersten waarbij een patiënt altijd ergens op de lijn tussen eenzijdige en dubbelzijdige aldosteron overproductie zit? Is het misschien zo dat er bij alle patiënten sprake is van ziekte van beide bijnieren, maar dat één bijnier ernstiger is aangedaan dan de andere? Echter als deze laatste hypothese klopt, dan zou dit betekenen dat het verwijderen van één bijnier niet genezend kan zijn. In de praktijk zien we echter bijna nooit dat de ziekte na verloop van tijd terugkomt. Het zou kunnen zijn dat dit is omdat we patiënten niet lang genoeg vervolgen. De meeste studies naar de uitkomst van een operatie stoppen immers binnen 5 jaar. Ook zou het kunnen zijn dat de ziekte zich in de overgebleven bijnier zo langzaam verder ontwikkelt dat dit nooit tot klachten leidt in de resterende levensjaren van de patiënt. Uiteraard kan het ook zo zijn dat bovenstaande hypothese niet klopt en dat er wel degelijk eenzijdig primair hyperaldosteronisme bestaat. In dat geval kunnen we ons afvragen of eenzijdige en tweezijdige ziekte wel op door hetzelfde mechanisme veroorzaakt wordt.

BLIJVEN PUZZELEN

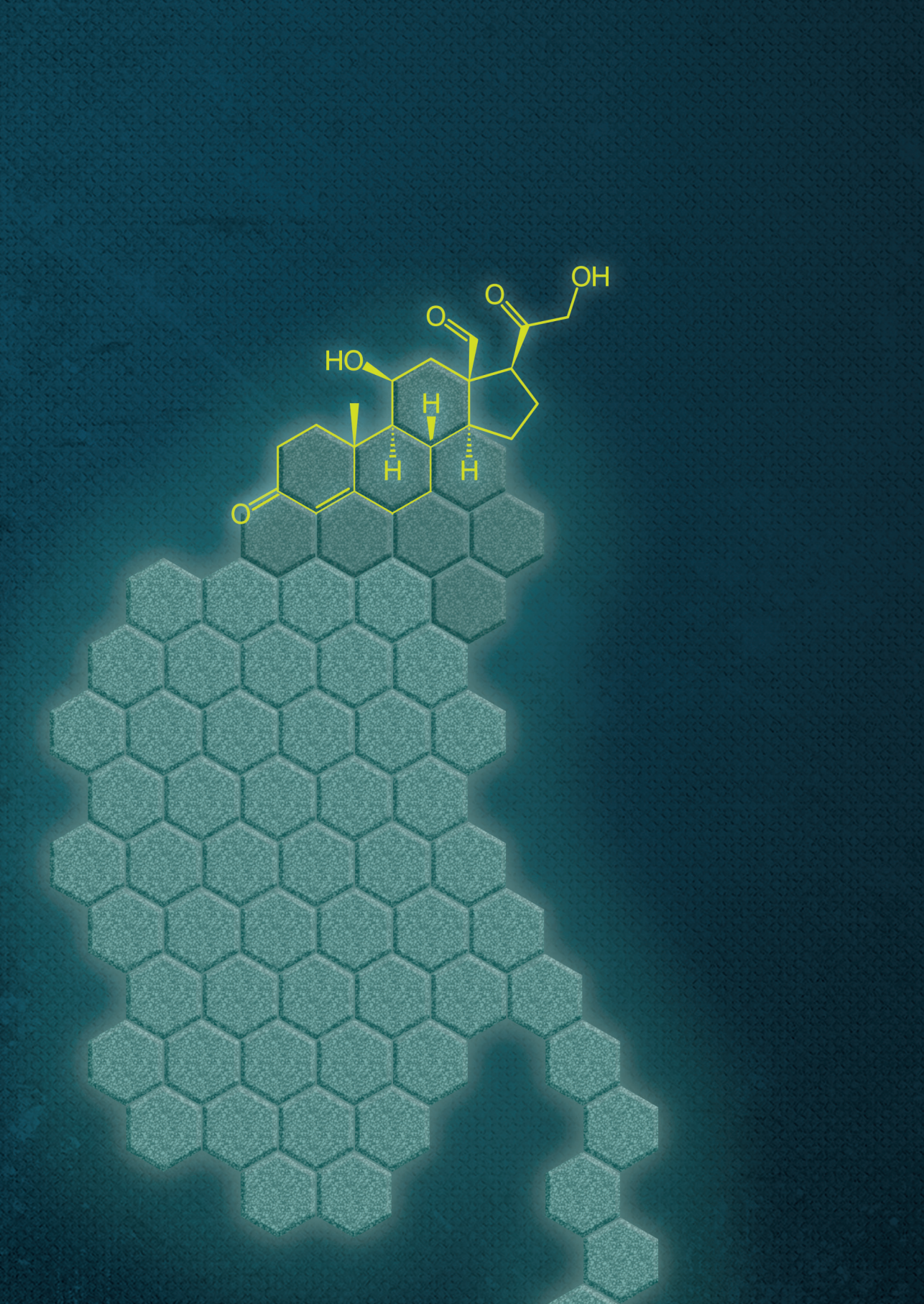
Met dit proefschrift wilde ik de bovenstaande drie vragen graag beantwoorden: Hoe vaak komt primair hyperaldosteronisme voor? Kun je het onderscheid tussen een eenzijdig adenoom of tweezijdige hyperplasie het best met een CT-scan of een bijniervenesampling maken? Bestaat die veronderstelde tweedeling tussen dat eenzijdige adenoom en die tweezijdige hyperplasie eigenlijk wel? Hoewel het nog niet mogelijk is om op deze vragen een klinkklaar antwoord te formuleren, zijn we wel weer een stapje verder in het beantwoorden hiervan.

Wat betreft de prevalentie van primair hyperaldosteronisme kunnen we stellen dat gebaseerd op de huidige studies het moeilijk vast te stellen is hoe vaak primair hyperaldosteronisme nu werkelijk voorkomt. De verschillen in de gerapporteerde prevalentiecijfers tussen de uitgevoerde studies lijken vooral te berusten op de verschillen in opzet van deze studies en de verschillende diagnostische strategieën die wereldwijd worden toegepast. We moeten die cijfers dus niet allemaal op één hoop gooien, maar proberen te begrijpen wat de verschillen tussen studies zou kunnen verklaren. Wat betreft het vraagstuk over de CT-scan en de bijniervenesampling blijkt dat patiënten met primair hyperaldosteronisme die op basis van CT-scan worden behandeld dezelfde behandeluitkomst hebben als patiënten die op basis van bijniervenesampling behandeld worden. De bijniervenesampling zoals deze momenteel wordt uitgevoerd lijkt dus niet in alle patiënten beter dan de CT-scan. Ten slotte: de klassieke tweedeling tussen “eenzijdig adenoom” en “dubbelzijde hyperplasie” is mogelijk een te simpele voorstelling van zaken. We zullen dus op een nieuwe manier moeten kijken naar de verschillende vormen van hyperaldosteronisme, wat mogelijk ook gevolgen zal hebben voor de diagnostiek en behandeling van deze aandoening.

Zo werkt wetenschap: nieuwe bevindingen beantwoorden sommige vragen, maar roepen ook weer nieuwe vragen op. Dat zet ons er toe aan om steeds dieper te graven en te blijven zoeken. Op het gebied van primair hyperaldosteronisme valt in ieder geval nog een heleboel te ontdekken.

REFERENTIES

1. Herder de Wd. Jerome W. Conn (1907-1981). Endocrinologie 2010:23-5.
2. Simpson SA, Tait JF, Bush IE. Secretion of a salt-retaining hormone by the mammalian adrenal cortex. Lancet (London, England) 1952;2:226-8.
3. Conn JW. Presidential address. I. Painting background. II. Primary aldosteronism, a new clinical syndrome. The Journal of laboratory and clinical medicine 1955;45:3-17.
4. Apostolopoulou K, Kunzel HE, Gerum S, et al. Gender differences in anxiety and depressive symptoms in patients with primary hyperaldosteronism: a cross-sectional study. The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry 2014;15:26-35.
5. Conn JW. The evolution of primary aldosteronism: 1954-1967. Harvey lectures 1966;62:257-91.
6. Berglund G, Andersson O, Wilhelmsen L. Prevalence of primary and secondary hypertension: studies in a random population sample. British medical journal 1976;2:554-6.
7. Swales JD. Primary aldosteronism: how hard should we look? British medical journal (Clinical research ed) 1983;287:702-3.
8. Funder JW, Carey RM, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. The Journal of clinical endocrinology and metabolism 2016;101:1889-916.
9. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. Journal of the American College of Cardiology 2005;45:1243-8.
10. Daunt N. Adrenal vein sampling: how to make it quick, easy, and successful. Radiographics : a review publication of the Radiological Society of North America, Inc 2005;25 Suppl 1:S143-58.
11. Degenhart C, Schneller J, Osswald A, et al. Volumetric and densitometric evaluation of the adrenal glands in patients with primary aldosteronism. Clinical endocrinology 2017;86:325-31.
12. Naruse M, Umakoshi H, Tsuiki M, et al. The Latest Developments of Functional Molecular Imaging in the Diagnosis of Primary Aldosteronism. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2017;49:929-35.
13. Buffolo F, Monticone S, Williams TA, et al. Subtype Diagnosis of Primary Aldosteronism: Is Adrenal Vein Sampling Always Necessary? International journal of molecular sciences 2017 Apr 17;18(4).
14. Sakakibara R, Sasaki W, Onda Y, et al. Discovery of Novel Pyrazole-Based Selective Aldosterone Synthase (CYP11B2) Inhibitors: A New Template to Coordinate the Heme-Iron Motif of CYP11B2. Journal of medicinal chemistry 2018;61:5594-608.


OH
O
O
HO
H
H
H
O

DANKWOORD

Mijn proefschrift had ik nooit kunnen volbrengen zonder de steun van mijn collega's, vrienden en familie. Graag wil ik een aantal van hen persoonlijk bedanken.

Beste Jaap Deinum. Wat hebben we samen een prachtig project opgezet! Graag wil ik je bedanken voor al het vertrouwen dat je in mij hebt gehad. Ik heb er bewondering voor hoe je het project hebt gestuurd: rustig en weloverwogen, maar tevens enthousiast en bevlogen. De begeleiding was continu perfect aangepast op mijn groei als arts-onderzoeker. Altijd heb ik alle vrijheid gevoeld tot eigen inbreng en initiatief. Ook heb ik veel geleerd van je maatschappijkritische opstelling en je prachtige uiteenzettingen over de misstanden in deze wereld. Dank voor al dit intellectueel erfgoed!

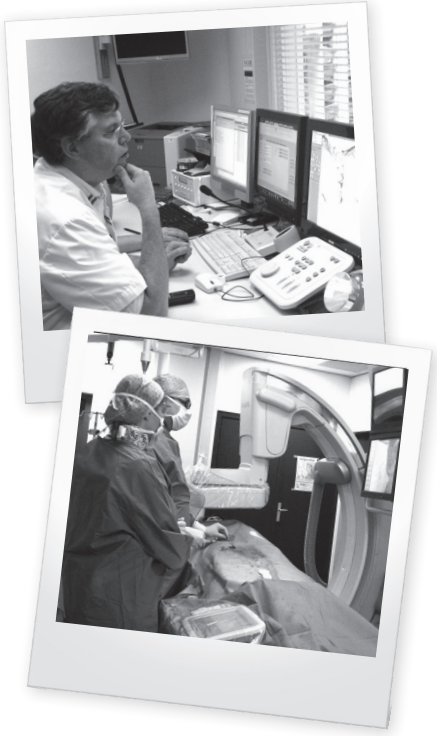
Beste Jacques Lenders. Passie voor het vak, een kritische blik en geen blad voor de mond, dat was precies wat we nodig hadden voor het slagen van de studies in dit manuscript. Je vurige betogen tijdens onze bespreking zetten mij steeds weer op scherp. Bedankt voor al je goede begeleiding en inbreng als promotor. Het volbrengen van mijn proefschrift voor je emeritaat is helaas (bij lange na) niet gelukt, maar ik ben blij dat je ook nadien erg betrokken bent gebleven en misschien zelfs nog meer tijd in de begeleiding hebt kunnen steken.

Beste Leo Schultze Kool en Gert Jan van der Wilt. Samen met Jacques vormden jullie een prachtig team van promotoren vanuit de verschillende medische disciplines. Samen wisten jullie alle onderzoeken in dit proefschrift vanuit verschillende invalshoeken te belichten. Gert Jan, graag wil ik je bedanken voor alle positieve en kritische feedback op onze onderzoeksprojecten en manuscripten waardoor we deze steeds weer naar een hoger niveau wisten te tillen. Leo, bedankt voor de begeleiding tijdens dit promotietraject. Met name voor je enthousiaste inzet om de bijniervenesampling in het RadboudUMC maar ook in Warschau tot een succes te maken. Beste Ad Hermus, na mijn onderzoeksstage

bij de endocrinologie heb je de goede begeleiding tijdens mijn promotietraject gewoon voortgezet. Dank voor je bijdrage aan de voortgang van de studies en van mijn proefschrift.

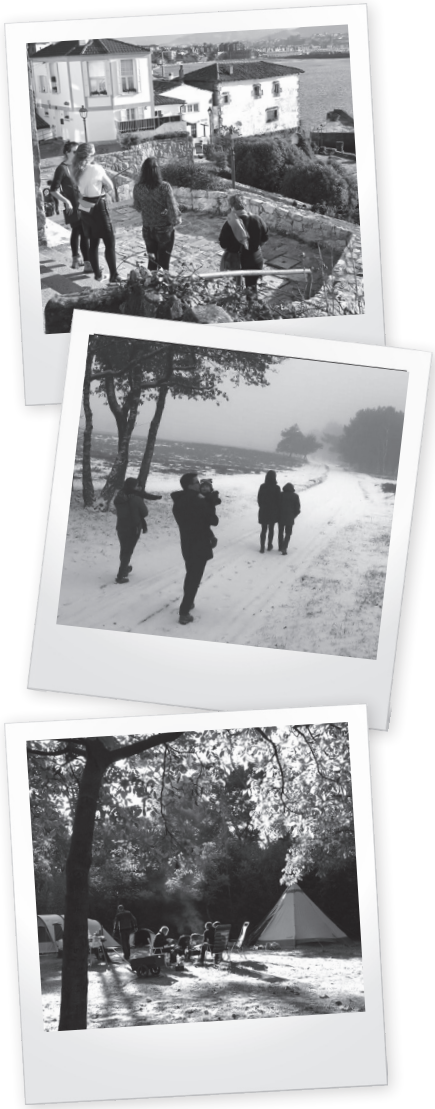
Geachte Spartanen. Graag wil ik alle patiënten die hebben deelgenomen aan de Spartacus studie enorm bedanken. Zonder jullie inzet hadden we dit project nooit kunnen volbrengen. Het was me een eer om voor velen van jullie de polidokter te zijn. Ook de artsen en researchverpleegkundigen uit de deelnemende centra wil ik van harte bedanken: Wilko Spiering, Corien Flint, Corina Joosten, Maarten Rookmaaker, Michiel Kerstens, Ton van den Meiracker, Evelien Jäger, Bert-Jan van den Born, Marianne Cammenga, Floris Vanmolkot, Jos Kooter, Arend Jan Woittiez, Sjoerd van Thiel, Mariette Kappers, Sanne van Wissen, Sytze van Dam, Marije ten Wolde, Natalie Smit en Peter Oomen. Dankzij jullie is het ons gelukt om het ambitieuze aantal van 200 geïncludeerde patiënten te behalen. Daarnaast mijn dank voor het warme onthaal dat ik steeds kreeg als ik langs kwam om te monitoren. Ik heb jullie ziekenhuizen steeds met veel plezier bezocht. A special thanks to our colleagues from the Institute of Cardiology in Warsaw: Aleksander Prejbisz, Sylwia Kołodziejczyk-Kruk, Jacek Kądziela and Andrzej Januszewicz. Amazing how you were able to establish well running sampling facilities within a few months which enabled us to include many Polish patients into the Spartacus trial.

Binnen het Radboud UMC wil ik alle leden van het Radboud Adrenal Centre van harte bedanken. Ook gaat mijn dank uit naar de afdeling endocrinologie en met name Henri Timmers die de bijnierbesprekingen op maandag altijd leidde; de afdeling radiologie en dan met name Mark Arntz, Sjoerd Jenniskens, de radiologielaboranten en Borka en Helma die samen met Leo Schultze Kool hebben gezorgd voor de goede logistiek en een prachtig slagingspercentage van de bijniervenesampling; de afdeling urologie in de persoon van Hans Langenhuijsen die het merendeel van de adrenalectomieën op zich heeft genomen; de afdeling pathologie en in het bijzonder Benno Kusters voor de urenlange sessies samen achter de microscoop en Bas Tops voor de DNA analyses;



de afdeling laboratoriumgeneeskunde, en dan vooral Fred Sweep, Teun van Herwaarden, André Brandt en Elma Prudon voor alle hormoonbepalingen voor de bijniervenesamplings en andere studies; de afdeling Health Evidence en dan voornamelijk Hans Groenewoud voor ondersteuning bij de statistiek en het bouwen van de Spartacus database en randomisatiewebsite; en uiteraard de afdeling vasculaire geneeskunde. Al tijdens mijn promotietraject voelde ik me erg welkom binnen jullie team en was de sfeer altijd positief. Het is gewoon een erg fijne club! Ik ben dan ook trots dat ik me sinds een jaar als fellow bij jullie heb mogen voegen. Anke en Ingeborg, jullie wil ik extra bedanken voor jullie inzet voor de Spartacus studie. De bijniervenesamplings en pre- en postoperatieve trajecten waren zonder jullie logistieke talenten en uitvoerende ondersteuning nooit zo succesvol geweest. Danielle, helaas hebben we, ondanks het gemeenschappelijke onderwerp van ons proefschrift, nooit als onderzoekers samengewerkt. Toch wil ik je bedanken voor het mooie afgelopen jaar als collega-fellow bij de vasculaire geneeskunde en het feit dat ik het hele “promotiegebeuren” een beetje bij je af heb mogen kijken. Tevens veel dank aan de opleiders van de algemeen interne geneeskunde, Jacqueline de Graaf en Gerald Vervoort voor de ruimte die ik binnen de opleiding heb gekregen voor het afronden van mijn proefschrift.

Ook wil ik graag mijn kamergenootjes uit de buitenhoek, Johanneke en Evertine, bedanken en alle onderzoekers met wie ik samen heb gewerkt aan de verschillende artikelen. Sabine, wat hebben we veel uren door al die artikelen voor het systematic review geploegd. Maar het resultaat mag er wezen! Bedankt voor alle vlijt en gezelligheid! Marja, hartelijk dank voor al je kennis en kunde ten aanzien van de kleuringen van de bijnierpreparaten. Heel veel succes bij je eigen promotie morgen! Marieke, bedankt voor het mooie case report en



je verdere uitwerking van de Spartacus data. Graeme Eisenhofer, many thanks for your terrific remote collaboration and supervision on our metanephrine project. Tevens wil ik graag Miranda bedanken voor de prachtige vormgeving van dit proefschrift.

Lieve Josien, Floor, Anouk, Noor en Lieke. Al ruim 30 jaar vriendinnen! Alle belevenissen op de basisschool, middelbare school, vervolgopleidingen, het werkende leven en de laatste jaren ook het gezinsleven hebben we al samen mogen delen. Wat bijzonder! Allemaal heel verschillend van karakter en ambities en daardoor juist zo'n prachtige, hechte groep. Het feit dat we ondertussen door heel Nederland zijn uitgewaaierd en elkaar daardoor minder vaak zien, heeft daar niets aan afgedaan. Ons zilveren jubileum hebben we al ruimschoots gehaald. Het kan niet anders dan dat dit ooit een platina of misschien zelfs eiken jubileum wordt!

Lieve Kim, Loes, Floor, Lotte, Inge, Annelies en Marlieke. Vanaf het eerste jaar van de geneeskundestudie, in 2003, heeft ons clubje zich langzaam gevormd. Het is bijna niet te bevatten dat dit alweer zo lang geleden is. Het voelt als de dag van gisteren. Over de jaren heen is iedereen steeds verder gegroeid en heeft een eigen plekje in de medische wereld gevonden. Ik ben er trots op dat we ondanks onze drukke banen in klinieken door heel Nederland, en zelfs Duitsland, nog steeds zo hecht en bij elkaar betrokken zijn.

Beste Karin, Stephan, Jaap, Rutger en consorten. Wat fijn als je collega's ook je vrienden worden! We moeten snel maar weer een kampeerweekendje plannen om bij een knus kampvuurtje bij te kletsen. Beste Laars-leden. Jullie zijn een prachtige vriendengroep en ondanks dat ik "slechts aanhang" ben, voelde ik me direct welkom. Fijn om te merken dat

IT-nerds zich net zo in hun IT-gespreksonderwerpen kunnen verliezen als internist-nerds dat in geneeskundige onderwerpen kunnen. Lieve Anne, ook al hebben we elkaar de laatste tijd minder vaak gezien, graag wil ik je bedanken voor je warme vriendschap. We moeten snel weer eens afspreken.

Beste Yoka, Ruud, Olaf, Henriette, Paavo, Wendela, Lilly, Pim en Pieter. Vanaf onze eerste kennismaking zeven jaar geleden, voel ik me helemaal welkom binnen jullie familie. Het is mooi om de hechte band die Justus met zijn broers en ouders heeft te zien. Yoka en Ruud, erg bedankt voor de goede zorgen voor Oscar en nu ook Vera. Het is elke keer weer een feestje voor hen als ze bij jullie mogen gaan spelen. Beste familie Waale en familie Dekkers. Twee kleine families, waarin iedereen elkaar goed kent. Helaas zie ik jullie als Brabantse "expat" niet heel vaak meer, maar de tamtams reiken gelukkig altijd vrij ver.

Lieve pap en mam. Al 35 jaar mijn steun en toeverlaat. Door jullie stimulans, vertrouwen en toewijding de afgelopen 35 jaar, sta ik hier vandaag. Jullie hebben me altijd aangemoedigd om het beste uit mezelf te halen, zonder daarbij enige druk op te leggen. Altijd hebben jullie me vrijgelaten in de keuzes die ik maakte en me daarin gesteund. Ook binnen mijn promotietraject bleven jullie mij stimuleren. Mam, de Flix-bus is rijk geworden van alle ritjes naar Malden om op mijn parttime dag op Oscar en nu ook Vera te passen, zodat ik mijn manuscript af kon maken. Oscar en Vera zijn helemaal dol op hun opa en oma, en terecht! Ik hoop dat Justus en ik net zulke goede ouders voor ze mogen worden als jullie altijd voor mij zijn geweest en nog steeds zijn! Ik hou van jullie!

Lieve Justus. Ik vind het moeilijk om onder woorden te brengen hoeveel je voor mij betekent. Alles! Al moet ik toegeven dat je dat "alles" de laatste jaren wel met de twee ondergenoemden moet delen. Je hebt me enorm gesteund tijdens mijn promotietraject en opleiding. Met je uitspraak "je hebt maar één hoofd en twee handjes" heb jij me met beide benen op de grond gehouden en gezorgd dat ik me niet over de kop werkte. Samen

hebben we ervoor gewaakt dat onze vrije tijd ook echt tijd voor elkaar was en deze gevuld met mooie reizen, uitjes, wandelingen, theater- en concertbezoekjes of gewoon een lekker filmpje op de bank. Mocht dit ooit veranderen, zet me dan alsjeblieft weer snel met beide beentjes terug op de grond. Ik hou van jou!

Lieve Oscar en Vera, jullie zijn het mooiste wat ons is overkomen. Oscar, ons bijdehand boefje, wat word je snel groot. Ik smelt elke keer weer als ik je met je vader op de bank zie ravotten of als je in de box klimt om je zusje een kusje te geven. Lieve, kleine Vera, pas enkele weken bij ons en nu al niet meer weg te denken uit ons gezinnetje. Met je twinkeloogjes en ondeugende lachje betover je iedereen. Boefjes, ik hou van jullie. En vergeet niet: lief zijn… niet draken

LIST OF PUBLICATIONS

1. Puar TH, Loh WJ, Lim DST, Loh LM, Zhang M, Foo FS, Lee L, Swee DS, Khoo J, Tay D, Kam C, **Dekkers T**, Velema M, Deinum J, Kek PC. Aldosterone-Potassium Ratio Predicts Primary Aldosteronism Subtype. *Submitted*
2. Bandell RAM, **Dekkers T**, Semmekrot BA, de Wildt SN, Fleuren HWHA, Warlé-van Herwaarden MF, Füssenich P, Gerrits GP, Kramers C. Macrolide prescription in Dutch children: compliance with guidelines. Eur J Clin Microbiol Infect Dis. 2019 Apr;38(4):675-681
3. Velema MS and **Dekkers T**, Hermus ARMM, Timmers HJLM, Langenhuijsen J, Arntz M, Kusters B, Eisenhofer G5, Lenders JWM, Deinum J. A pedunculated aldosterone-producing adenoma drained by an extra vein causing puzzling results of adrenal vein sampling. Clin Endocrinol (Oxf). 2018; august; 89(2). 242-244.
4. **Dekkers T**, Lafeber M, Kramers C. Dual Antithrombotic Therapy with Dabigatran after PCI in Atrial Fibrillation. N Engl J Med. 2018 Feb 1;378(5):484-5.
5. Velema M, **Dekkers T**, Hermus A, Timmers H, Lenders J, Groenewoud H, Schultze Kool L, Langenhuijsen J, Prejbisz A, van der Wilt GJ, Deinum J; SPARTACUS investigators. Quality of Life in Primary Aldosteronism: a Comparative Effectiveness Study of Adrenalectomy and Medical Treatment. J Clin Endocrinol Metab. 2018 Jan 1;103(1):16-24
6. Sze CWC, O'Toole SM, Tirador RK, Akker SA, Matson M, Perry L, Druce MR, **Dekkers T**, Deinum J, Lenders JWM, Eisenhofer G, Drake WM. Adrenal Vein Catecholamine Levels and Ratios: Reference Intervals Derived from Patients with Primary Aldosteronism. Horm Metab Res. 2017 Jun;49(6):418-423.
7. Arlt W, Lang K, Sitch AJ, Dietz AS, Rhayem Y, Bancos I, Feuchtinger A, Chortis V, Gilligan LC, Ludwig P, Riester A, Asbach E, Hughes BA, O'Neil DM, Bidlingmaier M, Tomlinson JW, Hassan-Smith ZK, Rees DA, Adolf C, Hahner S, Quinkler M, **Dekkers T**, Deinum J, Biehl M, Keevil BG, Shackleton CHL, Deeks JJ, Walch AK, Beuschlein F, Reincke M. Steroid metabolome analysis reveals prevalent glucocorticoid excess in primary aldosteronism. JCI Insight. 2017 Apr 20;2(8). pii: 93136.
8. van der Wilt GJ, **Dekkers T**, Lenders JW, Deinum J. Adrenal vein sampling versus CT scanning in primary aldosteronism - Authors' reply. Lancet Diabetes Endocrinol. 2016 Nov;4(11):886-887.

9. **Dekkers T**, Prejbisz A, Kool LJS, Groenewoud HJMM, Velema M, Spiering W, Kołodziejczyk-Kruk S, Arntz M, Kądziela J, Langenhuijsen JF, Kerstens MN, van den Meiracker AH, van den Born BJ, Sweep FCGJ, Hermus ARMM, Januszewicz A, Ligthart-Naber AF, Makai P, van der Wilt GJ, Lenders JWM, Deinum J; SPARTACUS Investigators. Adrenal vein sampling versus CT scan to determine treatment in primary aldosteronism: an outcome-based randomised diagnostic trial. Lancet Diabetes Endocrinol. 2016 Sep;4(9):739-46.
10. Käyser SC and **Dekkers T**, Groenewoud HJ, van der Wilt GJ, Carel Bakx J, van der Wel MC, Hermus AR, Lenders JW, Deinum J. Study Heterogeneity and Estimation of Prevalence of Primary Aldosteronism: A Systematic Review and Meta-Regression Analysis. J Clin Endocrinol Metab. 2016 Jul;101(7):2826-35.
11. Eisenhofer G, **Dekkers T**, Peitzsch M, Dietz AS, Bidlingmaier M, Treitl M, Williams TA, Bornstein SR, Haase M, Rump LC, Willenberg HS, Beuschlein F, Deinum J, Lenders JW, Reincke M. Mass Spectrometry-Based Adrenal and Peripheral Venous Steroid Profiling for Subtyping Primary Aldosteronism. Clin Chem. 2016 Mar;62(3):514-24.
12. Williams TA, Peitzsch M, Dietz AS, **Dekkers T**, Bidlingmaier M, Riester A, Treitl M, Rhayem Y, Beuschlein F, Lenders JW, Deinum J, Eisenhofer G, Reincke M. Genotype-Specific Steroid Profiles Associated With Aldosterone-Producing Adenomas. Hypertension. 2016 Jan;67(1):139-45.
13. Peitzsch M, **Dekkers T**, Haase M, Sweep FC, Quack I, Antoch G, Siegert G, Lenders JW, Deinum J, Willenberg HS, Eisenhofer G. An LC-MS/MS Method for Steroid Profiling during Adrenal Venous Sampling for Investigation of Primary Aldosteronism. J Steroid Biochem Mol Biol. 2015 Jan;145:75-84
14. **Dekkers T**, Deinum J, Schultzekool LJ, Blondin D, Vonend O, Hermus AR, Peitzsch M, Rump LC, Antoch G, Sweep FC, Bornstein SR, Lenders JW, Willenberg HS, Eisenhofer G. Plasma Metanephrine for Assessing the Selectivity of Adrenal Venous Sampling. Hypertension. 2013 Dec;62(6):1152-7
15. **Dekkers T**, ter Meer M, Lenders JW, Hermus AR, Schultze Kool L, Langenhuijsen JF, Nishimoto K, Ogishima T, Mukai K, Azizan EA, Tops B, Deinum J, Küsters B. Adrenal Nodularity and Somatic Mutations in Primary Aldosteronism: One Node Is the Culprit? J Clin Endocrinol Metab. 2014 Jul;99(7):E1341-51.

16. Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, Maniero C, Garg S, Bochukova EG, Zhao W, Shaikh LH, Brighton CA, Teo AE, Davenport AP, **Dekkers T**, Tops B, Küsters B, Ceral J, Yeo GS, Neogi SG, McFarlane I, Rosenfeld N, Marass F, Hadfield J, Margas W, Chaggar K, Solar M, Deinum J, Dolphin AC, Farooqi IS, Striessnig J, Nissen P, Brown MJ. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. Nat Genet. 2013 Sep;45(9):1055-60
17. **Dekkers T**, Arntz MJ, van der Wilt GJ, Schultze Kool LJ, Sweep FC, Hermus AR, Lenders JW, Deinum J. Single versus duplicate blood samples in ACTH stimulated adrenal vein sampling. Clin Chim Acta. 2013 Aug 23;423:15-7.
18. Wagenmakers MA, Netea-Maier RT, Prins JB, **Dekkers T**, den Heijer M, Hermus AR.Impaired quality of life in patients in long-term remission of Cushing's syndrome of both adrenal and pituitary origin: a remaining effect of long-standing hypercortisolism? Eur J Endocrinol. 2012 Nov;167(5):687-95.
19. **Dekkers T**, Deinum J. Adrenal venous sampling crucial in primary aldosteronism? Journal of Hypertension. 2012 Feb;30(2):433-5.

CURRICULUM VITAE

Tanja Dekkers werd op 18 september 1984 geboren in Eindhoven. Ze groeide op in het Brabantse dorpje Steensel. Na het gymnasium te hebben afgerond aan het Rythoviuscollege in Eersel, startte zij aan de opleiding geneeskunde in 2003 aan de Radboud Universiteit te Nijmegen. Tijdens de opleiding deed zij een tropen coschap in Rubya Hospital in Tanzania. Haar onderzoeksstage deed ze bij de afdeling Endocrinologie van het Radboud UMC. Hierbij richtte ze zich op de kwaliteit van leven bij het syndroom van Cushing. Na het behalen van haar artsendiploma bleef ze in de "bijnier business" hangen en startte ze een promotietraject op het gebied van primair hyperaldosteronisme. Uit dat promotietraject is dit proefschrift voortgekomen. Voor de Spartacus studie, beschreven in dit proefschrift, ontving zij de NVE/IPSEN publicatieprijs van de Nederlandse vereniging voor Endocrinologie, de ENSAT (european network for the study of adrenal tumors) award "clinical research on non-ACC adrenal tumors" en de prijs voor beste abstract (niet-oncologisch) bij de European Association of Urology.

Na zich drie jaar full time aan het onderzoek te hebben gewijd, begon zij in 2013 met de opleiding tot internist in het Radboud UMC. In 2015 verruilde ze het Radboud UMC voor het Canisius Wilhelmina Ziekenhuis om hier het perifere deel van de opleiding te volbrengen. In 2017 kwam zij terug naar het Radboud UMC om te starten met haar differentiatie bij de vasculaire geneeskunde.

In haar vrije tijd trekt Tanja er graag op uit in de natuur, in Nederland of ver daarbuiten, samen met haar vriend Justus, hun 2-jarige zoon Oscar en in juli geboren dochter Vera.

Institute for Health Sciences
Radboudumc

PHD PORTFOLIO

Name: PhD student: T. Dekkers	**PhD period:** 01-03-2010 – 1-7-2019
Department: Internal Medicine	**Promotor(s):** Prof. J.W.M. Lenders, Prof. G.J. van der Wilt, Prof. L.J. Schultze Kool.
Graduate School: Radboud Institute for Health Sciences	**Co-promotor(s):** Dr. J. Deinum

	Year(s)	ECTS
TRAINING ACTIVITIES		
A) Courses & Workshops		
ESH hypertension Summer School	2018	2.0
BROK: Good clinical Practice Course, Radboud University Nijmegen.	2016	0.1
Adrenal Master Class	2014	1.5
Academic writing. Radboud university Nijmegen	2012	3.0
SPSS	2011	1.0
PhD management course. Radboud university Nijmegen	2011	3.0
Quality of life measurement (HS11). NIHES, Erasmus University	2011	1.5
Biometrics. Radboud University Nijmegen	2011	2.5
Cost-effectiveness course: methods and principles (K72). VU.	2011	1
BROK: Good clinical Practice Course, Radboud University Nijmegen.	2010	1.5
Diagnostic research. NIHES, Erasmus University, Rotterdam	2010	1.5
Introductiecursus promovendi (NCEBP)	2010	1.5
Reference manager (RU medical library)	2009	0.05
B) Symposia & congresses		
Nederlandse internisten dagen, Sessie TOPpublicaties (Oral)	2017	1.0
European Association of Urology congress (Poster and oral)	2017	0.5

Dutch Society of Hypertension. (Oral)	2014	0.1
German Endocrine Society meeting (Oral)	2013	0.5
European society of hypertension (Poster)	2013	0.5
Dutch Society of Hypertension (Oral)	2012	0.4
European Society of hypertension. London (Poster)	2012	0.5
ENSAT meeting (Oral)	2012	0.5
Klinische Endocrinologie dagen 2011 (Oral)	2012	0.5
ENSAT meeting (Poster)	2011	0.5
European Society of Hypertension Oslo (Poster)	2010	0.5
Radboud adrenal center symposium	2010	0.5
C) Other		
Vascular Damage theme meetings	2010-2013	0.5
Radboud Adrenal Center meetings	2010-2013	0.5
TEACHING ACTIVITIES		
D) Supervision of internships		
Supervision of research internship student Technical Medicine	2013	
Supervision of research internship student Medicine	2012	
Supervision of research internship student Medicine	2010	
PHD AWARDS		
EAU. Prize for the Best Abstract (Non-Oncology)	2016	
NVE-Ipsen prize for best article in endocrinology.	2017	
ENSAT award clinical research on non-ACC adrenal tumors.	2015	
ESH poster prize	2013	
TOTAL		**28.15**

LIST OF ABBREVIATIONS

11ohase	11-hydroxylase
17ohase	17-hydroxylase
21ohase	21-hydroxylase
3HSD	3-hydroxysteroid dehydrogenase
A	Aldosterone
A/C	Aldosterone/ cortisol ratio
ACE	Angiotensin converting enzyme
ACTH	Adrenocorticotropic hormone
ADX	Adrenalectomy
Aldo synthase	Aldosterone synthase
APA	Aldosterone producing adenoma
APCC	Aldosterone producing cell cluster
ARR	Aldosterone-to-renin-ratio
AV	Adrenal vein
AVS	Adrenal Vein Sampling
BAH	Bilateral adrenal hyperplasia
C	Cortisol
CT	Computed Tomography Scan
DSA	Digital subtraction angiography
IMM	Inner mitochondrial membrane
IVC	Inferior vena cava
LAV	Left adrenal vein
MRA	Mineralocorticoid Receptor Antagonists
MRI	Magnetic resonance imaging
OMM	Outer mitochondrial membrane
P450scc	Cholesterol side chain cleavage enzyme
PA	Primary aldosteronism
RAV	Right adrenal vein
Star	Steroidogenic acute regulatory protein

Institute for Health Sciences
Radboudumc