Primary aldosteronism in Western Norway

Studies of diagnostic approach and treatment outcome

Marianne Aardal Grytaas

Thesis for the Degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2018

UNIVERSITY OF BERGEN

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2018

Date of defence: 06.09.2018

Year: 2018

Title: Primary aldosteronism in Western Norway

Name: Marianne Aardal Grytaas

Print: Skipnes Kommunikasjon / University of Bergen

Scientific environment

This thesis is based on research work carried out at the Department of Medicine, Section of Endocrinology, Haukeland University Hospital, Bergen, and at the Department of Clinical Science, University of Bergen. Main supervisor was Professor Kristian Løvås, and co-supervisors were Professor Eystein Sverre Husebye and MD Hrafnkell Baldur Thordarson. The late Professor Michael Brauckhoff was co-supervisor from 2013 to September 2014.

The research was funded by a PhD fellowship provided by the Western Norway Regional Health Authority.

Acknowledgements

First of all, I would like to express my heartfelt gratitude to my main supervisor Professor Kristian Løvås for his excellent guidance and for serving as an impeccable role model for scientific thought and conduct. His encouragement, support, vast knowledge, linguistic skills, patience and kind nature have been invaluable. I want to thank co-supervisor Professor Eystein S Husebye for his valuable inputs, in-depth academic knowledge and always enthusiastic and optimistic attitude. I am thankful to co-supervisor and head of the Section of Endocrinology Hrafnkell B Thordarson for his support and for facilitating research combined with clinical work. My gratitude also goes to Professor Terje H Larsen, who introduced me to cardiac magnetic resonance imaging research, and who gave me the opportunity to perform research with him.

The late Professor Michael Brauckhoff was co-supervisor from the start of this thesis until his death. I fondly remember his great enthusiasm for science and his care for each patient, and he is deeply missed.

I am very grateful for being part of Professor Husebye's research group. In particular I want to express my gratitude to Elisabeth T Halvorsen for her impeccable handling of study samples. A special thank you also goes to my office mate Marianne Øksnes for her friendship, positive personality and all our trusted office conversations, and to my friend and fellow researcher Grethe Å Ueland for our valuable private and scientific discussions. I greatly appreciate Martina M Erichsen for her enthusiastic and direct attitude, Marianne C Astor for sharing scientific experiences and frustrations, and Paal Methlie for his knowledge and inspiring scientific ideas. I thank Eirik Bratland, Anette S B Wolff, Lars E Breivik, Katerina Simunkova, Øyvind Bruserud, Bergithe E Oftedal, Alexander Hellesen, Elin Theodorsen, Hajirah Muneer, Ellen C Røyrvik, Sigrid Aslaksen and Haydee Artaza Alvarez for contributing to the stimulating and welcoming environment in the research group.

I would like to thank all my co-authors for their contributions, and especially Kristin Viste, Siri S Strømsøy, Kjersti Sellevåg and Jarle B Arnes. My thoughts and gratitude also go to the late Professor Jarle T Rørvik for his contribution.

The Hormone Laboratory has been an important partner in this scientific work, and I would in particular like to thank head of the Hormone Laboratory, Professor Jørn V Sagen, and Bjørg Almås for their cooperation. Likewise, the collaboration with the Section of Endocrine Surgery, especially endocrine surgeon Anette Heie, has been deeply appreciated. I would also like to thank Thomas Arnesen at the Department of Molecular Biology, University of Bergen.

I further want to express my gratitude to my colleagues at the Section of Endocrinology, Elinor Vogt, Sigfrid Reisegg, Anne Schønberg, Eirik Søfteland, Jan Inge Sørheim and Ida Kloster, for providing a great and inspiring work environment. I am also deeply grateful for the invaluable assistance provided by Nina Jensen and Mona Eliassen in taking blood samples from all the research patients.

Without the patients and healthy volunteers who have participated in these studies, this clinical research could not have been performed. I am grateful to them all.

To Elisabeth Kleppe and Mette N Vesterhus – I greatly appreciate our long friendship and good conversations, and I warmly thank them both for being my dear friends.

Lastly but most importantly I am forever grateful to my family. My parents Nina and Gunnar brought me up to believe that everything is within reach if I work for it, and I warmly thank them for providing me an upbringing full of love and support. I thank my sister Ingrid for always showing me her care, and for being the best sister in the world. Finally, my dear husband Håvard has given me unconditional love, interest, encouragement and support every day and through all times of scientific ups and downs. You are my solid anchor, and I am endlessly fortunate to have you. Our beloved children Ida, Ulrik and Martin – I thank them for continuously reminding me what is most important in life, and for the love and joy they bring me every day.

Abbreviations

ACE	Angiotensin converting enzyme
ACTH	Adrenocorticotropic hormone
ANP	Atrial natriuretic peptide
APA	Aldosterone-producing adenoma
ARR	Aldosterone-renin-ratio
AVS	Adrenal vein sampling
BAH	Bilateral adrenal hyperplasia
BP	Blood pressure
CMR	Cardiac magnetic resonance imaging
CMR1-CE	Cardiac magnetic resonance imaging, dynamic contrast enhancement
CMR2-T1	Cardiac magnetic resonance imaging, T1 mapping
CT	Computed tomography
DRC	Direct renin concentration
ECV	Extracellular volume
FH	Familial hyperaldosteronism
GFR	Glomerular filtration rate
GRA	Glucocorticoid-remediable aldosteronism
HT	Hypertension
IHA	Idiopathic hyperaldosteronism

LC-MS/MS	Liquid chromatography tandem mass spectrometry
LI	Lateralisation index
LV	Left ventricular
LV-EDV	Left ventricular end-diastolic index
MR	Mineralocorticoid receptor
NSAIDs	Non-steroidal anti-inflammatory drugs
PA	Primary aldosteronism
PET	Positron emission tomography
PHPT	Primary hyperparathyroidism
PRA	Plasma renin activity
PTH	Parathyroid hormone
RAAS	Renin-angiotensin-aldosterone system
ROI	Region of interest
SI	Selectivity index
SIT	Saline infusion test
ZG	Zona glomerulosa
ZF	Zona fasciculata
ZR	Zona reticularis
StAR	Steroidogenic acute regulatory protein
11β-HSD2	11β-hydroxysteroid dehydrogenase type 2

Contents

Summary

Primary aldosteronism (PA) is the most common cause of secondary hypertension (HT), accounting for 5 to 12% of all hypertensive individuals. Patients with PA experience increased cardiovascular, renal and metabolic complications and have impaired quality of life compared with essential HT. We aimed to determine the clinical and biochemical characteristics of the PA population in Western Norway, to identify the optimal diagnostic procedures, and to evaluate long-term treatment outcome after medical and surgical treatment.

Three clinical studies were conducted. We retrospectively identified all PA patients admitted to Haukeland University Hospital from 1998 to 2012, a total of 108 cases. The majority had unilateral PA and hypokalaemia, indicating that mostly patients with florid PA were detected. Cure rate of HT after adrenalectomy was low. Female sex, presence of somatic *KCNJ5* mutations and a histopathological adenoma were associated with cure of HT.

We also conducted an adrenal vein sampling (AVS) study, where we found that the use of intraprocedural point-of-care cortisol assay increased the AVS success rate substantially compared with historical controls.

In our cardiac magnetic resonance imaging (CMR) study, we found that left ventricular (LV) mass decreased rapidly after PA treatment, most pronounced in adrenalectomised patients. Dynamic contrast enhancement and T1 mapping did not reveal increase in myocardial fibrosis in PA compared with healthy subjects.

In conclusion, PA is probably grossly underdiagnosed in Norway, in particular bilateral PA, which often exhibits a mild phenotype. HT cure rate after adrenalectomy was low. Intraprocedural cortisol assay increased the success rate of AVS, and is now in routine clinical use at our centre. Specific PA treatment rapidly reduced LV mass, underlining the importance of correct diagnosis allowing for efficient treatment. Although cardiovascular risk is increased in PA, myocardial fibrosis may not represent a common clinical problem.

List of publications

Paper I

Grytaas MA, Strømsøy S, Rørvik JT, Arnes JB, Heie A, Arnesen T, Jørstad MD, Nedrebø BG, Jøssang DE, Jensen DK, Rørvik HD, Sagen JV, Mellgren G, Thordarson HB, Husebye ES, Løvås K. Clinical characteristics and long-term outcome of primary aldosteronism in a Norwegian population. *Hormone and Metabolic Research 2017 Nov;49(11):838-846*

Paper II

Viste K, **Grytaas MA**, Jørstad MD, Jøssang DE, Høyden EN, Fotland SS, Jensen DK, Løvås K, Thoradarson H, Almås B, Mellgren G. Efficacy of adrenal venous sampling is increased by point of care cortisol analysis. *Endocrine Connections 2013 Nov;2(4): 236-42*

Paper III

Grytaas MA, Sellevåg K, Thordarson HB, Husebye ES, Løvås K, Larsen TH. Cardiac magnetic resonance imaging of myocardial mass and fibrosis in primary aldosteronism. *Endocrine Connections 2018 Mar;7(3):413-424*

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1 Introduction

1.1 Normal physiology of the adrenals – historical perspectives

The anatomy of the adrenal glands was first described by Barholomeo Eustachius in 1563, but their functional role was not defined until the 19th century. Thomas Addison described both the clinical features and pathological findings in primary adrenal insufficiency (Addison's disease) in 1855. Shortly after Charles E Brown-Séquard by performing adrenalectomy in dogs, cats and guinea pigs, demonstrated that the adrenal glands were essential for life (1). Between 1937 and 1955 the different adrenocortical steroid hormones were isolated and synthesised. Sylvia A Simpson and James F Tait isolated aldosterone in 1953 (2). In 1954, the symptoms and signs of primary aldosteronism (PA) were described for the first time by Jerome Conn (3). More than three decades later, in 1987, Jeff Arriza cloned and characterised the mineralocorticoid receptor (MR) (4). Aldosterone and cortisol have similar binding affinity for the MR, but cortisol circulates at much higher concentrations than aldosterone. The tissue specificity of the MR to aldosterone was therefore a mystery until Christopher RW Edwards and John W Funder in 1988 simultaneously showed that tissue specific expression of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β- HSD2) conferred specificity of the MR to aldosterone and not cortisol (5, 6).

1.2 Aldosterone synthesis and regulation

Aldosterone is an adrenal steroid hormone that is synthesised in the outermost layer of the adrenal cortex, the zona glomerulosa (ZG). The other main types of hormones synthesised in the adrenal cortex are the glucocorticoid cortisol in the zona fasciculata (ZF), and the sex steroids, mainly androgens, synthesised in the innermost layer of the adrenal cortex, the zona reticularis (ZR). The precursor of all steroid hormones produced by the adrenal cortex is cholesterol, with zone specific expression of the different enzymes. The biosynthetic pathways of the steroidogenesis of aldosterone, cortisol and androgens are shown in Figure 1.

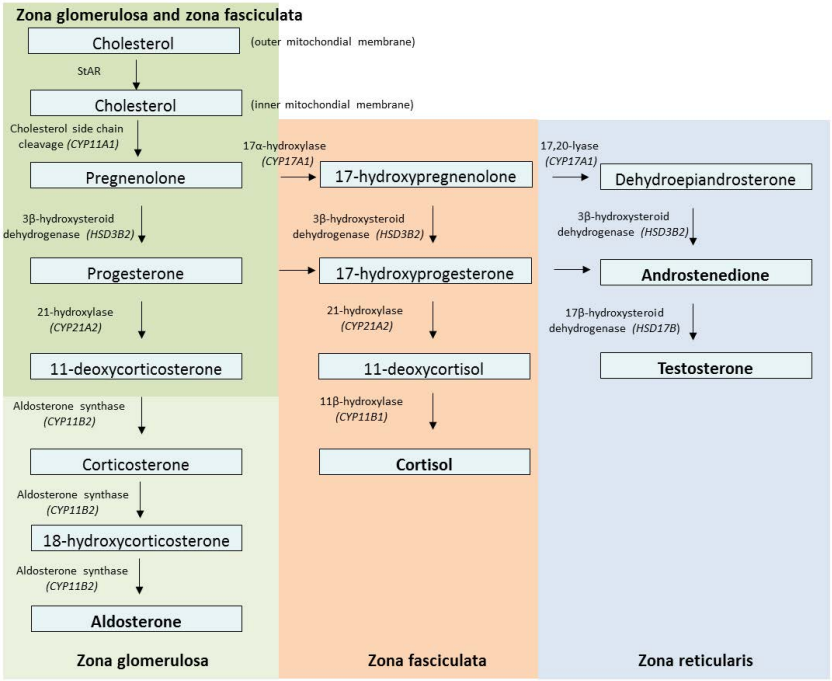

Figure 1 Adrenal steroidogenesis. *Modified from Stowasser et al, Physiol Rev. volume 96, 2016, and others.*

The initial and rate-limiting step of all adrenal steroidogenesis is the transport of intracellular cholesterol from the outer to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR), for conversion to pregnenolone. In the endoplasmatic reticulum pregnenolone is converted to progesterone, and further to deoxycorticosterone. The final three steps of aldosterone biosynthesis occur in the mitochondria, where deoxycorticosterone undergoes 11-hydroxylation, 18-hydroxylation and 18-oxidation, all three steps catalysed by aldosterone synthase, encoded by *CYP11B2*. Aldosterone synthase is expressed exclusively in the ZG. The enzyme is highly homologous with 11β-hydroxylase (encoded by *CYP11B1*), expressed in ZF and responsible for the final steps of cortisol biosynthesis (7).

18-Hydroxycortisol and 18-oxocortisol are hybrid steroids with structural characteristics of both cortisol and aldosterone. They are both produced by aldosterone synthase using the cortisol precursor 11-deoxycortisol as substrate. Production of 18-hydroxycortisol and 18-oxocortisol is normally extremely low, due

to functional zonation of the adrenal cortex, with 11-deoxycortisol present in the ZF and aldosterone synthase expressed in the ZG (8, 9).

The main stimulatory regulator of aldosterone synthesis is angiotensin II via the renin-angiotensin-aldosterone system (RAAS) (Figure 2), but elevation of plasma potassium and adrenocorticotropic hormone (ACTH) also stimulate aldosterone synthesis.

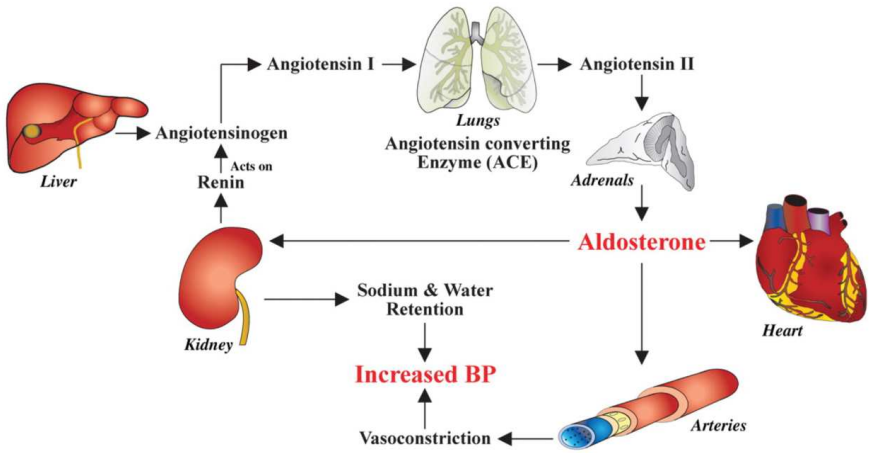

Figure 2 Renin-angiotensin-aldosterone system. *Patel et al: Role of radiology in the management of primary aldosteronism, Radiographics 2007; 27(4):1145-1157. Reprinted with permission.*

Renin is an enzyme produced by the juxtaglomerular cells of the kidney. It causes cleavage of angiotensinogen originating in the liver, to the inactive peptide angiotensin I. This is the rate-limiting step of the RAAS. Angiotensin converting enzyme (ACE), predominantly expressed in the lungs, further cleaves angiotensin I to angiotensin II, the active hormone. Binding of angiotensin II to type 1 angiotensin II receptors of the membrane of ZG cells stimulates aldosterone synthesis. Angiotensin II also mediates arteriolar vasoconstriction, leading to increase in systemic vascular resistance and systemic blood pressure (BP).

Principal stimulators of renin secretion include: 1) reduced perfusion pressure sensed by baroreceptors in the wall of the afferent arteriole of the glomerulus, 2) reduction of sodium chloride delivery to the cells of the macula densa in the early distal tubuli,

and 3) sympathetic activation of the renin-secreting juxtaglomerular cells. Thus, renin secretion is stimulated in clinical situations with sodium and/or volume depletion, acute drop in BP, stress or change of posture to the upright position, or renal artery stenosis. Moreover, pharmacological blockage of the RAAS by ACE inhibitors or angiotensin II receptor blockers also increases renin. Conversely, renin is inhibited by sodium retention or extracellular volume expansion, by renal conditions associated with abnormal retention of sodium, by aging and chronic kidney disease, which reduce the renin-producing capacity. Also pharmacological agents reducing sympathetic activation (e.g. β-adrenergic blockers) reduce renin. Furthermore, angiotensin II exerts negative feedback on renin secretion, by way of arteriolar vasoconstriction and sodium reabsorption via aldosterone secretion. Renin secretion follows a diurnal rhythm, with a peak of secretion between 2 a.m. and 8 a.m. (10).

In addition to the systemic RAAS, local RAAS operate in various tissues, including the heart, vasculature, adrenal glands, central nervous system, skin, intestine and adipose tissue. Here, local angiotensin II may cause both autocrine and paracrine physiological and pathophysiological effects (11, 12).

Besides stimulation by RAAS, aldosterone synthesis is stimulated by increase in plasma potassium both acutely and in the long term. Conversely, hypokalemia reduces aldosterone secretion. The responsiveness of ZG cells to potassium levels is retained even in conditions where aldosterone synthesis is autonomous, that is, not sensitive to angiotensin II levels. ACTH secreted by the pituitary gland may also weakly stimulate aldosterone secretion acutely, but transiently. Thus, stress-induced rise in ACTH may cause transient rise in aldosterone. ACTH exhibits a diurnal rhythm, with a peak between 5 a.m. and 9 a.m. (13). Under physiological circumstances, aldosterone synthesis shows a circadian rhythm parallel to, but preceding that of cortisol, with the most prominent aldosterone secretion occurring at night during recumbency. The underlying regulatory mechanisms are still unknown (14, 15).

Apart from the major regulators angiotensin II, potassium and ACTH, several other regulators may cause minor effect on aldosterone secretion, such as atrial natriuretic peptide (ANP), estrogens, vasopressin and parathyroid hormone (PTH) (16-19).

1.3 Aldosterone effects

Aldosterone's main action is in the kidney, where it stimulates sodium reabsorption in the epithelial cells of the distal nephron. Water is retained with the osmotically active sodium ions, causing extracellular volume expansion and increase in blood pressure. Sodium reabsorption is accompanied by increased potassium and hydrogen ion excretion. Similar actions of aldosterone occur in epithelial cells of the distal colon and sweat glands. These aldosterone effects are mediated by the intracellular MR. The MR has similar binding affinity for and can also be activated by cortisol, which is present in plasma concentrations about 1000-fold greater than those of aldosterone. However, in epithelial cells as in the kidney the enzyme11β- HSD2 inactivates cortisol to the inactive metabolite cortisone, and prevents a mineralocorticoid excess state (5, 6, 20) .

The MR is also present in a range of non-epithelial tissues including endothelial cells, vascular smooth muscle, adipocytes, macrophages, cardiomyocytes, and in the central nervous system (21). The MR in non-epithelial tissues, with the exception of blood vessels, lack 11β- HSD2, thus normal cortisol levels occupy the majority of MR, exerting an inhibitory effect on MR activity under normal circumstances (10, 22). However, under conditions of tissue inflammation and hypoxia, cortisol becomes an MR agonist.

The classical effects of aldosterone are normally seen after hours, and are mediated by MR-mediated gene transcription. In addition, rapid non-genomic effects of aldosterone have been demonstrated in the kidneys, the heart and the vascular wall that involve different signaling pathways (23, 24).

1.4 Primary aldosteronism, definition and epidemiology

PA is a pathological condition where the secretion of aldosterone from the adrenal glands is excessive in relation to the body's sodium and volume status, and autonomous of its normal regulation by the RAAS. Its main clinical manifestation is hypertension (HT), with or without concomitant hypokalaemia. PA was first described by Jerome Conn in 1954, through studies of a 34-year old hypertensive woman with florid signs and symptoms indicative of excessive aldosterone (3). She had a 7-year history of muscle spasms, temporary paralysis, tetany and weakness, a 4-year history of HT, and she presented with HT, severe hypokalaemia and metabolic alkalosis. Conn suspected that her symptoms and signs were caused by excess secretion of aldosterone, and planned for bilateral adrenalectomy. However, during surgery, a large tumour in the right adrenal gland was encountered and removed, and the contralateral adrenal gland was left intact. Postoperatively, both the patient's metabolic abnormalities and clinical signs were almost totally reversed. PA is therefore also called Conn's syndrome, in recognition of Jerome Conn.

Although Conn himself predicted PA to be a relatively common disease, until the early 1990s, PA was considered to be rare, accounting for less than 1% of all hypertensive patients. However, after the introduction of aldosterone-renin-ratio (ARR) as a screening method in 1981 (25), it has become evident that PA is a highly prevalent cause of secondary HT, accounting for between 5-12% of all HT, with the majority of patients being normokalaemic. Nonetheless, wide variations in prevalence (1-30%) are reported in different studies (26-28). The prevalence of PA depends both on the population examined, the degree of HT and the stringency of the diagnostic criteria. Reported prevalence is around 4-6% in stage 1 HT in primary care patients and about 11% in referral centres, while among patients with resistant HT prevalence numbers up to 20-24% have been reported (27, 29-32).

Recently, PA has also been described in normo- and prehypertensive cohorts in Asia, Europe and USA. (33-35). One was a longitudinal study which demonstrated that normotensive individuals with confirmed PA had an increased risk of developing HT

at five years, compared with controls without PA (34). Another study examining normotensive individuals with suppressed renin levels, found that 14% had autonomous aldosterone secretion that fulfilled confirmatory criteria for PA, although ARR levels were not different from those who did not fulfill the diagnostic criteria (35). Thus, renin-independent autonomous aldosteronism represents a continuum ranging from low-renin in normotension, through low-renin HT to classical PA.

1.5 Subtypes and genetics in PA

The two most common subtypes of PA are aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA). APA is usually unilateral, accounting for about 28 - 50%, whereas IHA mostly has bilateral ZG hyperplasia, also termed bilateral adrenal hyperplasia (BAH), and accounts for about 50-70% (26, 27, 29). However, these traditional main subtypes reveal a wide diversity in adrenal morphology. In an adrenal with APA, the remaining cortex may contain additional, smaller nodules, and the surrounding ZG may show diffuse hyperplasia. Unilateral hyperplasia without APA may also occur (36). In IHA, the hyperplasia may be diffuse, micro- or macronodular (10). Very rare subtypes of PA are aldosterone-producing carcinoma (<1%) and ectopic aldosterone-producing adenoma or carcinoma (<0.1% of all PA) (37).

Recently, major insights have emerged into the pathogenesis and genetic causes of PA Familial forms of hyperaldosteronism (FH) are rare, but at least four different types exist. FH-I, also called glucocorticoid-remediable aldosteronism (GRA), was first described in 1966 (38). It is inherited in an autosomal dominant fashion, and is caused by a chimeric *CYP11B1/CYP11B2* gene responsive to ACTH rather than angiotensin II, first elucidated in 1992 (39). GRA has a presumed prevalence of 0.7-1.0% of all PA (7). FH-II, first described in 1991, is also autosomal dominant, but the genetic basis is still unclear, and it is clinically indistinguishable from non-familial PA (7, 40). It is diagnosed when two or more members of the same family are affected. Prevalence estimates of FH-II vary from 3% to 7% of all PA. In 2008, FH-III was described, and the causative germline mutation in the *KCNJ5*-gene encoding

the potassium channel Kir 3.4 was found in 2011 (41, 42). Affected families reveal a phenotypic heterogeneity, ranging from early-onset treatment-resistant HT and profound hypokalaemia, to milder phenotypes (7). A germline mutation in the *CACNAID* gene encoding the calcium channel subunit was discovered in 2013 in two children with PA and neuromuscular abnormalities (43). FH-IV, first described in 2015, is due to mutations in the *CACNAIH* gene, which encodes a voltage-gated calcium channel (44). Affected patients all presented with HT by the age of 10.

Much more common are somatic mutations in APAs. Pathogenetic somatic mutations in *KCNJ5* were first reported in 2011 (42). Discoveries of disease-causing somatic mutations in the sodium/potassium ATPase *ATP1A1*, the calcium ATPase *ATP2B3* genes, and in the *CACNA1D* gene, all followed in 2013 (43, 45). Functional characterisations revealed that the mutated variants found in APAs caused elevation of intracellular calcium in adrenocortical cells, followed by aldosterone synthase overexpression and aldosterone overproduction. Recently, somatic mutations in the *CTNNB1*gene encoding β-catenin in the Wnt signaling pathway have been reported both in APAs and in other adrenocortical tumours, but the role of *CTNNB1*mutations in APA pathogenesis is still unclear (46, 47).

Somatic mutations in *KCNJ5* are found in 34-54% of APAs in Caucasians patients, and even more frequently in Asian populations. Patients with *KCNJ5* mutations are more often female, younger and have more florid PA. The tumours are larger than patients without *KCNJ5* mutations, and tend to have higher proportions of clear ZF-like cells. Somatic mutations in *ATP1A1*, *ATP2B3* and *CACNAID* genes are more frequent in males, with small APAs predominantly containing ZG-like cells. Somatic *ATP1A1* gene mutations have been found in 1-6% of APAs, mutations in *ATP2B3* in 1-3% and mutations in the *CACNAID* gene in 1-9%. *CTNNB1*mutations are found in 2-5% of APAs, often in older patients with a shorter duration of HT, and a lower HT cure rate than *KCNJ5* mutation carriers and those without identified mutations (47-57). However, genotype-phenotype correlations have been inconsistent across different studies.

Recently, functional histopathology with immunohistochemistry has uncovered a significant histological complexity. Specific monoclonal antibodies against the aldosterone synthase were first introduced in 2014 (58). APAs may demonstrate intra-tumour heterogeneity with wide variation in functional histopathological features, exhibiting a wide complexity of aldosterone synthase staining and co-expression of various steroidogenic enzymes. In addition, aldosterone synthase-positive micronodules or aldosterone-producing cell clusters (APCC) with strong immunoreactivity for aldosterone synthase are frequently present, and harbour somatic mutations in genes associated with APA, although not *KCNJ5* mutations (59-64). This variation in histopathological features of adenomas and concurrent presence of APCCs has led to the hypothesis that many cases of unilateral PA might represent bilateral asymmetric hyperplasia with steroidogenically active nodules due to somatic mutations (36).

Most patients with IHA are angiotensin II responsive, defined as a rise of plasma aldosterone at least 50% during 2-3 hours of upright posture after overnight recumbency, or during a one hour infusion of angiotensin II. APAs were initially thought exclusively angiotensin II-unresponsive, but later studies have shown that up to 50% of APAs are angiotensin II-responsive. Differences in cellular composition between angiotensin II-responsive and -unresponsive APAs have been found in some, but not all studies. Most consistently, angiotensin II-unresponsive APAs are composed predominantly of ZF-like cells, whereas angiotensin II-responsive APAs contain more ZG-like cells (65-67).

1.6 Clinical manifestations and end-organ damage

The main clinical manifestation of PA is HT. Hypokalaemia, previously considered a major characteristic of PA, was found in only 9-37% in large patient cohorts (26, 27). Higher proportions were reported in retrospective studies (68). Hypokalaemia is most common in APAs (27). Marked hypokalaemia may cause muscle weakness and cramps. The patients may experience polyuria and/or nocturia, which is caused by a

hypokalaemia-induced renal concentration defect (37). Nocturia is also common in normokalaemic patients, possibly due du a rise in ANP levels (10, 69).

In addition to the effects on BP, chronic exposure of aldosterone excess causes deleterious effects in multiple organs, in particular the heart, kidneys and blood vessels.

1.6.1 PA and cardiovascular damage

Several clinical studies conducted in the general population have provided evidence of association between aldosterone levels and cardiac left ventricular (LV) hypertrophy and geometric remodeling. In the Framingham offspring study, the ARR was identified as a key correlate of concentric and eccentric LV hypertrophy (70). Similarly, in a study of patients with at least one risk factor for cardiovascular disease and preserved LV ejection fraction, aldosterone levels were positively correlated with increased LV mass index and wall thickness (71). A direct relationship between aldosterone levels and LV hypertrophy has also been found in several studies of patients with essential HT (72, 73) .

In PA, echocardiographic and applanation tonometry studies have provided evidence of increased LV mass and LV hypertrophy greater than in essential HT. Similarly, increased diastolic dysfunction, endothelial dysfunction, carotid intima-media thickness, arterial wall stiffness, femoral pulse wave velocity and myocardial and carotid artery ultrasonic backscatter signals indicative of fibrosis have been found (74-81). Newly diagnosed PA patients have a marked increase of cardiovascular events, including cerebral stroke, myocardial infarction, atrial fibrillation and heart failure. Cardiovascular mortality is increased compared with matched patients with essential HT (82-88). A recent meta-analysis found no differences in cardiovascular events between patients with bilateral and unilateral PA (87). However, in a large multicenter study from Japan, hypokalaemia, unilateral PA, and a baseline aldosterone level above 350 pmol/L (125 pg/mL) were all associated with the highest risk of cardiovascular comorbidities (88). Thus, the most florid cases of PA experience the most cardiovascular events.

The myocardium comprises several cell populations (Figure 3). Cardiomyocytes occupy 75% of its structural place. In a pressure- and volume-overloaded heart, growth of cardiomyocytes causes left LV hypertrophy. The cardiac interstitium comprises endothelial cells lining the coronary and lymphatic vasculature and endocardium, vascular smooth muscle cells found in epicardial and intramyocardial coronary arteries and arterioles, cardiac fibroblasts, macrophages and mast cells. The fibroblasts are responsible for the synthesis of fibrillary collagens type I and III. Activation of cardiac fibroblast leads to abnormal accumulation of collagen which causes reactive interstitial fibrosis. These processes may lead to increased stiffness and pump dysfunction, first apparent during diastole. This reactive fibrosis differs from the focal replacement fibrosis (cardiac "scarring") caused by cardiomyocyte necrosis (89, 90).

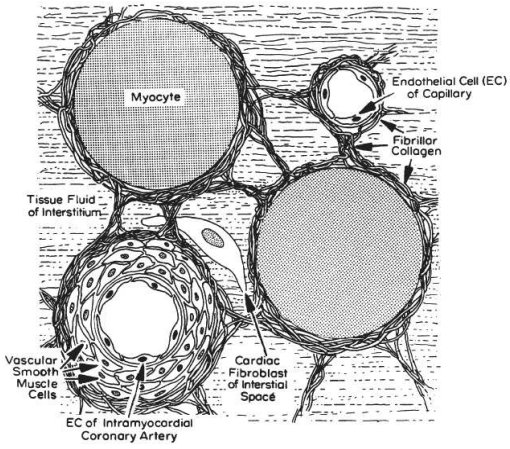

Figure 3 Schematic representation of myocardium and its (cardio)myocyte and nonmyocyte cells and tissue fluid. *Weber and Brilla; Pathological Hypertrophy and Cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system, Circulation 1991; 83:1849-1865. Reprinted with permission.*

The deleterious cardiac effects of aldosterone excess were first demonstrated in a landmark experimental animal study by Christian G Brilla and Karl T Weber in 1992, followed by several other animal studies. In these studies, chronic aldosterone infusion in the setting of a high-salt diet induced oxidative stress and caused myocardial and perivascular inflammation, accumulation of fibrillary collagen and subsequent interstitial and perivascular fibrosis independently of the BP effect (91-94). These effects were attenuated by treatment with MR antagonists.

The cardiac responses to aldosterone are thought to be mediated by activation of the MR, which is present in the cardiomyocyte. However, expression of MR in fibroblasts is controversial, and MR stimulation may promote fibroblast collagen synthesis via paracrine effects (12). In human PA, echocardiographic videodensitometric and ultrasonic backscatter signal analyses have shown alterations in myocardial textures compared with essential HT, suggestive of increased collagen deposition (79-81). The increased frequency of sustained arrhythmias seen in PA, especially atrial fibrillation, is presumably caused by cardiac fibrosis and/or LV hypertrophy (82, 83).

1.6.2 Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging (CMR) is the recommended imaging modality for characterisation of myocardial tissue (95). It provides a highly accurate non-invasive assessment of cardiac and vascular structure and function. In addition, CMR contrast enhancement techniques can detect irreversible myocardial focal fibrosis. The contrast agent gadolinium is prevented from crossing cell membranes due to its large molecular size. Following intravenous bolus administration, gadolinium accumulates in the extracellular space of the myocardium, and slowly disappears as it is cleared from the blood by the kidneys. The T1 relaxation time is a measure of the recovery of the nuclear spin magnetisation after a radiofrequency pulse in the CMR scanner, and is a key source of soft tissue contrast in CMR. Gadolinium shortens T1 relaxation time proportionally to its concentration in the tissue. Expansion of the extracellular space leads to a higher concentration of gadolinium, and will appear bright on inversion-recovery T1-weighted sequence. However, the most commonly used contrast enhancement technique, late gadolinium enhancement, requires a comparison between affected and unaffected myocardium. It is therefore less sensitive in detecting diffuse myocardial fibrosis affecting the whole myocardium, an early form of fibrosis which may be reversible. Several contrast enhancement quantification techniques do exist, but there is no universally accepted method, and sufficient validation for routine clinical use has not been performed (96, 97).

However, newer CMR T1 mapping techniques enable quantitative assessment of tissue composition and the detection of both focal and diffuse myocardial fibrosis, either by native T1 mapping or calculation of extracellular volume (ECV) percentage. ECV after gadolinium administration is used as a surrogate marker of the extracellular space, which is assumed to reflect diffuse myocardial fibrosis (95, 97).

Two CMR studies both applying contrast enhancement techniques revealed results consistent with increased myocardial fibrosis in PA (98, 99). However, only one of those applied a quantitative contrast enhancement method (99). No CMR studies have so far applied T1 mapping in human PA. Thus, the degree of cardiac fibrosis induced by PA in humans has not been extensively studied and is still incompletely known.

1.6.3 PA and renal damage

Excessive aldosterone may also affect renal function. Renal parenchymal damage in renal biopsies of PA patients was first demonstrated in 1977 (100). PA patients demonstrate higher urinary albumin excretion rates and more often microalbuminuria, a marker of early renal involvement and a predictor of cardiovascular risk, than in essential HT. Whether the increased albumin excretion is caused by the haemodynamic load or represents a structural response to aldosterone/salt imbalance involving endothelial dysfunction and glomerular damage is not fully elucidated. The effects of PA on glomerular filtration rates have shown divergent results, with some studies showing glomerular hyperfiltration in untreated PA, while others found lower GFR compared with essential HT (101-104).

1.6.4 PA and effects on glucose metabolism

An association between aldosterone overproduction and abnormal glucose metabolism may also exist. In the Framingham offspring study, higher aldosterone concentrations were associated with future development of the metabolic syndrome (105). Both the metabolic syndrome and diabetes mellitus have been found more prevalent in PA than in essential HT in some, but not all studies (106-109). Possible mechanisms include both aldosterone-mediated impaired first-phase insulin secretion

from the pancreas, and increased insulin resistance caused by excess aldosterone (110-112). Moreover, concurrent glucocorticoid excess may have a role in altering glucose metabolism in PA, actualised by the recent finding that glucocorticoid excess in PA was closely linked with parameters of metabolic risk (113).

1.6.5 PA and other complications

Obstructive sleep apnoea is highly correlated with HT, and particularly treatment resistant HT. A particularly high prevalence of PA has been found in patients with obstructive sleep apnoea (114, 115). Aldosterone may worsen obstructive sleep apnoea by promoting accumulation of fluid within the neck area in the supine position, increasing upper airway resistance. Conversely, intermittent nocturnal hypoxia may active the RAAS (116).

Coincident PA and primary hyperparathyroidism (PHPT) was reported in several studies, with increasing evidence supporting a bidirectional interaction between PTH and aldosterone (117, 118). The MR is expressed in the parathyroid gland, although the mechanisms by which aldosterone stimulates PTH are not exactly known (119). Similarly, PTH receptors are present in the adrenal cortex, with PTH causing release of both aldosterone and cortisol (120). Patients with PHPT have increased risk of HT and cardiovascular disease and patients with PA have lower bone mineral density and higher fracture rates than non-PA, which may be features of this relationship (121-124).

In addition to the target organ effects discussed above, aldosterone excess may also cause psychological effects. The MR is present in the brain, and accumulating evidence suggests that aldosterone may act selectively on the MR in relevant mood-regulating brain areas, without competing with cortisol (125). Additionally, severe HT may in itself adversely affect quality of life (126). PA is associated with depression, anxiety disorders and impaired quality of life, with female PA patients having lower quality of life compared with men (127-130).

1.7 PA and concomitant glucocorticoid excess

Concomitant PA and autonomous cortisol secretion may occur, although thought to be relatively rare (131, 132). If present, it may influence the clinical work-up, treatment and prognosis of PA. Recently, concomitant glucocorticoid hypersecretion in PA was found more frequently than expected, when measured as 24h urinary glucocorticoid output. In that study, the cortisol and total glucocorticoid metabolite excretions in PA were at least as high as in patients with autonomous cortisol secretion. Interestingly, for unclear reasons, few of the PA patients showed a pathological response to the overnight dexamethasone suppression test or increased late-night salivary cortisol, but plasma ACTH was in the low normal range. In addition, several surrogate parameters of metabolic risk correlated with the glucocorticoid output, but not mineralocorticoid output, indicating that glucocorticoid cosecretion contributes to the associated metabolic risk in PA. Glucocorticoid excess may also account for the increased risk of osteoporotic fractures and depression in PA (113).

1.8 Aldosterone and sodium

Dietary sodium intake strongly influences the adverse effects of aldosterone excess, both in experimental and clinical studies. An interaction between sodium and aldosterone was first demonstrated in 1992, when rats given chronic aldosterone infusion developed myocardial inflammation and subsequent fibrosis only in the setting of a concomitant high-sodium diet (91). Similarly, in populations with chronic sodium deficiency, extraordinarily high aldosterone levels are present but not associated with BP or cardiovascular damage (133). In patients with essential HT, a significant relationship between LV mass and daily urinary sodium was found both before and after medical treatment that inhibited the RAAS (134). In human PA a high-sodium diet is associated with both greater LV mass and worsened proteinuria. Reduction in LV mass after specific PA treatment is greater when dietary sodium intake is reduced (135-137).

1.9 Diagnostic workup

Clinical guidelines for case detection, diagnosis and treatment of PA were published by the Endocrine Society in 2008, and updated in 2016 (138, 139). The diagnostic workup comprises three phases: screening (case detection), confirmatory testing and subtype differentiation (Figure 4).

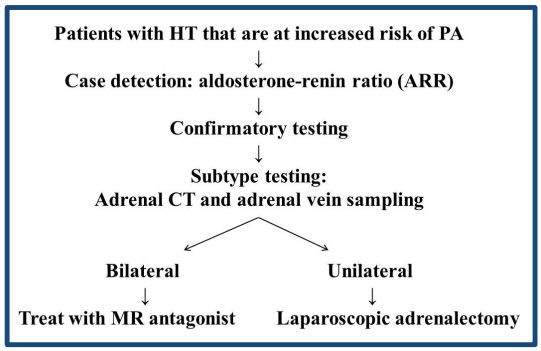

Figure 4 Algorithm for the detection, confirmation, subtype testing and treatment of PA. *Modified from Funder et al: The Management of Primary Aldosteronism: Case Detection, Diagnosis and Treatment: An Endocrine Society Clinical Practice Guideline, JCEM 2016, 101(5): 1889-1916.*

1.9.1 Screening

Screening is recommended in the patient groups shown in Figure 5, all considered to have increased risk of PA.

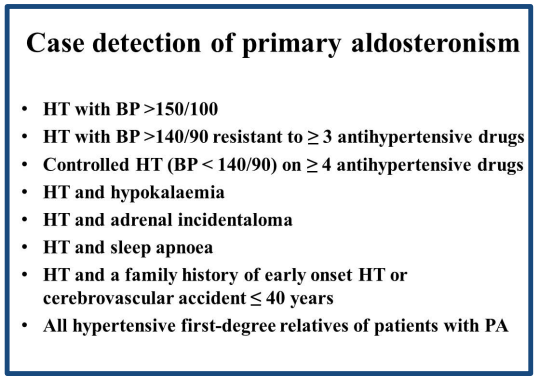

Figure 5 Patient groups in which case detection for PA is recommended. *Modified from Funder et al: The Management of Primary Aldosteronism: Case Detection, Diagnosis and Treatment: An Endocrine Society Clinical Practice Guideline, JCEM 2016, 101(5): 1889-1916*

The ARR is recommended as the preferred screening test, as it is more sensitive for detection of PA than both potassium and aldosterone levels, and more specific than isolated renin measurements (25, 140). Both aldosterone and renin are affected both by posture and time of day. ARR testing should be performed in the morning,

minimum with two hours out of bed, and then seated for 5-15 minutes. If possible, antihypertensive medication interfering on the RAAS system and non-steroidal anti-inflammatory drugs (NSAIDs) should be withdrawn before testing, and replaced with non-interfering medication, as recommended in the guidelines (Table 1) (139). Products derived from licorice root that inhibit the enzyme 11β-HSD2, should additionally be withdrawn for at least four weeks. Verapamil slow-release and α-adrenergic blockers have minimal effects on the ARR and can be used to control BP in this period. Recently, moxonidine was shown not to interfere significantly with the ARR, and may therefore also be used during screening (141). Potassium status is important: hypokalaemia suppresses aldosterone and must be replenished. Sodium intake should be unrestricted during testing. Other factors that may influence the ARR are estrogen-containing medication, gender, menstrual cycle phase, selective serotonin reuptake inhibitor antidepressants, renal failure and old age (142-146) .

Medications	Effect on renin levels	Effect on aldosterone levels	Effect on ARR	Recommended period of withdrawal
β-adrenergic blockers	↓↓	↓	↑	2 weeks
Central α-agonists	↓↓	↓	↑	2 weeks
Potassium-wasting diuretics	↑↑	→↑	↓	4 weeks
Potassium-sparing diuretics (MR antagonists, amiloride)	↑↑	↑	↓	4 weeks
ACE inhibitors	↑↑	↓	↓	2 weeks
Angiotensin II receptor blockers	↑↑	↓	↓	2 weeks
Calcium channel antagonists (dihydropyridines)	↑	→↓	↓	2 weeks
Renin inhibitors	↓↑*	↓	↑↓*	2 weeks
NSAIDs	↓↓	↓	↑	2 weeks

Table 1 Medication that may lead to false-positive or false- negative ARR results, and recommended period of withdrawal. **Renin inhibitors lower plasma renin-activity (PRA), but increase direct renin-concentration (DRC). ARR increases when PRA is used, and is lowered when DRC is used. Modified from Funder et al: The Management of Primary Aldosteronism: Case Detection, Diagnosis and Treatment: An Endocrine Society Clinical Practice Guideline, JCEM 2016, 101(5): 1889-1916*

Cut-off values for positive ARR depend on the assay. Renin may be measured as direct renin concentration (DRC), or as the intrinsic angiotensin I formation capacity of plasma renin, termed plasma renin activity (PRA). Accurate and highly reproducible assays of both renin and aldosterone are essential for reliable ARR results and all further stages of the diagnostic workup. Concern has been raised about the reliability and reproducibility of current available automated chemiluminescence immunoassays for aldosterone and DRC, which have replaced the more laborious aldosterone and PRA radioimmunoassays (10, 147). The development of new, highly reliable liquid chromatography tandem mass spectrometry (LC-MS/MS) methodology for aldosterone and recently for PRA represents a major step forward (148, 149). Furthermore, methods to measure aldosterone and renin in the same sample to simplify the screening procedure are in development (139).

1.9.2 Confirmatory testing

When ARR is positive, a confirmatory test should be performed to ascertain or exclude a diagnosis of PA. A confirmatory test should, if PA is present, demonstrate evidence of ongoing aldosterone production in the face of suppressed renin. Four different procedures are in use: saline infusion test (SIT), fludrocortisone suppression test, captopril challenge test and oral sodium loading. There is insufficient evidence to recommend one test over the others (150, 151). In Norway and in Europe SIT is mostly used. Here, two liters of 0.9% saline is infused intravenously over four hours, with the patient recumbent from one hour before and during the whole test. Under normal circumstances the saline volume load should suppress the RAAS. Inadequately suppressed post-infusion aldosterone value confirms PA. Again, medication interfering on the RAAS-system should, if considered safe, be withdrawn and exchanged with non-interfering medication (Figure 1). The optimal cut-off value for a positive SIT post-infusion aldosterone level, representing the best trade-off between sensitivity and specificity is still debatable, but 140 pmol/L is commonly used (139, 152, 153). Two additional modified confirmatory tests were recently proposed; a dexamethasone-enhanced fludrocortisone suppression test, eliminating the effect of stress-induced ACTH on aldosterone secretion, found a very high

prevalence of PA of 31% (154). Similarly, a seated SIT was shown to be more sensitive than the recumbent SIT (155).

In the setting of spontaneous hypokalaemia, plasma renin below detection level and aldosterone levels above 550 pmol/L no further confirmatory testing is needed (139).

1.9.3 Subtype testing

When PA is confirmed, all patients should undergo adrenal computed tomography (CT) to exclude adrenal carcinoma and to assist the interventional radiologist and surgeon in the event of future adrenal surgery. However, for subtype evaluation adrenal CT has several limitations. The visualised adenomas may be non-secreting, and small secretory adenomas may be overseen or misinterpreted as nodular hyperplasia, or nodular hyperplasia may be misinterpreted as several small APAs. Several studies have shown low sensitivity and specificity of CT when compared with adrenal vein sampling (AVS, see below); concordance rates between CT and AVS vary from 40% to 79% (156-159). Therefore, AVS is recommended for subtype differentiation in all patients where surgery is feasible and desired, to determine if the patient has unilateral PA that will benefit from unilateral adrenalectomy. Only patients younger than 35 years with spontaneous hypokalaemia, marked aldosterone excess (aldosterone above 831 pmol/L) and with adrenal CT showing a solitary unilateral adenoma, may proceed directly to unilateral adrenalectomy without AVS (139).

During AVS (Figure 6), both adrenal veins are approached by percutaneous catheterisation of the femoral vein. Blood is then obtained from both the adrenal veins and from the inferior vena cava, and analysed for concentrations of cortisol and aldosterone. The procedure may be performed with stimulation from the synthetic ACTH derivate cosyntropin (Synacthen®). Cosyntropin is given continuously or as bolus, to maximise cortisol secretion from both adrenals, to minimise pulsatile or stress-induced variations in the secretions of cortisol and aldosterone, and to increase aldosterone secretion from an APA (160). If performed without consyntropin, both adrenals should be sampled simultaneously and preferably in the morning.

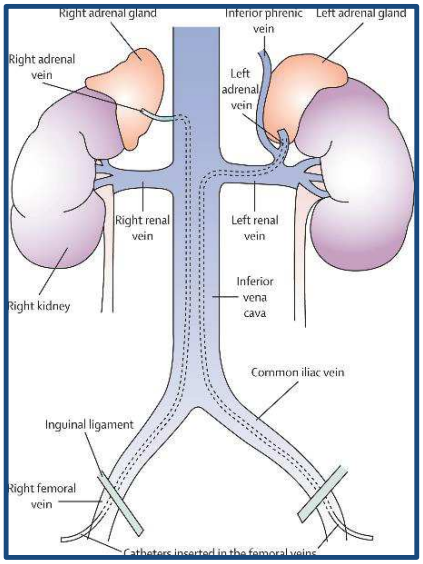

Figure 6 Schematic drawing of the AVS catheterisation procedure of the left and right adrenal veins, with catheters introduced via the femoral vein. *Monticone et.al: Adrenal vein sampling in primary aldosteronism: towards a standardised protocol, the Lancet Diabetes & Endocrinology, volume 3, no 4, p 296-303, April 2015. Reprinted with permission.*

Adrenal vein samples are often obtained from near the orifice of the adrenal vein, due to difficulties in placing the catheter tip within the small adrenal veins. Dilution from non-adrenal vein blood may then occur. To correct for such dilution and to secure the adequacy of the cannulation, cortisol is measured simultaneously with aldosterone. Adrenal vein cannulation is normally considered successful if the selectivity index (SI), i.e. the cortisol gradient of the ratio adrenal vein/inferior vein is above 3-5 with cosyntropin, or above 2-3 without cosyntropin. The lateralisation index (LI) is defined as the aldosterone/cortisol ratio in one gland compared with the aldosterone/cortisol-ratio in the opposite gland. With cosyntropin, a LI above 4 is considered to represent lateralisation to one adrenal gland. LI between 3-4 represents an overlap zone, whereas a LI below 3 is consistent with bilateral aldosterone secretion. Without cosyntropin, a LI above 2-4 is recommended as cut-off for lateralisation. No consensus exists about the optimal cosyntropin stimulation protocol, and there is no conclusive evidence to recommend cosyntropin stimulation to unstimulated AVS (160-163). Moreover, recent studies have shown 9-28% disconcordance for lateralisation results when comparing unstimulated with stimulated results during the same AVS procedures (164-166). Some centres also interpret contralateral suppression, defined as aldosterone/cortisol ratio from the non-dominant adrenal gland lower than the peripheral aldosterone/cortisol ratio, as an

additional criterion for lateralisation. However, no outcome studies show unequivocally that contralateral suppression is a useful diagnostic criterion (164, 167, 168).

For unstimulated AVS, where the SI may be falsely low, plasma metanephrine, 11-deoxycortisol, androstenedione and 17-α-hydroxyprogesterone have all been suggested as better indicators than cortisol, if cortisol fails to confirm bilateral successful cannulation (169-171).

As for screening and diagnostic testing, withdrawal and replacement of antihypertensive agents interfering with the RAAS should also be performed before AVS (Table 1). However, in patients with treatment-resistant HT withdrawal of several hypertensive agents may not be medically opportune, and angiotensin II-blockers and ACE-inhibitors may then be used as long as renin is suppressed. With low or suppressed renin-levels, it is considered unlikely that these medications will stimulate a non-dominant contralateral gland sufficiently to confuse the interpretation of the AVS (160). In patients with uncontrolled hypokalaemia and uncontrolled HT, even MR antagonists may be continued during AVS as long as renin values remain low, without obscuring lateralisation results (172).

AVS is a technically challenging procedure, especially for the right adrenal vein, which is short, small in calibre, may have an angulated path and normally drains directly into the inferior vena cava in an acute angle. This makes it difficult both to identify, cannulate and withdraw blood samples from (173). The success rates of the procedure differ substantially between centres, and increase with the experience of the interventional radiologist and by implementing standard operating procedures to increase accuracy (156, 174). The introduction of a rapid intraprocedural cortisol assays was first reported in 2000, with the first AVS study applying a intraprocedural cortisol assay in patients with PA published in 2007 (175, 176). Here, after collecting samples from the putative left and right adrenal vein and from the inferior vena cava, blood samples are immediately analysed with the intraprocedural cortisol assay, either in the laboratory or at point-of-care in the immediate vicinity of the radiologist

operating suite. While the analyses are being performed, the catheters are removed from the patient, but the catheter sheaths are left in place. The results from the intraprocedural cortisol assay ensure feedback within 20-60 minutes of whether the adrenal cannulation was successful or not, allowing for immediate resampling of improperly collected adrenal samples. A few studies have followed, all showing significantly increased AVS success rates when applying intraprocedural cortisol assays (177-180). However, all the studies used different AVS study protocols, and only in one of those studies cortisol was analysed using a point-of-care instrument (175).

Segmental AVS, in which blood is collected from the intra-adrenal tributaries of the adrenal vein instead of from the central adrenal vein, may provide more precise localisation of both the intra-adrenal source of aldosterone oversecretion and of unaffected adrenal segments. Thus, segmental AVS may be useful in identifying patients eligible for adrenal sparing surgery, but requires a highly experienced radiologist and is more expensive than standard AVS (181).

In the event of failed cannulation of an adrenal vein, a recent study found that if the aldosterone/cortisol-ratio from the successfully cannulated adrenal vein divided by the aldosterone/cortisol ratio from the peripheral vein was below 0.5, this identified patients with contralateral unilateral disease (182).

Complication rate after AVS is low; at centres with experienced radiologists less than 2.5% (139). In a large study, the rate of adrenal vein rupture was 0.61% (162). Adrenal vein hemorrhage may subsequently occur, but normally does not affect adrenal function (183).

As mentioned above, concomitant glucocorticoid secretion may occur in PA (113, 132). In such cases, concomitant aldosterone and cortisol secretion from an APA could confound both confirmation of successful cannulation and interpretation of AVS by masking lateralisation. Plasma metanephrine has been suggested to yield correct lateralisation ratios when cortisol co-secretion is suspected (184).

Even though AVS is considered the gold standard to distinguish between unilateral and bilateral forms of PA, it has several limitations, as discussed above. It is a technical challenging, invasive procedure which demands dedicated experienced radiologists, success rates vary highly, and it has high costs and is not widely available in all centres. Furthermore, different protocols are in use at different centers, i.e. use of bolus or continuous cosyntropin or none, applying sequential or simultaneous cannulations, and criteria for defining successful cannulation and lateralisation (139). The stringency of criteria used has been shown to greatly impact the reproducibility of AVS results, and different criteria translate into heterogeneous classification that influences management decisions (185, 186). In 2016, a large prospective randomised multicentre study (the SPARTACUS trial) compared AVS with CT-determined treatment outcome. This study showed no significant differences in intensity of antihypertensive medication or health-related quality of life after one year, challenging the current recommendations that AVS should be performed in all PA patients (187). The results from the SPARTACUS trial were met with broad interest, but have also triggered extensive discussions and controversies (188).

^{11}C-metomidate is a positron emission tomography (PET) radiotracer which acts as a potent inhibitor of adrenal steroidogenic enzymes. It is selectively accumulated in APAs, and ^{11}C-metomidate PET-CT has been shown as a sensitive and specific non-invasive alternative to AVS for subtype differentiation of PA. Its use is limited by the short half-life of ^{11}C-metomidate which requires PET centres with access to an on-site cyclotron (189, 190).

The potential roles of serum and urine measurements of the aldosterone precursor 18-hydroxycorticosterone (Figure 1), and the hybrid steroids 18-hydroxycortisol and 18-oxocortisol, have also been evaluated in the diagnosis and subtyping of PA. Levels of 18-hydroxycortisol and 18-oxocortisol are extremely low in healthy individuals, but are greatly elevated and particularly valuable in the diagnosis of GRA (191). Several studies have shown that these steroid assays are also elevated in non-familiar PA, in particular in APAs, and may therefore contribute in both diagnostic workup and subtype differentiation of PA (192-197).

1.10 Treatment of PA

PA can be targeted with specific treatments and is potentially curable in unilateral cases. Unilateral laparoscopic adrenalectomy is the recommended treatment in patients with unilateral PA. Lifelong medical treatment with MR antagonists is recommended in bilateral disease or in unilateral disease if surgery is not feasible or desired (139).

1.10.1 Surgical treatment

Laparoscopic surgery is the gold standard for adrenal surgery due to its low morbidity and short hospital stay (198, 199). It can be performed with transperitoneal or retroperitoneal approach, and total adrenalectomy is normally performed (Figure 7). Partial adrenalectomy is an alternative. However, a patient with unilateral PA may have multiple adjacent nodules, with unknown source of aldosterone oversecretion, and HT may therefore persist after partial adrenalectomy (200). The mean operative time was 2-3 hours in a series of high-volume referral centres, with a 3.4% conversion rate to open surgery, and an overall mortality rate of 0.4% (201) . In a systematic review, the mean complication rate was 4.7% (202). Bleeding is the most frequent severe complication of laparoscopic adrenalectomy. Patients are observed in hospital for about three days, with BP and potassium levels monitored. Potassium supplements and MR antagonists are withdrawn immediately postoperatively, other antihypertensive therapy is reduced when appropriate. A generous sodium diet during the first weeks after surgery should be recommended, to avoid hyperkalaemia that may develop from hypoaldosteronism due to chronic suppression of the contralateral adrenal gland (139, 203). A recent consensus document recommends that a post-surgical assessment of BP and potassium level should be performed within the first three months, whereas final outcome including BP, potassium, aldosterone and renin levels should be assessed 6-12 months post-adrenalectomy and reassessed yearly (204).

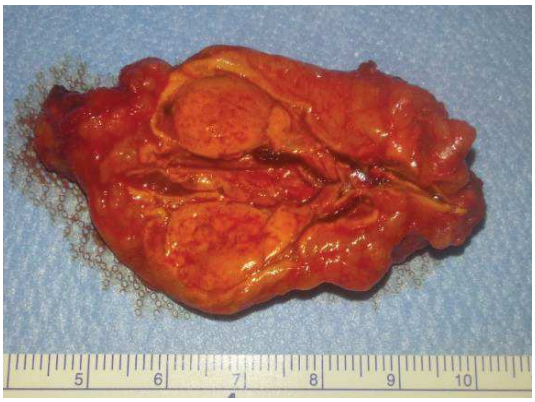

Figure 7 Resected adrenal with a single adenoma from a 57-year old female PA patient with AVS-lateralisation (courtesy of endocrine surgeon Anette Heie).

The published cure rates of HT after unilateral adrenalectomy vary widely, from 20-70% of PA patients in different reports, albeit with significant improvement of HT and resolution of hypokalaemia in the remainder (157, 202, 204-211). Factors associated with cure of HT are lack of family history of HT, preoperative use of only two or fewer antihypertensive drugs, short duration of HT, high preoperative urinary aldosterone levels, young age, female sex and no sign of LV hypertrophy (204, 209, 210, 212-214). Histopathological examinations of resected adrenals show a single adenoma in 43-77% of the cases, and multinodular or diffuse hyperplasia in the remaining. Some, but not all studies have found an association between a histopathological single adenoma and cure of HT (62, 210, 215, 216).

Development of postoperative hypoaldosteronism with hyperkalaemia due to chronic ZG suppression in the remaining adrenal gland has been reported in 5% of adrenalectomised PA patients, and may require long-term fludrocortisone replacement treatment (203). In the study described above, where increased urinary cortisol output in PA was found, 29% of the patients demonstrated an inappropriately low response to cosyntropin stimulation post-adrenalectomy, indicative of a compromised cortisol reserve. Thus, adrenal insufficiency postoperatively may occur more frequently than previously thought (113).

Biochemical cure after adrenalectomy is indicated by resolution of hypokalaemia and normalisation of the ARR. A post-adrenalectomy confirmatory test may also be

performed, at least in the event of persistent elevated ARR. Biochemical cure rates vary in the range 83-100% (204).

1.10.2 Medical treatment

In patients with bilateral PA, lifelong treatment with a MR antagonist that inhibits aldosterone-induced activation of the MR, is recommended. Spironolactone, developed in the late 1950s, is the drug of choice, with a recommended starting dose at 12.5 to 25 mg daily in a single dose, and a maximum dose 100 mg per day (139, 217-219). However, spironolactone is an unselective MR antagonist that also binds to the androgen and progesterone receptors, where it acts as an androgen antagonist and progesterone agonist. It may therefore cause adverse effects such as impotence, gynaecomastia, mastodynia and menstrual abnormalities (220). Eplerenone, released in 1987, is a more selective MR antagonist with fewer side effects (221). It has only 50% of the MR antagonist potency of spironolactone and a shorter half-life, and should be given twice daily, with a recommended starting dose at 25 mg x 2. Both spironolactone and eplerenone efficiently reduce BP in PA, but spironolactone may have an antihypertensive effect superior to eplerenone (220).

Agents acting by blocking the epithelial sodium channels of the distal tubuli (e.g. amiloride) are also beneficial in reducing HT and ameliorating hypokalaemia in PA, but are not recommended as monotherapy as they do not prevent the deleterious effects of aldosterone excess in other organs.

Many PA patients require supplemental antihypertensive agents used in combination with MR antagonists to lower BP adequately. Little evidence exists to favour one class of agents over other, but ACE inhibitors, angiotensin II receptor blockers, calcium channel antagonists and thiazide diuretics are all commonly used in PA (10, 139).

Spontaneous remission of IHA after long-term medical MR antagonist treatment has been reported (222, 223). It has been postulated that this remission may be caused by

reduced vascular and adrenal sensitivity to angiotensin II, or that spironolactone may exert a direct inhibitory action on adrenal steroidogenesis (224).

In carefully selected patients with bilateral PA, unilateral adrenalectomy may be beneficial if medical therapy has failed because of intolerance or inadequate BP response (225).

1.10.3 Sodium restriction

In patients with bilateral PA, due to the deleterious combined effects of high aldosterone levels and an inappropriate sodium status, dietary sodium restriction should be recommended (15).

1.10.4 New approaches to medical treatment

A new class of non-steroidal MR antagonists was recently discovered, which exhibits similar in vitro potency to spironolactone, but without effects on androgen and progesterone receptors (226, 227). These drugs, termed third-generation MR antagonists, are now tested on other patient groups, and may be available for use in PA in a few years. Another attractive option would be drugs that could inhibit aldosterone synthase. The current recommended treatment for PA with MR antagonists activates the RAAS, which leads to high levels of aldosterone that may overcome the MR blockade, requiring escalation of doses. In addition, increased aldosterone levels may be responsible for non-genomic aldosterone effects (228, 229). Therefore, selective aldosterone synthase inhibitors are eagerly anticipated.

1.11 Effects of treatment on organ damage

In addition to the antihypertensive effect seen both after adrenalectomy and after medical treatment with MR antagonists, specific PA treatment also causes beneficial effects on cardiovascular and renal outcomes.

1.11.1 Cardiovascular outcomes

Spironolactone treatment was first shown to reduce both morbidity and mortality when added to standard therapy among patients with severe heart failure in the large RALES trial in 1999. The EPHESUS trial in 2001 showed a similar beneficial effect on morbidity and mortality with the addition of eplerenone to optimal medical therapy in patients with acute myocardial infarction and heart failure (230, 231). In PA, several echocardiographic long-term follow-up studies have shown significant decrease in LV mass following both adrenalectomy and MR antagonist treatment. The reduction in LV mass seems to occur earlier after adrenalectomy than in those treated medically (75, 207). However, in a CMR study of PA patients treated with MR antagonists, significant decreases in both LV mass and LV end-diastolic volumes were found already after three months (232). A meta-analysis of echocardiographic studies found that the reduction in LV mass was similar after adrenalectomy as after medical MR antagonist treatment, although BP reduction was greater after adrenalectomy (233). Furthermore, the increased risk of cardiovascular events found in PA at diagnosis, aligns with that of essential HT at long-term follow-up after specific PA treatment (83, 207). However, a recent large retrospective longitudinal study found that PA patients treated with MR antagonists had higher incidence of cardiovascular events and death than patients with essential HT (234). The higher risk appeared to be limited to PA patients whose renin activity levels remained suppressed on MR antagonist therapy. Achieving non-suppressed renin levels, either by increasing MR antagonist dose or by lowering sodium intake (235), may therefore be a treatment target to reduce cardiovascular risk. In contrast, patients with surgical treated PA had lower risk of incident cardiovascular events than in essential HT. To our knowledge, no CMR studies have been published evaluating cardiac fibrosis after treatment of PA.

In low-renin HT, MR antagonist treatment has been shown to be equally effective as in PA, both in reducing BP and LV hypertrophy (236).

1.11.2 Renal outcomes

Improvement of albuminuria is also seen after specific PA treatment, but also a fall in glomerular filtration rate (GFR). These findings indicate that renal dysfunction in PA is characterised by reversible glomerular hyperfiltration, with elevated albuminuria being a marker of a dynamic rather than structural renal defect (102, 103).

1.11.3Metabolic outcomes

Data on effects on glucose metabolism after PA treatment are sparse. In one study, insulin resistance was restored to normal after adrenalectomy and MR antagonist treatment. In another study, first phase insulin reaction in intravenously glucose tolerance test increased significantly after adrenalectomy (110, 237). In a recent large national health insurance database study, PA patients who underwent adrenalectomy had a reduced risk of diabetes mellitus compared with essential HT, whereas PA patients treated with MR antagonists were at increased risk. This difference may be caused by high circulating levels of aldosterone in MR antagonist treated patients, causing non-genomic deleterious effects including insulin resistance (238). However, it has also been postulated that concurrent glucocorticoid excess found in PA, removed by adrenalectomy but remaining unopposed by MR antagonist treatment, may be the main cause of increased metabolic risk in the latter group (239). The same hypothesis may apply to the long-term increased rate of osteoporotic fracture risk found in female PA patients treated with MR antagonists, but not after adrenalectomy (124).

1.11.4 Effect of treatment on quality of life

Quality of life improves after specific PA treatment (128, 129). In a recent prospective study, a larger improvement in quality of life was seen after adrenalectomy than in patients treated medically with MR antagonists (130).

1.11.5 Effect of treatment on mortality

Recently, reduced long-term all-cause mortality after adrenalectomy was demonstrated, as compared with patients treated with MR antagonists and patients with essential HT. For MR antagonist treated PA, a defined daily dose of 12.5-50 mg spironolactone was associated with lower mortality compared with lower or higher doses (238, 240).

1.12 Summary

To summarise, based on epidemiological studies, PA is still grossly underdiagnosed in most countries, probably also in Norway (29, 31). However, no studies of PA in Norway had been published at the start of this PhD work in 2013. Based on evidence described above, both surgery and medical treatment with MR antagonists efficiently decrease the excess organ complications found in untreated PA. However, recent evidence suggests superior effect of adrenalectomy compared with MR antagonist treatment both on BP reduction, diabetes, fracture risk, quality of life and mortality. These findings emphasise the need for correct diagnosis and subtyping. AVS is the gold-standard for subtyping and treatment decision, but is a technical challenging procedure with varying success rates without the aid of intraprocedural cortisol assays. The increased cardiovascular risk in untreated PA may partly be due to myocardial fibrosis. CMR is the recommended imaging modality for myocardial tissue characterisation, and new CMR T1 mapping techniques enable quantitative assessment of diffuse myocardial fibrosis. However, to our knowledge, no studies applying CMR T1 mapping techniques have been performed in human PA, and the degree of myocardial fibrosis in PA is still incompletely known.

The last literature search was performed February 12th 2018.

2 Aims

Overall objective

- To determine the clinical and biochemical characteristics of the PA population in Western Norway, identify the optimal diagnostic procedures and evaluate the long-term treatment outcome after surgical and medical treatment.

Specific aims

- Describe the epidemiology and patient characteristics, diagnostic approach, somatic mutational status and long-term treatment-outcome of PA in Western Norway **(paper I).**
- Determine the success rate of AVS using intraprocedural point-of-care cortisol analysis **(paper II).**
- Apply cardiac magnetic resonance imaging to assess myocardial mass, function and fibrosis in PA patients before and after specific PA treatment **(paper III).**

3 Materials and methods

The work is based on three clinical studies all conducted at the tertiary centre Haukeland University Hospital in Bergen, Norway. Haukeland University Hospital is one of only two university centres in Norway that perform AVS. It therefore serves as a tertiary referral centre for all PA patients admitted for subtype testing from the Western and Middle parts of Norway, and to some extent also for the Northern parts of the country.

3.1 Study design and patients

Paper I

In this study, all patients who had been investigated for PA during the period 1998-2012 at Haukeland University Hospital were retrospectively evaluated. AVS was introduced at Haukeland University Hospital in 1998, representing the starting point of recommended subtyping of our PA patients. Inclusion criteria were verified PA after confirmatory testing, or highly likely PA including elevated ARR and severe or treatment resistant HT with or without hypokalaemia. Clinical characteristics, diagnostic work-up and treatment were retrieved from patient records. Adrenal imaging and histological slides of adrenal tissue were reevaluated, and analysed for somatic mutations in genes known to cause PA. All patients still alive by August 2014 were invited to a follow-up visit at our out-patient clinic, for evaluation of clinical and biochemical outcome.

Paper II

Here we compared AVS results performed with and without the use of intraprocedural point-of-care cortisol assay. Twenty-one consecutive patients with confirmed or a strong suspicion of PA who were planned for AVS were included. Their AVS were performed in the period February-April 2013, applying intraprocedural point-of-care cortisol assay during the AVS procedure. Interfering antihypertensive medication was changed 2-4 weeks before the procedure, according

to guidelines, if considered safe. The previous 21 AVS procedures without the use of intraprocedural cortisol assay were used as controls.

Paper III

This study assessed cardiac mass and fibrosis in PA before and after specific PA treatment, compared with normotensive healthy subjects, applying CMR. In the period October 2013-June 2014, fifteen patients with newly confirmed PA (PA1) and 24 age-and sex-matched healthy subjects were included for baseline CMR examinations, performed with dynamic contrast enhancement (CMR1-C1). Then, at least one year after start of specific treatment, both the PA1 group and the healthy subjects were invited to a follow-up CMR with T1 mapping technology (CMR2-T1), which had replaced the original CMR scanner used at baseline. In order to obtain T1 mapping also from a group of newly diagnosed PA, an additional 17 patients with newly confirmed PA (PA2) were recruited, of whom 16 were included in the study. The CMR2-T1 sub-study was performed between June 2015 and March 2016. A study flow chart for the different study groups participating in CMR1-CI and CMR2-T1 is shown in Figure 7.

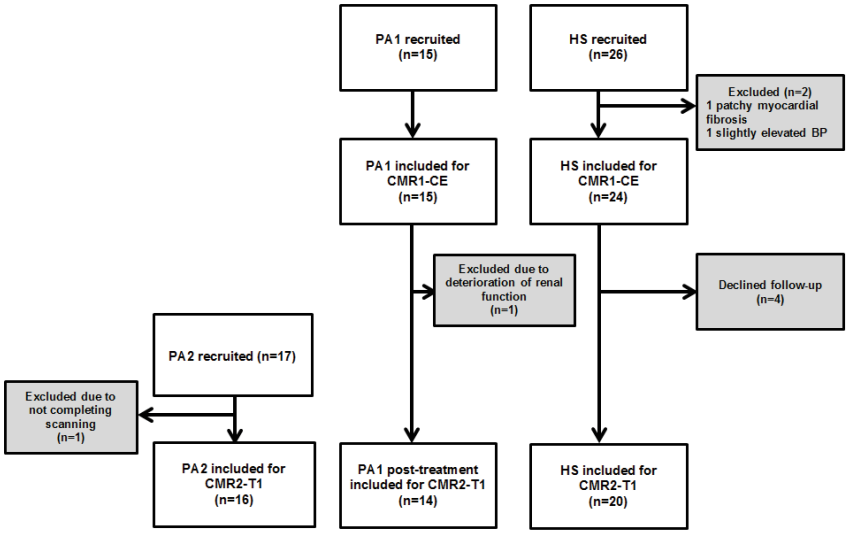

Figure 7 Study flow chart for the three study groups in Paper III; PA1, HS and PA2. *Abbreviations: PA1, primary aldosteronism group 1; HS, healthy subjects; PA2, primary aldosteronism group 2; CMR1-CE, cardiac magnetic resonance imaging with dynamic contrast enhancement; CMR2-T1, cardiac magnetic resonance imaging with T1 mapping*

3.2 Laboratory assays for aldosterone, renin and cortisol

Hormone measurements for case detection and diagnostic testing in regional PA patients, and all hormone measurements performed during AVS procedures, were analysed at the Hormone Laboratory of Haukeland University Hospital. The assays for both aldosterone and renin were changed during the study period of **Paper III,** with replacement of plasma renin activity (PRA) with direct renin concentration (DRC)**.**

From the1990s until December 2014, plasma aldosterone was measured using the radioimmunoassay Coat-a-Count® Aldosterone (Siemens) **(Paper I, II** and **III).** The aldosterone assay was in December 2014 replaced with the chemiluminescent immunoassay LIAISON® Aldosterone (DiaSorin) **(Paper III).** Aldosterone was measured as pmol/L with both methods. The normal reference range was modified from 110-860 pmol/L in the first assay, to 70-1086 pmol/L in the new assay (upright position).

From the 1990s until February 2016, renin was analysed as PRA, using the radioimmunoassay Gammacoat® (DiaSorin), given as µg/L/t **(Paper I, II** and **III).** From February 2016 onwards, the assay was replaced with the chemiluminescent immunoassay LIAISON® Direct Renin (DiaSorin) measuring DRC, given as mIE/L **(Paper III).**

For the ARR, a value above 750 pmol/µg/h was considered elevated with renin measured as PRA (**Paper I, II** and **III**) (138). For ARR calculated with DRC, the cut-off value depended on whether aldosterone was below or above 300 pmol/L. With aldosterone below 300 pmol/L an ARR above 50 pmol/mIE was considered elevated, whereas for aldosterone above 300 pmol/L, the cut-off for elevated ARR was set at 35 pmol/mIE **(Paper III).**

Cortisol was until February 2003 measured using AutoDELFIA® automatic immunoassay system (PerkinElmer life sciences) **(Paper I).** From February 2003 until May 2013 cortisol was analysed using the automated immunoassay IMMULITE

2000 (Siemens) **(Paper I and II).** For Paper II, cortisol was also analysed with an inhouse LS-MS/MS assay. From May 2013 onwards, cortisol was measured using this LS-MS/MS assay **(Paper III).**

Intraprocedural cortisol **(Paper II)** was analysed at point-of-care using the AIA-360 Cort-pac system (TOSOH Bioscence). Lithium/heparin tubes were centrifuged immediately, and the samples were then analyses for cortisol undiluted and at 1/20 and 1/39 dilution. The first results were available 20 minutes after the samples were loaded into the analyser, with further samples analysed every 1.5 minutes. With sample centrifugation and dilution included, results were available 25-30 minutes after the samples were obtained.

3.3 Confirmatory testing

Recumbent saline infusion test (SIT) was the confirmatory test in use at Haukeland University Hospital. The patients stayed recumbent for one hour before and during the infusion of two liters of 0.9% saline given intravenously over four hours. Blood samples were drawn before and at the end of the infusion. A positive SIT was defined as a post-infusion plasma aldosterone level above140 pmol/L (**Paper I, II** and **III**) (138, 152).

In cases where patients were admitted from other Norwegian hospitals with the captopril challenge test used as confirmatory test, an aldosterone suppression of less than 30% of baseline values was considered positive **(Paper I)** (138).

3.4 Adrenal vein sampling

All AVS procedures **(Paper I, II** and **III)** were performed within the Department of Radiology at Haukeland University Hospital, and were performed with sequential cannulation of both adrenal veins during continuous cosyntropin (Synacthen®) infusion 50 µg/h, by dedicated radiologists. Samples were collected from the putative left and right adrenal vein and from the inferior vena cava.

For **Paper II,** blood samples were immediately analysed with the intraprocedural point-of-care cortisol assay, situated in the room next to the radiologist operating suite. While the analyses were performed, the patient remained in the radiology suite, and the catheter sheaths were left in place until the results of the intraprocedural cortisol assay were available. If these results indicated incorrect catheter position from one or both adrenals, a new attempt of cannulation of the adrenal vein was performed, with repeated samples drawn.

The cut-off for correct catheter position (selectivity index) was defined as a ratio of adrenal vein cortisol to peripheral cortisol level above 5. A lateralisation index (aldosterone/cortisol-ratio from dominant to contralateral adrenal vein) above 4 was defined as cut-off for unilateral PA (156, 161).

3.5 Adrenal imaging

For **Paper I**, all CT and MRI examinations were re-evaluated by an experienced radiologist. Axial scans (3 mm) were used for evaluation of both modalities. Normal adrenals were classified as a limb junction less than 8 mm and both limbs less than 5 mm. Hyperplasia was defined as the junction and limbs equal to or exceeding 8 and 5 mm. Macronodular hyperplasia was defined as hyperplasia with one or more nodules, and a tumour was defined as a focal lesion exceeding 10 mm in diameter (241).

3.6 Histopathology and genetic analysis

In order to obtain a uniform histopathological evaluation, all available original histological slides and biopsy reports for **Paper I** were reviewed by one pathologist. Microscopically, the specimens were classified as histopathological normal tissue, adenoma(s), hyperplasia with or without macronodules, or uncertain.

For molecular genetic analysis, one block from each nodular lesion or from the most nodular area, and an additional block from normal adrenal tissue were selected for molecular genetic analysis. From 2009 onwards, tissue samples for genetic analysis

were collected directly after adrenalectomy from both macroscopic tumour tissue or, when no tumour was present, from the most thickened area of the adrenal cortex, and from macroscopically unaffected adrenal gland, and stored at -80°.

Genomic DNA was extracted from shaves of archived formalin-fixed paraffin-embedded tissue, or isolated from fresh tumour and non-tumour tissue, using QIAamp DNA FFPE Tissue Kit, according to the manufacturer`s instruction. DNA regions where mutations in the genes *KCNJ5*, *ATP1A1*, *ATP2B3*, *CACNAID* and *CTNNB1* had previously been identified were amplified by PCR using gene-specific primers. No patients were tested for germline mutations.

3.7 Clinical follow-up

All patients included in **Paper I** who were still alive by 2014, were invited to a clinical follow-up at the outpatient clinic of Haukeland University Hospital to assess clinical and biochemical outcome. In order to get a correct measure of their BP outcome, the patients were asked not to stop or change interfering antihypertensive medication prior to the visit. BP was measured manually in a standardised manner, with the patient having being seated for at least five minutes, with three repetitive measurements, using the mean of the second and third measurement. Blood samples were drawn between 8.30 a.m. and noon, with the patient in the seated position for the last five minutes. Cure of HT was defined as BP below140/90 without the aid of antihypertensive medication. Improved HT was defined as the same or reduced BP compared with baseline, and decrease of at least one antihypertensive medication. Persistent HT was defined as unchanged or increased BP with either the same or increase in antihypertensive medication. Biochemical cure after adrenalectomy was defined as ARR below 750 pmol/µg/h.

Medically treated patients who had not had AVS or had a non-representative AVS, were offered a new AVS with intraprocedural cortisol analysis.

3.8 Cardiac magnetic resonance imaging and analysis

In **Paper III,** the baseline sub-study of CMR with dynamic contrast enhancement (CMR1-CE) was performed with a 3T GE Signa Excite scanner (Milwaukee, WI, USA). This scanner was replaced in June 2014 with a 3T Siemens Magnetom Skyra (Erlangen, Germany) with T1 mapping technology, which was used for the T1 mapping sub-study (CMR2-T1).

For both scanners, images were obtained during breath-hold with ECG-triggering. LV mass, volumes and function were analysed from short-axis CINE views, with manual tracing of endo-and epicardial borders in all slices in end-diastole and end-systole, excluding papillary muscles. The software Segment, version 1.9 R3697 was used to calculate values for LV mass, end-diastolic volume, end-systolic volume, stroke volume, cardiac output and ejection fraction, and the values were indexed to body surface area.

All post-image analyses were performed by two different investigators, and the mean of their results was applied for statistical analyses.

Estimation of dynamic contrast enhancement

For the contrast enhancement image acquisition in CMR1-CE, a protocol earlier published by Su et al. was adopted and slightly modified (99). Mid-ventricular short axis views were obtained before and 2,4,6,8,10,12 and 14 minutes after administration of 0.2 mmol/kg body weight of a gadolinium based contrast agent (Gadovist 1 mmol/mL). Three non-overlapping regions of interest (ROI) were placed both in the interventricular septum and within the LV lumen from pre- and post-contrast images, and the average signal intensities (SI) both from the interventricular septum and lumen were calculated. Adopted from Su et al, contrast enhancement values for the different time-points post-contrast were then defined as the following ratio:

$$\text{Contrast enhancement value} = \frac{SI\ post.contrast\ myo - SI\ native\ myo}{SI\ post.contrast\ blood - SI\ native\ blood}$$

SI post.contrast myo = the signal intensity (SI) of myocardium after contrast, SI native myo = the SI of myocardium before contrast, SI post.contrast blood = SI within the lumen after contrast, and SI native blood = SI within the lumen before contrast

Estimation of myocardial fibrosis from T1 mapping

For the T1 mapping images in CMR2-T1, an inversion recovery based MOLLI sequence was used. A pre-contrast T1 mapping mid-ventricular short-axis view was performed before administration of Gadovist 0.15 mmol/kg body weight. Further T1 mapping images were then performed at 10, 15 and 20 minutes post-contrast. The syngo.via software (Siemens, Erlangen) was applied for T1 mapping calculations. A ROI covering most of the interventricular septum and one ROI within the LV lumen were drawn from pre- and post-contrast T1 mapping views. Following a recent consensus statement by the Society for Cardiovascular Magnetic Resonance, the extracellular volume (ECV) % was calculated as a surrogate marker for the extracellular space using the following formula (95):

$$\text{ECV (\%)} = \frac{\frac{1}{T1\ post.contrast\ myo} - \frac{1}{T1\ native\ myo}}{\frac{1}{T1\ post.contrast\ blood} - \frac{1}{T1\ native\ blood}} \text{ x (100 – haematocrit)}$$

T1 post.contrast myo = SI of myocardium after contrast, T1 native myo = SI of myocardium before contrast, T1 post.contrast blood = SI within the LV lumen after contrast, and T1 native blood = SI within the lumen before contrast.

3.9 Statistical analysis

All parameters were expressed as median and ranges. Student's t-test and Mann-Whitney U-test were used to compare normally and non-normally distributed continuous data between two groups, and Wilcoxon Signed Rank Test for within-group not normally distributed data. The Chi-square test or Fisher`s Exact Probability test were used as appropriate for comparison of categorical data. Trend lines for biochemical correlations in **Paper II** were fitted using linear regression. In

Paper III, one-way ANOVA was used to compare continuous data between three groups, and Bland-Altman plots were used to analyse the agreement between measurements performed by two investigators. For all statistical tests, a p-value less than 0.05 was considered statistically significant.

3.10 Ethics

All studies were approved by the Regional committee for medical and health research ethics for Western Norway (2012/1702, 2012/1856 and 2013/742). Written informed consent was in study I obtained from all adrenalectomised patients and from all patients still alive who met to follow-up in 2014. In study II and III written consent was obtained from all patients.

4 Results

4.1 Paper I

We retrospectively identified 108 patients (73 males and 35 females) with a confirmed or highly suspected PA, admitted to Haukeland University hospital in the period 1998-2012. The included patients had a median 10 year history of HT before their diagnosis. A very large proportion (92/108) had a history of hypokalaemia. We found that PA had been verified by confirmatory testing in 83/108 of these patients. Confirmatory testing had been performed in a higher proportion of the patients after publication of clinical guidelines in 2008. The majority of our cohort, 95/108, had performed AVS as subtype testing. AVS was representative in 70/95, with unilateral PA found in the majority (50/70). However, the proportion with bilateral PA increased over the study period. The patients with AVS-confirmed unilateral PA had lower serum potassium and higher post-SIT aldosterone levels compared with those with bilateral PA.

Comparison of AVS results with adrenal imaging showed low concordance; only 27/65 had concordant results on adrenal imaging and AVS. Even in the age subgroup 35- 40 years, one patient with an adrenal adenoma found by CT imaging had AVS lateralisation to the opposite adrenal.

Sixty-eight of the 108 patients were adrenalectomised, of whom 48 had AVS-confirmed lateralisation. The remaining patients were operated based on CT/MRI imaging, either alone (n=8) or with non-representative AVS results (n=12). Forty patients were medically treated, twenty of these had bilateral PA confirmed with AVS.

Sixty-five pathology specimens of adrenal tissue were available for histopathological evaluation. Forty-seven of these specimens were defined adenomas, and the remaining hyperplasia. *KCNJ5* mutational status could be determined in 64 specimens, with mutations in *KCNJ5* found in 19. In two patients, *KCNJ5* mutations were found in one nodule only of nodular hyperplasia. *KCNJ5* mutations were

associated with female sex, a florid PA phenotype and a higher cure-rate of HT than patients without *KCNJ5* mutations. Due to old age and low quality of some of the adrenal specimens, only 26/65 specimens were adequate for analysis of the *ATP1A1*, *ATP2B3* and *CACNAID* genes. Of the tested specimens pathogenetic mutations in *ATP1A1* were detected in 4/39 specimens, all adenomas. Mutations in *ATP2B3* (2/39) and *CACNAID* (4/39) were found both in adenomas and nodular hyperplasia. Sixteen specimens were analysed for *CTNNBI*, all tested negative.

Seventy-three patients met to a clinical follow-up visit in 2014, fifty-two adrenalectomised and 21 medically treated. Twelve patients were deceased. Of the remaining 23 alive patients who did not meet, fifteen declined the invitation, and the remaining patients were unavailable or unable to meet. We found similar BP reductions in the adrenalectomised and medically treated patients, but the former used fewer antihypertensive drugs and had higher potassium levels; all were normokalaemic without potassium supplements after adrenalectomy. Cure of HT after adrenalectomy was achieved in 11/52. Female sex, presence of *KCNJ5* mutation and a histopathological adenoma were associated with cure of HT. Eight of 48 adrenalectomised patients with available ARR results had persistently elevated ARR consistent with no biochemically cure, all with AVS-confirmed lateralisation before adrenalectomy. Seven of these had a pathology specimen available, of which four showed a histopathological adenoma, the remaining hyperplasia.

Only 16/21 medically treated patients used MR antagonists at follow-up. All medically treated patients without representative AVS results were offered a repeat AVS with intraprocedural cortisol analysis. Three of nine patients who accepted were later diagnosed with AVS-lateralisation, and were subsequently adrenalectomised.

4.2 Paper II

Here we demonstrated that intraprocedural point-of-care cortisol assay increased the AVS success rate from 10/21 patients in a historical cohort, to 17/21 patients in the study group. The increased success rate was due to a significant increase in

representative samplings from the right adrenal vein. In the study group, 13/21 AVS procedures were bilaterally representative after the first samples, whereas four patients achieved representative samples after a first resampling. Seven of the PA patients in the study group had previously undergone an unsuccessful AVS, compared with three in the historical cohort. Throughout the study period the success rate of representative first set of samples increased, without the need of repeated samples. Thirteen of the successful 17 samples in the study group showed AVS-lateralisation, whereas 6/10 successful samples in the historical cohort lateralised.

During the study period, the mean time from the patients arrived at the radiology suite until the first set of samples was drawn was 88 minutes. The first point-of-care cortisol results were available 25-30 minutes after the samples were obtained. Mean duration for the whole AVS procedure was 138 minutes.

The intraprocedural point-of-care cortisol assay showed satisfactory quality compared with the routine immunological assay.

One patient in the study group experienced adverse effects with persistent abdominal pain and was readmitted to hospital. CT revealed a small amount of fluid around a diffusely oedematous right adrenal gland, treated conservatively without sequelae.

4.3 Paper III

In this CMR study we reported the results of both LV mass and fibrosis assessment in PA, determined before and after specific treatment. In paper III we found that LV mass index in the PA1 group was significantly higher than in healthy subjects at baseline, but decreased rapidly after specific PA treatment, with a median of 18 months since adrenalectomy or start of medical treatment with MR antagonists. The decrease in LV mass appeared despite only small non-significant reductions in BP. When the adrenalectomised (n=8) and MR antagonist treated (n= 6) PA1 patients were analysed separately, the adrenalectomised patients displayed a larger, highly significant reduction in LV mass index, whereas the medically treated patient showed a trend towards, but no significant reduction in LV mass index.

No evidence of myocardial fibrosis in PA was found in the newly diagnosed PA1 group when measured with CMR with dynamic contrast enhancement (CMR1-CE) from 2 to 14 minutes post-contrast, and compared with healthy subjects. Similarly, subsequent T1 mapping results from the CMR2-T1 scanner showed no increase in ECV as a surrogate marker of interstitial fibrosis in either PA1 (now treated) or the PA2 group (newly diagnosed), compared with healthy subjects. On the contrary, slightly lower ECV values were found in the newly diagnosed PA2 than in controls at both 10 and 20 minutes post-contrast, and in the treated PA1 at 20 minutes post-contrast.

Left ventricular end-diastolic index (LV-EDV) showed a non-significant trend of reduction after specific treatment (PA1 group). When comparing the adrenalectomised with MR antagonist treated patients, a significant reduction in LV-EDV was found in the medically treated patients only.

5 General discussion

Over the past few decades PA has become recognised as the most common cause of secondary HT. The diagnosis may be tricky, but the condition is easily managed and potentially curable. PA causes excessive risk of organ complications if not diagnosed and treated correctly. Notwithstanding this knowledge, the diagnosis, treatment and outcome of the PA population in Norway have not previously been addressed. This doctoral thesis is based on researcher initiated clinical studies of the PA population in Western Norway, with the overall aim to determine the characteristics of a large Norwegian PA population. Specifically, we aimed to evaluate the diagnostic procedures in use and treatment outcomes, including effects on the myocardium of the disease and its treatment.

5.1 PA epidemiology and characteristics

When we first chose to conduct these studies of patients with PA, no scientific publication existed on PA patients in Norway. **Paper I** is a benchmark study, which represents a starting point for the evaluation of current practice and for improvement.

In Paper I, we identified 108 PA patients diagnosed between 1998 and 2012. The incidence and prevalence of PA in the population at large are not well known, and are mostly given as proportions among selected groups of hypertensive patients. Following the revised Endocrine Society guidelines, approximately 50% of patients with HT belong to categories at high risk of PA, and should therefore be screened for PA (32, 139). In large prospective studies, a PA prevalence at 6% among HT patients in primary care and 11% in referred hypertensive patients were found (27, 29). However, in a recent survey among primary care physicians in Italy and Germany, only 7-8% of patients with HT were screened for PA (242). Similarly, another study, also from Italy, showed that only 1.9% of expected patients with PA were diagnosed with PA in the period 2000-2015, and 1% of expected adrenalectomies were performed (243). **In Paper I,** presumably all diagnosed PA patients in the three counties of Western Norway, harbouring a population of 1.0 million in 2012,

underwent diagnostic workup at our centre in the years 1998-2012. If we assume that 20-30% of the adult population in Norway is diagnosed with HT, the number of unidentified PA patients in Western Norway during the study period might have been several thousand. We therefore believe that likewise to Italy and Germany, PA is grossly underdiagnosed in Norway, with the majority of PA patients never screened or diagnosed. Education of both primary care physicians and specialists in referral centres to order more ARR testing of their hypertensive patients at risk of PA, should be encouraged.

The diagnosis inevitably depends on diagnostic criteria, i.e. cut-off levels for aldosterone and renin levels for screening and diagnostic testing. However, neither hormone assays nor recommended cut-of levels are standardised, and large variations exist between centres. Additionally, due to circadian variation with primarily nocturnal aldosterone hypersecretion, a day-time blood sample may not reflect the aldosterone hypersecretion (15). Still, as of today, no other screening test superior to ARR exists. Evidence is increasing that a continuum of autonomous aldosterone hypersecretion exists beyond the limits of what is today defined as PA, with individuals with low-renin HT experiencing a similar beneficial effect of MR antagonist treatment as those with IHA. This raises the question as to whether distinguishing between PA and non-PA is the most important, or rather only diagnosing those with an APA, who should be offered surgical intervention.

The very high proportions of hypokalaemia and unilateral PA found in our PA cohort indicate that mostly PA patients with the more severe phenotypes were diagnosed and admitted for AVS. This seems especially evident during the first years of the study period, before PA guidelines were published in 2008 (138, 244, 245). However, our findings clearly demonstrate that there has been significant development over the study period in Norway as elsewhere. One important example is the increased proportion of patients undergoing AVS, which increases the precision of the subtyping and, consequently, the therapy. Additionally, bilateral PA in our cohort increased during the study period, pointing to an increased focus and ability also to detect bilateral PA with milder phenotypes. It is likely that the observed increase in

incidence reflects increased diagnostic awareness, but true change and regional differences due to external factors (lifestyle, environment) cannot be ruled out.

Like several other PA studies, we found more men than women diagnosed with PA (186, 187, 246). However, there are no data showing that PA is more prevalent in males than females, and the female dominance in harbouring the common *KCNJ5* somatic mutation indicates a possible female preponderance in APAs (15). Thus, our findings probably reflect a referral bias with an increased attention to diagnose and refer male patients for exploration of possible underlying causes of HT. It should be remembered that female patients are more likely than men to achieve complete or partial clinical success after adrenalectomy (204).

5.2 Subtype diagnostics

The principal challenge after the diagnosis is made is to determine whether the disease is localised to one side, and thus can be treated with adrenalectomy.

Adrenal AVS has become the gold standard procedure for detecting lateralisation in PA. This recommendation by Endocrine Society Guidelines was based on several studies evaluating surgical clinical outcome of surgically confirmed APAs, showing that imaging was not reliable compared with AVS in distinguishing unilateral from bilateral aldosterone excess (156, 247). However, AVS is expensive, invasive and a technical challenging procedure and is only available in specialised centres. Non-functioning adrenal adenomas are uncommon in younger patients. Therefore, young patient below 35 years of age, with spontaneous hypokalaemia, marked aldosterone excess and unilateral adrenal adenoma on adrenal imaging may therefore proceed directly to adrenalectomy without AVS (139).

In **Paper I,** the proportion of patients who underwent AVS increased in the period after 2008 compared with the earlier period, pointing towards an increased focus and ability to apply AVS for subtyping of PA after the guidelines were published in 2008.

In **Paper I** our results corroborate other studies in that the concordance rate between adrenal imaging (CT and MRI scans) and AVS was low. In fact, our concordance rates were lower than those found both in a previous systematic review and in a study by the Mayo clinic (157, 159). In this way, we clearly show that AVS has contributed to significant improvement in diagnostic quality and, hence, treatment safety. Based on abnormal imaging alone, a large proportion of the patients in our cohort would either have had the wrong adrenal removed, been unnecessarily adrenalectomised, or not been offered adrenalectomy. In accordance with the recommendations in the latest Endocrine Society Guidelines, we also found that even in the age group 35-40 years, the wrong adrenal would have been removed without AVS.

However, the debate concerning the accuracy of adrenal imaging versus AVS in diagnosing unilateral PA has recently been actualised, after the randomised SPARTACUS trial showed no difference in BP outcome in patients whose treatments decisions were based on CT imaging compared with AVS (187). However, the SPARTACUS cohort has been criticised for not being representative of PA. Only patients with drug-resistant HT or hypokalaemia, consistent with a severe phenotype, and thus less likely to achieve remission of HT, were included. Secondly, the choice of daily defined dose of antihypertensive treatment as primary end-point has been considered suboptimal, as it does not reflect biochemical cure of PA after adrenalectomy. A third area of dispute is the comparison made between AVS versus CT-based MR antagonist therapy, since patients with APA also respond well to medical therapy. Additionally, the sample size has been criticised for not being adequate to prove non-inferiority of CT scanning compared with AVS to determine indication for adrenalectomy (188, 248).

The rate of bilaterally successful AVS found in **Paper I** was in the middle range compared with other reports (156, 174). However, introduction of the intraprocedural point-of-care cortisol assay in 2013, as studied in **Paper II,** showed a significant improvement compared with a historical cohort. The increased success rate was

caused by an increase in successful cannulation of the right adrenal vein, which, due to anatomical reasons it the most difficult to cannulate.

Interestingly, our study also demonstrated a learning effect for the radiologists, with improved successful sampling at first attempt at the end of the study period compared with in the beginning. A similar training effect was found in another study (179). These findings emphasise the importance of AVS procedures being performed in high-volume centres by dedicated, experienced radiologist.

Including **Paper II**, several prospective studies have demonstrated increased success rates with intraprocedural cortisol assays, independent of different AVS protocols, and with low complication rates (175, 177-180). All these studies, including **Paper II,** are referred in the updated Endocrine Society guidelines. However, to this date, no randomised trials have been published to conclusively determine if this approach improves the success rate of AVS, but a randomised trial (I-Padua) is now ongoing (249).

PA is associated with significant cardiovascular morbidity. Identification of unilateral PA with subsequent adrenalectomy causes greater BP reduction and lowers risk of cardiovascular disease compared with medical MR antagonist treatment (233, 234). However, the economic costs should be considered before screening procedures and subtype diagnostics are implemented to identify patients with surgically correctable PA. A cost-effectiveness analysis was therefore performed, which found that CT scanning followed by AVS was a cost-effective strategy to identify patient with unilateral PA among patients with resistant HT, compared with medical treatment alone (250). Still, access to AVS is limited. If far larger groups of patients with PA will be diagnosed in the future, this will necessitate new and easier accessible subtype diagnostics.

One such method may be PET-CT. In cases where AVS cannulation is not successful, functional imaging with ^{11}C-metomidate PET-CT offers an alternative method of subtyping, but the application is limited due to short half-life of the ^{11}C-metomidate which requires an on-site cyclotron (189). At our centre we have sent three selected

patients with unsuccessful AVS to ^{11}C-metomidate PET-CT (Addenbrooke's Hospital in Cambridge, UK). One of those patients was adrenalectomised based on ^{11}C-metomidate PET-CT showing lateralisation, with complete biochemical cure. However, a molecular tracer with longer half-life than ^{11}C-metomidate and capable of binding specifically to aldosterone synthase is desirable (251).

Another path may be steroid profiling. The development of LC-MS/MS technology with simultaneous measurements of multiple steroids in one blood sample including the hybrid steroids 18-hydroxycortisol and 18-oxocortisol have shown promising results in determining correct subtype in recent retrospective series (194, 197). These results need to further be confirmed in prospective studies where steroid profiling guides diagnostic decision making.

5.3 Genetics

From 2011 onwards, starting with the discovery of the somatic *KCNJ5* mutations, the genetic basis of APAs has been elucidated. About 60% of APAs in the Western world harbour somatic mutations in genes known to be associated with APA (252). In our PA cohort in **Paper I** we found slightly lower frequencies of *KCNJ5* mutations than reported in two large European multi-centre studies (50, 51). We found the same association between mutations in *KCNJ5* and female sex as found in those studies and in a large meta-analysis (55). Similarly to the meta-analysis, *KCNJ5* mutations were associated with a more florid phenotype, indicated by higher post-SIT aldosterone levels. However, unlike those studies, we found no association between *KCNJ5* mutations and young age, which could be due to study size.

Similar to a recent Dutch study, we found somatic *KCNJ5* mutations in one nodule of nodular hyperplasia in two of 19 patients, as opposed to adenomas in the remaining (215). The origin of *KCNJ5* mutations is still unknown, but they obviously differ from the other somatic mutations. Tumours with *KCNJ5* mutations histologically display mostly ZF-like cells, in contrast to more ZG-like cells in tumours with other somatic mutations. Additionally, *KCNJ5* mutations have not been detected in the

aldosterone-producing cell clusters APCCs, which may be precursors for some APAs and are thought to play a key role in PA development (252, 253).

Further studies of immunohistochemistry and genetics may elucidate the pathophysiology of PA in more detail, which could eventually lead to new diagnostic and therapeutic approaches.

5.4 Treatment outcome

The ultimate aim of case detection and correct subtyping of PA is to ensure targeted treatment depending on whether the PA is unilateral or bilateral, to ensure a best possible outcome. Specific PA treatment with either adrenalectomy or MR antagonist treatment lowers BP values, lowers cardiovascular, metabolic and renal complications, improves quality of life and may biochemically cure unilateral PA, compared with non-specific antihypertensive treatment alone (83, 102, 110, 130, 234). Most likely, the outcomes depend on both the reduction of BP and aldosterone levels.

In our study cohort in **Paper I,** both HT cure rate (11/52) and biochemical cure rate (8/48) after adrenalectomy were in the low range compared with other reports (157, 204, 205, 208, 210). The low HT cure rate may have been caused by a relatively long-follow up time after adrenalectomy, allowing for age-related coexisting HT. Long-standing exposure to excess aldosterone may have caused structural vascular remodelling, contributing to sustained HT after adrenalectomy. However, our results did not show this, despite the association between long-standing HT and low cure rate that has been shown in several other studies (210, 213). Not all our patients had PA subtype determined with AVS, and may theoretically have been incorrectly adrenalectomised. However, we found no difference in HT cure rate between adrenalectomised patients with and without AVS-confirmed unilateral disease. All the operated patients with persistently elevated ARR at follow-up had AVS-confirmed lateralisation before adrenalectomy, but nearly half had histopathological hyperplasia. Those patients presumably therefore had asymmetrical bilateral

aldosterone production rather than true unilateral PA. Conversely, and similar to several other studies, we found female sex, a histopathological verified adenoma and the presence of *KCNJ5* mutations to be associated with cure of HT (209, 214, 215). The better outcome in females is not understood, but may reflect a vasoprotective role of estrogens before menopause.

5.5 Myocardial changes in PA

CMR offers an accurate, non-invasive 3-dimensional assessment of cardiac structure, function and tissue characteristics. Former echocardiographic studies have shown that PA is associated with increased LV mass and LV hypertrophy compared with essential HT with similar BP levels, with a significant decrease in LV mass both after adrenalectomy and after long-term medical treatment with MR antagonists (74, 75, 207).

In **Paper III,** using CMR we similarly found a significant regression in LV mass after relatively short time (median 18 months) of specific PA treatment. The regression in LV mass occurred despite small non-significant reductions in BP, underscoring the importance of reducing the aldosterone excess in treating organ complications. As several of the patients had already been adrenalectomised or initiated MR antagonist treatment in the months preceding the baseline CMR evaluation, the true effect of PA treatment on LV mass was probably larger than found in our study. Our results are in accordance with another CMR study showing significant reduction in LV mass in suspected PA both 3 and 6 months after MR antagonist treatment, and underscores that also after MR antagonist treatment, effects on LV mass measured by CMR occur earlier than former echocardiographic studies have been able to detect (232).

Based on animal studies and previous imaging studies in humans, the medical literature suggests that myocardial fibrosis is a clinical problem in PA. In **Paper III,** surprisingly, we found no evidence of increased myocardial fibrosis in PA patients compared with healthy subjects, either with CMR dynamic contrast enhancement

technique or with CMR T1 mapping. Moreover, the T1 mapping of both the treated and untreated PA groups (PA2 and PA1) showed a tendency towards lower ECV values than the healthy subjects, indicating an increased intracellular fraction of the LV with more pronounced cardiomyocyte hypertrophy relative to the extracellular volume expansion.

Thus, our results contrast experimental animal studies where aldosterone infusion combined with high sodium intake caused histopathological myocardial interstitial fibrosis (91). However, human myocardial fibroblasts may differ from rodents when exposed to excess aldosterone, and the levels of aldosterone and sodium given in rodents under experimental conditions may not reflect the exposure in human PA. An association between sodium intake and the detrimental cardiovascular and renal effects of aldosterone excess have been demonstrated both in animal studies and human PA (91, 135-137). Dietary sodium intake was not measured in our study participants, nor was it recorded whether or not the PA patients had been given specific dietary counselling in order to reduce sodium intake.

Only one small human post-mortem study in five patients with suspected PA has been performed, showing histological fibrosis in several organs; the myocardium, lungs and pancreas (254). The patients of that study were approximately ten years older than our patients, and it has been shown that aging is associated with progressive fibrosis (255).

Additionally, echocardiographic videodensitometric and ultrasonic backscatter signal analyses found alterations in myocardial texture in PA, suggestive of increased collagen deposition and possible fibrosis (79-81). However, CMR is the recommended imaging modality for myocardial tissue characterisation. Two former CMR studies, both applying contrast enhancement techniques, found evidence of myocardial fibrosis measured as higher contrast enhancement in PA when compared with essential HT and healthy subjects (98, 99). One of those studies (98) measured contrast enhancement as the presence of a non-infarct diffuse pattern of late gadolinium enhancement, which is considered a less optimal method to evaluate

diffuse myocardial fibrosis (97). We applied the same formula for dynamic contrast enhancement as in the other study (99). Our contrasting results despite applying the same quantification technique may reflect the fact that none of the existing dynamic contrast enhancement quantification is accepted as an optimal universal quantification technique (96, 97). The discrepancy of our results may also have been caused by possible clinical differences in PA subtypes, ethnicity, gender distribution, severity of PA phenotype or severity of HT. Possible differences in dietary sodium intake in various studies may represent another confounding factor.

Ultimately, the fact that the increased incidence of cardiovascular events found in untreated PA are reduced to the same level as in essential HT after specific treatment, supports that irreversible myocardial fibrosis is not present (83).

To our knowledge, our study is the first to apply CMR T1 mapping with quantitative assessment of ECV for evaluation of diffuse myocardial fibrosis in human PA. We thus applied the gold standard parametric mapping technique for evaluation of diffuse myocardial fibrosis, as recommended in a newly published consensus statement (95). Therefore, based on our results, myocardial fibrosis may not be a significant clinical problem in PA if diagnosed and treated within time frames commonly used in clinical practice. However, larger human CMR T1 mapping studies should be performed to explore possible differences in myocardial texture between PA and essential HT; between unilateral and bilateral PA; between low-sodium diet and unrestricted sodium diet; between different PA age groups; and before and after specific treatment.

5.6 Methodological considerations

The main limitation of **Paper I** is the retrospective design, with retrospective collection of baseline data. Not all data were accessible for all patients, and not all included patients had a confirmed PA. Thus, some of the patients included without confirmatory testing might not have had a genuine PA, which might have brought a bias to the results. We did however use strict criteria for inclusion. Additionally, not

all patients had subtype determined by AVS. The high proportion of patients with hypokalaemia and unilateral disease may also have affected the representativeness of our study cohort.

The retrospective design with old age and low quality of some of the pathology specimens entailed that not all the adrenal specimens could be determined for somatic mutations other than *KCNJ5*. A significant proportion of specimens could not be determined for mutations in *ATP1A1*, *ATP2B3* and *CACNAID* mutational status, which most likely influenced the prevalence of these mutations found in our study cohort.

However, the one-centre design is a strength, with all AVS procedures performed by a few radiologists at the same site with the same criteria for selectivity index and lateralisation index during the whole study period. Similarly, all the adrenal images and histopathological slides were reevaluated by one radiologist and one pathologist, both experienced in their fields. Additionally, all outcome data for **Paper I** were prospectively collected by the same physician, under standardised conditions.

In **Paper II**, the previous AVS procedures used as historical controls had all been performed by the same radiologists as during the study period. This was done in order to minimise the risk of bias related to different radiologists during the study and control period. However, our study design with a historical control group cannot definitely prove the hypothesis that intraprocedural cortisol assays increases AVS success rates, and the results from the ongoing randomised I-Padua study are therefore awaited (249)

The time span between AVS procedures with intraprocedural point-of care cortisol was shorter than for the previous AVS procedures serving as controls, which might have caused an enhanced training effect and therefore may represent a potential confounder.

Paper III was planned as a pilot CMR study for assessment of myocardial fibrosis, and relatively small sizes of all study groups may have had the potential for false

negative conclusions. However, it is important that also trials with negative results are published, to avoid a publication bias. For the PA patients, due to CMR contraindications, no patients with a history of coronary arterial bypass surgery were included, neither were patients with chronic atrial fibrillation, due to potential difficulties with CMR image acquisition. Exclusion of PA patients with those comorbidities might have caused a selection bias towards a healthier subgroup of PA patients included in the study. Patients with paroxysmal or radiofrequency ablated treated atrial fibrillation were included.

The lack of a control group with essential HT poses a further limitation to **Paper III,** especially where differences between PA and controls were found. A supplementary control group of patients with essential HT could have discriminated between aldosterone excess or haemodynamic effects of HT *per se* as causes of differences between PA and healthy subjects.

We originally planned for both a control group of healthy subjects and a control group with essential HT. However, at our University hospital there is no HT outpatient clinic, and such patients had to be recruited from the cardiology and nephrology outpatient clinics, where patients without either severe heart disease of renal failure were hard to find. The dead-line of the recruitment period was also limited by the replacement of the original CMR scanner in June 2014. By then we had only managed to recruit and perform CMR in four patients with essential HT, mostly recruited from private cardiologists. Due to the low number these patients were excluded from further study analysis.

The intraprocedural point-of-care cortisol assay applied in **Paper II** was chosen due to its relatively short time required to complete analyses. Additionally, the instrument was small enough to fit on to the limited amount of bench space available in the vicinity of the radiologist. Comparison of the intraprocedural point-of-care assay was performed with the routine immunological assay employed in the laboratory, and with cortisol measured by LC-MS/MS. Correlation analyses showed satisfactory quality compared with both these methods.

The design of **Paper III** with two different CMR scanners used for the baseline CMR1-CE and the follow-up CMR2-T1 sub-studies poses a potential limitation to this study, in that direct comparisons of these two methods simultaneously were not possible. For the dynamic contrast enhancement quantification used for sub-study CMR1-CE, no universally accepted optimal technique exists (97), which may limit these results. However, in sub-study CMR2-T1, we applied T1 mapping with ECV quantification, which is the gold standard parametric mapping technique (95). On the other hand, the application of two different CMR techniques both showing similar results strengthens our results, indicating that this particular group of PA patients did not have more cardiac fibrosis than the healthy controls.

The post-image analyses were only blinded for one of two investigators, as these analyses were performed by the same investigator that had included all the patients and controls and created the identification key. However, Bland-Altman plots found deviations of less than 10% between the values obtained by the investigators, which was considered acceptable.

6 Conclusion and future perspectives

6.1 Conclusion

Paper I

- One hundred and eight diagnosed PA patients were identified in Western Norway from 1998-2012, reflecting potentially several thousand undiagnosed individuals.
- The majority of PA patients had unilateral disease and a history of hypokalaemia, indicating that particularly normokalaemic and bilateral PA remained undiagnosed.
- The proportion with bilateral PA increased during the study period, pointing towards an increased ability to detect bilateral disease.
- Concordance between adrenal imaging and AVS was low.
- Somatic *KCNJ5* mutations were found in 30% of adrenal specimens, and were associated with female sex and a florid PA phenotype.
- Cure rate of HT after adrenalectomy was low. Female sex, a verified adenoma and *KCNJ5* mutations were associated with cure of HT.

Paper II

- Intraprocedural point-of-care cortisol analysis improved the success rate of AVS.
- A training effect among the radiologists performing AVS with intraprocedural cortisol analysis was observed, reducing the need for repeated samples.
- Correlations between the intraprocedural cortisol assay and the routine cortisol assays were good.

Paper III

- Specific PA treatment rapidly reduced LV mass measured by CMR, most prominent after adrenalectomy.
- No evidence of increased myocardial fibrosis was found in PA compared with healthy subjects, measured by CMR dynamic contrast enhancement or T1 mapping with ECV quantification.
- Both treated and untreated PA patients had lower T1 mapping ECV values compared with healthy subjects, indicating more pronounced cardiomyocyte hypertrophy than fibrosis in PA.

Taken together, our findings reflect that most likely PA is still grossly underdiagnosed in Norway, in particular the often less florid bilateral IHA. The current diagnostic work-up is laborious and requires expert centres. However, after introduction of intraprocedural point-of-care cortisol analysis permanently introduced at our centre in 2013, the success rate of AVS has increased substantially. Delayed diagnosis and high morbidity and mortality are common, but decrease rapidly with correct treatment. Cardiac fibrosis may not represent a common clinical problem in PA, if diagnosed and treated in due time. Continuous emphasis on identification of patients with PA is needed, so that early diagnosis and targeted treatment can be initiated in far larger groups of patients.

6.2 Future perspectives

Several major challenges must be faced in order to optimise the future management of PA. Primary care physicians should be educated and convinced to screen for PA in hypertensive patients at risk for PA. However, currently wide variations exist in the diagnostic criteria and management of PA between centres. Hence, there is a need for harmonisation of screening procedures and diagnostic testing with standardised assays in order to minimise these variations. Furthermore, it would be desirable if the diagnostic work-up could be simplified without the need of a strict drug withdrawal

regime or without the need of AVS, restricted to specialised centres, as subtype testing in all patients. This will be increasingly important if the number of patients with PA escalates as predicted.

To this end, we are studying whether analysis of aldosterone levels and rhythm in subcutaneous fluid might have merit. We have developed an ambulant microdialysis technique allowing for dynamic measurement of hormone levels in subcutaneous fluid collected through a microdialysis catheter, and analysed by LC-MS/MS. This sampling can be done continuously for 27 hours, thus enabling characterisation of the circadian and ultradian rhythms of subcutaneous free aldosterone levels. After initial technical difficulties and extensive testing for two years, we have so far successfully sampled 13 PA patients with this technique, and included them in our ongoing project "Aldosterone rhythm in PA and healthy people". This study is part of a larger multicenter study (the Ultradian study) involving the Endocrinology Departments at Karolinska University Hospital Stockholm, the University Hospital of Bristol, and the University Hospital in Athens. The Ultradian study is part of the EU Framework Programme for Research and Innovation Horizon 2020.

There is increasing evidence that PA also exists in normotensive individuals and that PA represents a continuum. As patients with low-renin HT show the same beneficial effects of MR antagonists both on BP and organ complications as patients with overt PA, MR antagonist treatment might become first choice treatment also in these patient. Finally, the pathophysiological mechanisms leading to bilateral IHA constituting the majority of PA patients, should be further elucidated with genetic and immunohistochemical studies. This might open new paths for diagnostics and therapy.

Since the beginning of this PhD work in 2013, I have prospectively registered all newly diagnosed PA patients attending Haukeland University Hospital. We have created an infrastructure for systematic diagnostics, follow-up, and collected blood, urine and adrenal tissue in biobanks. Furthermore, we have strengthened our collaboration with other units (Stavanger University Hospital, Haugesund Hospital,

St. Olav's Hospital, and Tromsø University Hospital). So far, more than 200 PA patients have been registered, with an increased proportion of AVS-confirmed bilateral PA compared with in our retrospective cohort from Paper I (unpublished data). This infrastructure, with ongoing registration and bio-banking, provides a solid platform for future research on PA that may provide further insight into PA and improve the care of these patients.

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Papers I - III

I

II

Endocrine CONNECTIONS

Efficacy of adrenal venous sampling is increased by point of care cortisol analysis

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Abstract

Primary aldosteronism (PA) is a common cause of secondary hypertension and is caused by unilateral or bilateral adrenal disease. Treatment options depend on whether the disease is lateralized or not, which is preferably evaluated with selective adrenal venous sampling (AVS). This procedure is technically challenging, and obtaining representative samples from the adrenal veins can prove difficult. Unsuccessful AVS procedures often require reexamination. Analysis of cortisol during the procedure may enhance the success rate. We invited 21 consecutive patients to participate in a study with intra-procedural point of care cortisol analysis. When this assay showed nonrepresentative sampling, new samples were drawn after redirection of the catheter. The study patients were compared using the 21 previous procedures. The intra-procedural cortisol assay increased the success rate from 10/21 patients in the historical cohort to 17/21 patients in the study group. In four of the 17 successful procedures, repeated samples needed to be drawn. Successful sampling at first attempt improved from the first seven to the last seven study patients. Point of care cortisol analysis during AVS improves success rate and reduces the need for reexaminations, in accordance with previous studies. Successful AVS is crucial when deciding which patients with PA will benefit from surgical treatment.

Key Words
- adrenal
- cardiovascular

Endocrine Connections
(2013) **2**, 236–242

Introduction

Primary aldosteronism (PA) is a common cause of secondary hypertension; the prevalence is 2–15% in selected cohorts of hypertensive patients (1, 2, 3, 4, 5). Patients with PA have higher cardiovascular mortality and morbidity than controls with essential hypertension, possibly due to the presence of mineralocorticoid receptors in the heart and large vessels (2, 6). In ∼30–50% of the patients, the disease is unilateral, caused by for instance aldosterone-secreting adenomas, whereas the rest have bilateral disease (2, 6, 7, 8). The clinical management of PA depends on whether the disease is lateralized. Most patients with unilateral adenomas are either cured or have significant improvement of their hypertension after adrenalectomy (9). If the adrenal hypersecretion of aldosterone is bilateral, or a patient is unwilling to undergo surgery, medical treatment with a mineralocorticoid receptor antagonist is recommended (10).

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DOI: 10.1530/EC-13-0063

Selective adrenal venous sampling (AVS) is the recommended method to determine whether aldosterone hyper production is lateralized (10). When recommended diagnostic cutoff values are used, the sensitivity and specificity for detecting unilateral disease are 95 and 100%, respectively. In comparison, adrenal computed tomography (CT) has sensitivity and specificity at 78% and 72–75% respectively (7, 11). During AVS, blood samples are drawn from the right and left adrenal veins as well as a peripheral vein. The ratio between cortisol measured in the samples taken from the putative adrenal veins and the peripheral blood sample is most commonly used to determine whether the sample is representative for adrenal vein blood (12). A recent study has suggested, however, that metanephrine might be a better marker of correct catheter placement (13). The procedure is technically challenging, and reported success rates range from 8 to 97% (11, 14, 15, 16). It is particularly demanding to obtain a representative sample from the right adrenal vein due to anatomic reasons (16). Although the left adrenal vein drains to the left renal vein, and hence is more easily identified, the right adrenal vein mostly drains directly into the vena cava and with a steep angle. The procedure might also be complicated by the collapse of the adrenal vein due to the gentle vacuum applied to obtain the sample.

In traditional AVS protocols, the cortisol levels in the blood samples are determined after the patient has left the examination room. Recently, four prospective studies have shown increased success rate when the sampling procedure was guided by intra-procedural rapid assay measurements of cortisol (17, 18, 19, 20). A retrospective study has showed that diagnostic centers that introduced such measurements when revising their AVS protocols had more improvement over time in success rates than diagnostic centers that did not (14). The published studies were all small, and used different study protocols. The cortisol gradient required to deem a sample representative of adrenal vein blood ranges from two- to fivefold. In the studies that used cosyntropin to stimulate cortisol production, the required cortisol gradient ranges from three- to fivefold. The number of procedures performed by each radiologist is variable, and different instruments have been used. Only one study analyzed cortisol using a point of care instrument (20). This study showed proof of concept of intra-procedural cortisol measurements, but included five patients and no control group. In the three studies including retrospective controls, the samples were analyzed in the main laboratory (17, 18, 19) or two patients were examined sequentially to minimize the total time used for the procedure (17, 18, 19).

We wanted to determine the success rate of AVS using intra-procedural, point of care cortisol analysis and compare with a historical AVS series, applying recommended criteria for sample selectivity (12).

Subjects and methods

Patients

Patients planned for AVS at the Department of Medicine, Haukeland University Hospital were sent invitation to participate in the study. The previous 21 AVS procedures were used as controls. All patients in the study period provided written consent. The study was approved by the Regional Ethics Committee for Medical Research (REK-Vest #2012-01856) and is registered at Clinicaltrials.gov with accession code NCT01761344. One patient experienced adverse effects after the procedure, and has provided written consent for this to be reported.

Adrenal venous sampling

AVS was conducted sequentially under continuous cosyntropin infusion at a dose of 50 μg/h. The infusion was started 30 min before the procedure was initiated. For most procedures a Simmons 2/3 catheter was used to draw samples from the left adrenal vein whereas Hook, Simmons 2/3, or Shepherds hook catheters were used to draw samples from the right adrenal vein. A side hole was made in some catheters to decrease the vacuum at the tip of the catheter, and a gentle vacuum was applied to draw samples. For some procedures, a 0.014 inch floppy tip wire was inserted to prevent the adrenal vein from collapsing due to the applied vacuum. One to five blood samples were drawn from the external iliac vein and the putative left and right adrenal veins. The samples were transferred to Li/Heparin tubes for the point of care cortisol assay and serum tubes for routine assays. The sheath was not removed before the results of the point of care cortisol assay were available. If the assay revealed that the samples were not representative, repeated samples were drawn. The procedure was terminated after the third attempt to obtain representative samples or when the radiologist terminated due to increased risk of complications.

Intraoperative cortisol analysis

Cortisol was analyzed at point of care using the AIA-360 Cort-pac system (TOSOH Bioscience, Tokyo, Japan) according to the protocol provided by the manufacturer. The lithium/heparin tubes were centrifuged immediately

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DOI: 10.1530/EC-13-0063

at 4440 *g* for 2 min. The samples were analyzed for cortisol undiluted and at 1/20 and 1/39 dilution (in cortisol-free serum provided by the assay supplier). We used a cutoff ratio of 5:1 between adrenal venous sample and peripheral sample to determine whether the sample was representative for adrenal blood as described in reference (12).

Routine analysis

Serum cortisol was analyzed using Immulite 2000 (Siemens, Erlangen, Germany) according to the supplier's protocol and an inhouse multisteroid LC-MS/MS assay (21). The Siemens Immulite system automatically dilutes samples at 1/5 if the signal exceeds 1380 nmol/l, and samples were analyzed at 1/10, 1/20, and 1/40 sequentially. For LC-MS/MS analysis, all samples were diluted 1/50 in steroid-depleted serum (SunnyLab, Sittingbourne, UK) using an automated pipetting system (Hamilton Microlab Star, Bondzau, Switzerland). Aldosterone was detected using a RIA assay (Coat-a-count Aldosterone, Siemens). As criteria for lateralization, we used aldosterone:cortisol ratio in adrenal venous sample on one-side four times greater than that on the other side (12).

Statistical analysis

P values were determined by applying two-tailed Fisher's exact test, Student's *t*-test, or Wilcoxon's signed-rank test. A *P* value of <0.05 was considered statistically significant. Trend lines for biochemical correlations were fitted using linear regression.

Results

Patients

The clinical characteristics of the patients in the study cohort and controls are shown in Table 1. Notably, most of the patients had been diagnosed with hypertension several years before AVS was carried out. The prevalence of hypokalemia, as defined by serum potassium values below the reference limits or use of oral potassium supplements, was higher than what has been reported from several other diagnostic centers (6, 22, 23). Some patients had developed hypokalemia several years before the diagnosis of PA was made (not shown). Among the study patients, seven had previously undergone an unsuccessful AVS, the corresponding number in the historical series was three. The two groups were not significantly different with regard to age, ratio between women and men, and number of patients who had undergone an unsuccessful AVS previously. The relative contribution from the two radiologists who conducted the procedures was similar in the two groups.

Table 1 Clinical and procedure characteristics of patients examined with and without point of care cortisol analysis.

	Historical controls	Study population
Women/men	7/14	9/12
Radiologist 1/2	12/9	8/13
Patients with previous nonrepresentative AVS	3	7
Age (years) (median and range)	54 (39–77)	55 (30–69)
Days since the radiologist's previous procedure (median and range)	28 (2–147)	7 (1–110)
Samples taken at primary attempt (median and range)	4 (3–6)	5 (4–9)
Months of known hypertension (median and number of patients)	96 ($n=15$)	150 ($n=19$)
Patients with hypokalemia[a] (%)	76	81
Blood pressure at admission (median)	164/102	162/101
Number of antihypertensive used[b] (mean and range)	3 (2–5)	3 (1–6)
Lateralized/bilateral disease	6/4	13/4

[a]Patients were considered hypokalemic if they used potassium supplements and/or had a blood potassium level below the reference range upon admission to AVS.
[b]Number of antihypertensive used before drug adjustment for procedure.

Adrenal venous sampling

Representative adrenal venous samples were obtained bilaterally in 17 of the 21 study patients (81%), whereas the procedure was successful in only ten of the 21 historical controls (48%) (Fig. 1A). The increased success rate was due to a significant increase in correct sampling from the right adrenal vein (Fig. 1B; $P=0.0431$). The success rate of left AVS was unchanged (Fig. 1C). The first set of samples was representative for 13 of the study patients, and the first resampling resulted in representative samples from four additional patients, for three of which resampling from the right adrenal vein was required. Of the four unsuccessful procedures, renewed sampling was done twice in two patients, whereas the procedure was terminated due to increased risk of complications after the second sampling in two of the patients. Seven of the study patients had previously undergone an unsuccessful procedure. The procedure was successful for six of these (not shown).

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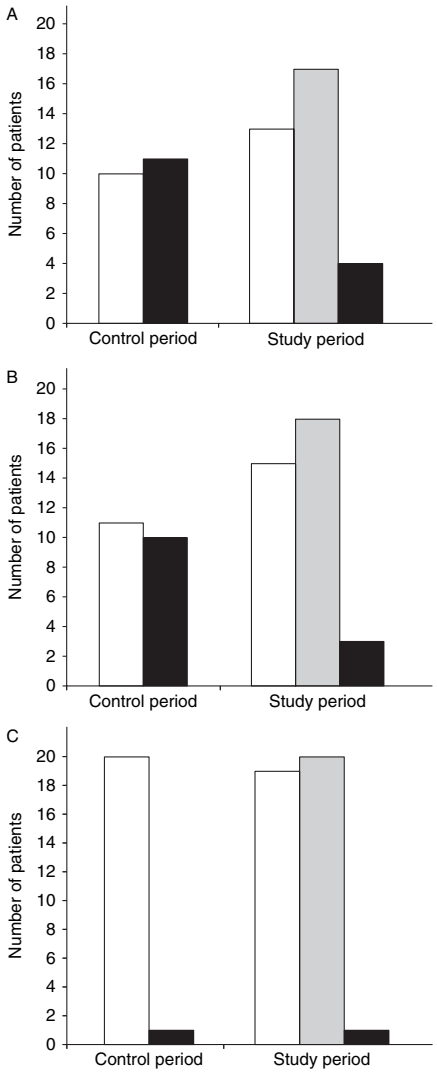

Figure 1
Number of patients whose first set of samples was representative for adrenal venous blood (white columns), representative samples were obtained before the procedure was terminated (gray columns) or representative samples were not obtained (black columns). (A) Data for both adrenal veins, (B and C) Data for right and left adrenal veins respectively.

We also observed an increased success rate without repeated sampling throughout the study period. Whereas two of the seven first procedures were successful without renewed sampling, six of the final seven patients did not require repeated sampling (Fig. 2). The mean time from patient arrived the radiology suite until procedure was terminated was 138 min. The mean timespan before the first set of samples was drawn was 88 min (data not shown). In the procedures where the first set of samples was representative, the mean timespan from the acquisition of the last sample till the procedure was completed was 18 min. One of the patients in the study group was readmitted to hospital due to persistent pain. CT did not show ongoing bleeding, but a small amount of fluid was observed around a diffusely edematous right adrenal gland. The patient was treated conservatively, and was discharged without sequelae. No other adverse effects were noted in the study population or the historical series.

Assay performance

The technical specifications of the point of care cortisol assay used during the AVS procedures are presented in the Supplementary Section, see section on supplementary data given at the end of this article. Briefly, the cortisol concentrations measured by the intraoperative cortisol assay correlated well with that obtained using a routine immunological cortisol assay (Fig. 3 and Supplementary Figure 1C–E, see section on supplementary data given at the end of this article) or an LC-MS/MS protocol (Supplementary Figure 1F). The average bias between the routine immunological assay and the rapid cortisol assay was −11%.

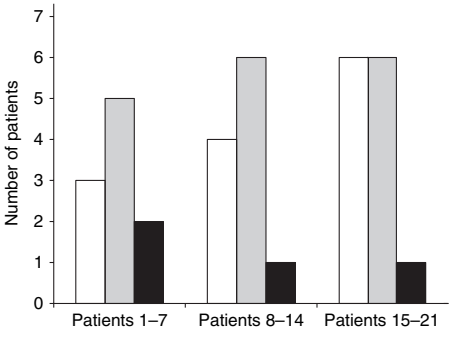

Figure 2
Number of patients whose first set of samples was representative for adrenal venous blood (white columns), representative samples were obtained before the procedure was terminated (gray columns), or where representative samples were not obtained (black columns) as a function of study number.

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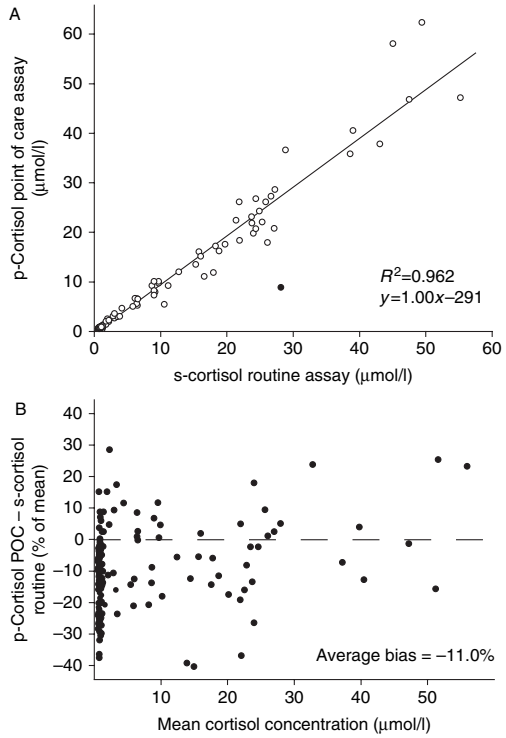

Figure 3
Comparison of results of point of care plasma cortisol and routine serum cortisol. (A) Correlation between p-cortisol in the point of care assay and s-cortisol in the routine assay. Open circles were included in the correlation analysis, closed circles were considered outliers. (B) Difference between the two assays as a percentage of the mean cortisol concentration.

Discussion

We found that after implementation of intra-procedural point of care cortisol analysis, the success rate of AVS procedures increased from 48% in the control period to 81% in the study period. This was due to an increase in the success rate of right AVS. There was a trend toward increased number of successful procedures without the need of resampling over the study period. The increased success rate can be attributed both to the opportunity to draw a new set of samples, as was done successfully in four patients, as well as a training effect for the radiologists. All previously published studies have shown significant increased success rate after repeated sampling (Table 2) (17, 18, 19). The trend toward increased success of initial sampling throughout the study period reproduces the results of Betz *et al.* (19). In our study, there was, however, a higher proportion of patients undergoing their second AVS among the first seven of the study patients. The uneven distribution of these patients might constitute a confounder to the observed training effect. However, there was no significant difference between the successful sampling of patients undergoing first and repeated procedures (not shown). We therefore find this to be an unlikely confounder. It is difficult to differentiate between the training effect due to shorter time interval between procedures in the study period and the effect of the point of care cortisol analysis. This also applies to the study by Betz *et al.* (19), where the intervention group was examined over a shorter time period than the controls. A shorter time span between procedures may thus represent a potential confounder in both studies.

Of the previously published studies on intra-procedural cortisol measurements during AVS, only the study reported by Rossi *et al.* (17) required a fivefold cortisol gradient for successful sampling. In that study, a single radiologist performed all 25 AVS. In the current study, all the 21 procedures were performed by two radiologists. We used an approach where the length of the procedure was kept at a minimum, and the patient was not moved to recovery before resampling, as described in two previous studies (17, 18). This approach was chosen to

Table 2 Prospective studies of intra-procedural cortisol analysis during AVS. The cortisol selectivity ratio is the ratio of cortisol between adrenal vein sample and peripheral sample required to deem the samples representative.

First author/ reference	Historical control (success/total)	First set of samples (success/total)	After repeated samples (success/total)	Use of ACTH	Cortisol selectivity ratio	Assay	Number of radiologists	Serious complications (historical/ intervention)[a]
Betz (19)	26/47	25/46	39/46	No	>2	LIAISON-Kit	3	0/1
Rossi (17)	16/25	19/25	23/25	Yes	>5	LIAISON-Kit	1	0/0
Auchus (18)	22/30	27/30	29/30	Yes	>3	Avida Centaur	5	0/0
Presented study	10/21	13/21	17/21	Yes	>5	Tosoh AIA 360	2	0/1
Total	74/123	84/122	108/122					0/2

[a]Complications are considered serious if they required re-admission to hospital or prolonged hospitalization.

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DOI: 10.1530/EC-13-0063

limit patient discomfort. Despite this, the radiology suite was only occupied for a mean 50 min after the first set of samples was drawn, 18 min if the first set of samples was representative. In comparison, Rossi *et al*. used 200 min to examine two patients if both procedures were successful.

Although there are no randomized trials showing effect of intra-procedural cortisol assay during AVS, four prospective studies, including this study, have shown increased success rate upon implementation of cortisol measurements (Table 2) (17, 18, 19). The increased success rate is independent of whether cosyntropin is used, the cortisol gradient required to deem the sample representative, the assay employed, and the number of procedures conducted by the individual radiologist in the study period. No published trials showing negative results have been identified. In total, including this study, two patients have had serious complications and required prolonged hospital stay or readmission to hospital. Neither patient has experienced further sequelae. Of the 123 patients subjected to AVS with intra-procedural cortisol analysis, this gives a complication rate of 1.6%, which is not significantly higher than what is reported for the procedure in general (7).

All published prospective studies are from centers with intermediate initial success rates (range 48–73%). The accumulated evidence for utilizing intra-procedural cortisol assays during AVS at centers with success rates $<75\%$ is compelling. Implementing the assay increases the quality of patient care by reducing the need for repeated procedures. The rate of complications is not significantly increased compared to protocols without point of care cortisol measurements.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/EC-13-0063.

Declaration of interest
Access to the TOSOH AIA-360 bench analyzer was provided free of charge from Alere Norway. The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements
The authors are grateful to Ralf Kellmann and Otto Bårholm for assistance with LC-MS/MS analysis.

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Received in final form 16 October 2013
Accepted 28 October 2013

http://www.endocrineconnections.org
DOI: 10.1530/EC-13-0063

Supplementary section:

Description of the point of care cortisol assay

TOSOH-AIA360 was chosen as the point of care instrument due to the relatively short time required to complete analysis, and as the instrument would fit on the limited amount of bench space available. TOSOH-AIA360 utilizes fluorescence enzyme immunoassay as the mode of detection and sufficient analytical quality had been shown in a previous study (1). After the samples were loaded into the analyzer the first results were available after 20 minutes, with one sample analyzed every 1.5 minutes. With sample centrifugation and dilution, the results were available 25-30 min after the sample was obtained. The results of the point of care assay were compared with the routine assay employed in the laboratory (Siemens Immulite 2000). The CV% of the point of care assay using one lot of reagents was 7,5-13,2 % using controls provided by the supplier and 4,3-5,4 % using Li-Heparin plasma spiked with cortisol. The CV% of diluted plasma samples were 13-20%.

The correlation between cortisol measured in plasma and serum on the point of care instrument was linear (figure S1, panel A), with an average bias of -7.4 % (95% KI for bias: -10.5% to -4.5 %). Serum samples run on the point of care instrument and the routine instrument showed linear correlation (figure S1, panel B) without significant bias (95 % KI -1.1% to 15.3%). Plasma cortisol measured by the point of care assay correlated well with the serum values of cortisol measured by the routine immunological assay (figure 3a, figure S1, panels C-E). Although the bias was comparable between undiluted samples and samples diluted 1/20 (-13 % and -14 % respectively), the observed bias was lower for samples diluted 1/39 (-1,8%). This last point needs to be taken into account when evaluating these samples, and might be due to increased inaccuracy of the pipette at lower volumes as well as a decreased effect of the plasma matrix in more dilute samples. The direct measurements of cortisol in serum by LC-MS/MS also correlated well with point of care plasma measurements of cortisol (figure S1, panel F). The average bias was 2,6 % (n.s). The concentration of cortisol in plasma samples was not determined by LC-MS/MS.

As a conclusion the assay had satisfactory quality compared to the routine methods employed. The results from highly diluted samples must be assessed with care.

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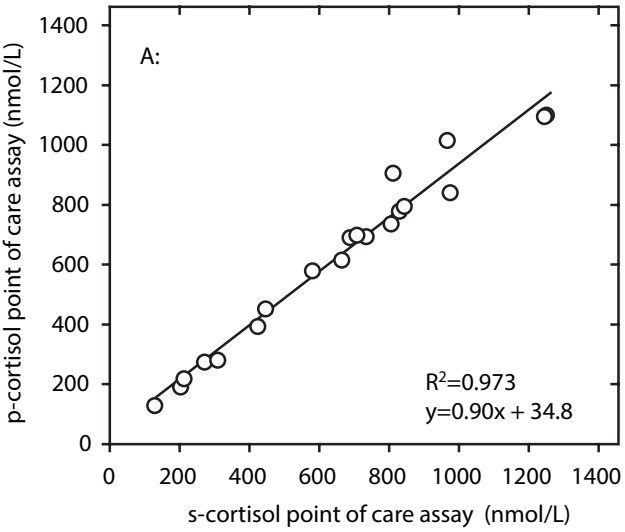
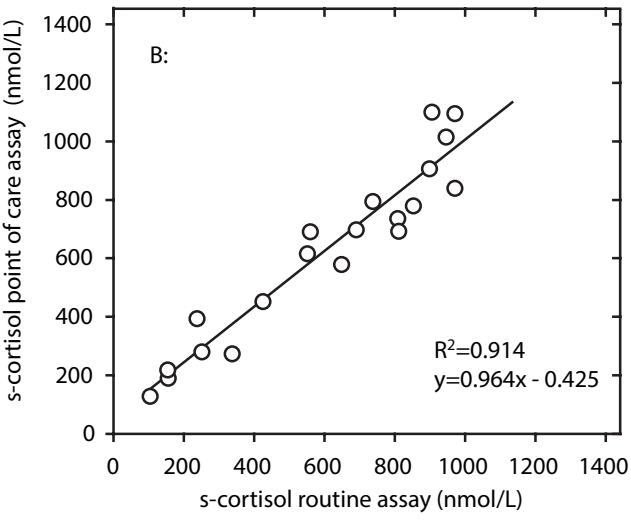
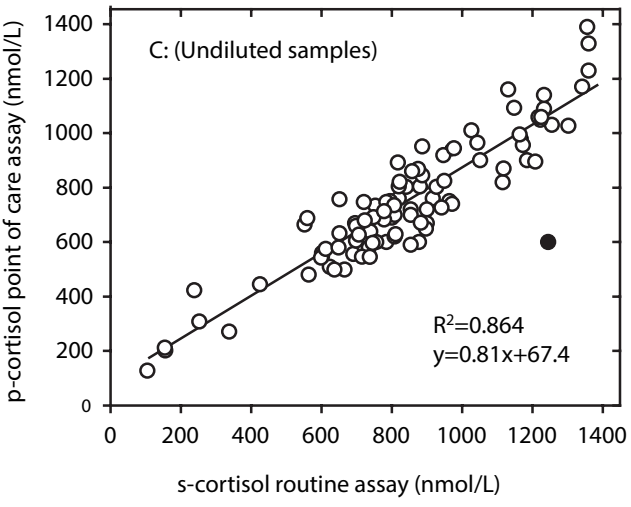
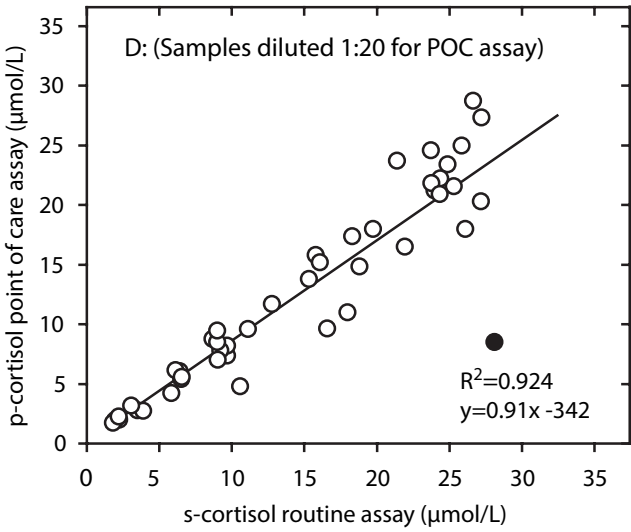
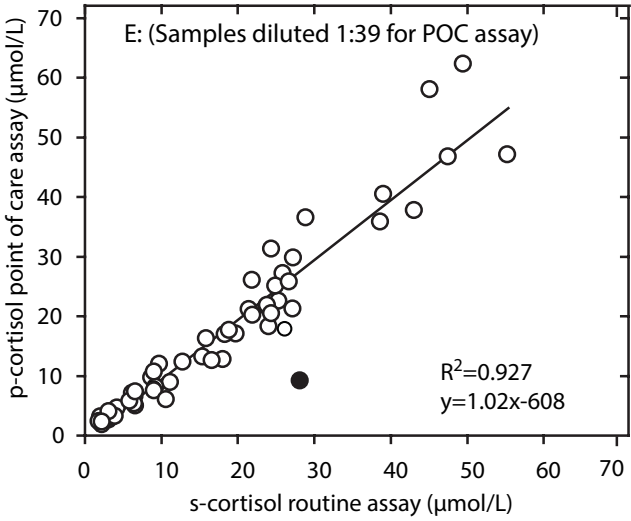

A:
p-cortisol point of care assay (nmol/L)
s-cortisol point of care assay (nmol/L)
R²=0.973
y=0.90x + 34.8


B:
s-cortisol point of care assay (nmol/L)
s-cortisol routine assay (nmol/L)
R²=0.914
y=0.964x - 0.425


C: (Undiluted samples)
p-cortisol point of care assay (nmol/L)
s-cortisol routine assay (nmol/L)
R²=0.864
y=0.81x+67.4


D: (Samples diluted 1:20 for POC assay)
p-cortisol point of care assay (µmol/L)
s-cortisol routine assay (µmol/L)
R²=0.924
y=0.91x -342


E: (Samples diluted 1:39 for POC assay)
p-cortisol point of care assay (µmol/L)
s-cortisol routine assay (µmol/L)
R²=0.927
y=1.02x-608

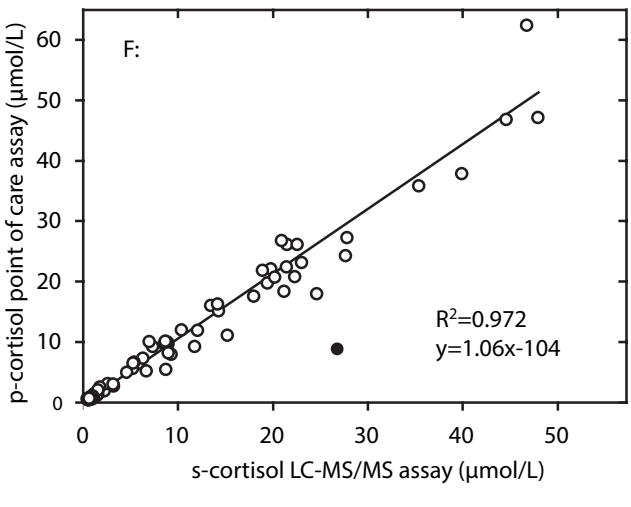

F:
p-cortisol point of care assay (µmol/L)
s-cortisol LC-MS/MS assay (µmol/L)
R²=0.972
y=1.06x-104

FIGURE S1

III

RESEARCH

Cardiac magnetic resonance imaging of myocardial mass and fibrosis in primary aldosteronism

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Abstract

Background: Primary aldosteronism (PA) is associated with increased cardiovascular morbidity, presumably due to left ventricular (LV) hypertrophy and fibrosis. However, the degree of fibrosis has not been extensively studied. Cardiac magnetic resonance imaging (CMR) contrast enhancement and novel sensitive T1 mapping to estimate increased extracellular volume (ECV) are available to measure the extent of fibrosis.
Objectives: To assess LV mass and fibrosis before and after treatment of PA using CMR with contrast enhancement and T1 mapping.
Methods: Fifteen patients with newly diagnosed PA (PA1) and 24 age- and sex-matched healthy subjects (HS) were studied by CMR with contrast enhancement. Repeated imaging with a new scanner with T1 mapping was performed in 14 of the PA1 and 20 of the HS median 18 months after specific PA treatment and in additional 16 newly diagnosed PA patients (PA2).
Results: PA1 had higher baseline LV mass index than HS (69 (53–91) vs 51 (40–72) g/m^2; $P<0.001$), which decreased significantly after treatment (58 (40–86) g/m^2; $P<0.001$ vs baseline), more with adrenalectomy ($n=8$; −9 g/m^2; $P=0.003$) than with medical treatment ($n=6$; −5 g/m^2; $P=0.075$). No baseline difference was found in contrast enhancement between PA1 and HS. T1 mapping showed no increase in ECV as a myocardial fibrosis marker in PA. Moreover, ECV was lower in the untreated PA2 than HS 10 min post-contrast, and in both PA groups compared with HS 20 min post-contrast.
Conclusion: Specific treatment rapidly reduced LV mass in PA. Increased myocardial fibrosis was not found and may not represent a common clinical problem.

Key Words
- primary aldosteronism
- myocardial fibrosis
- left ventricular mass index
- cardiac magnetic resonance imaging
- T1 mapping

Endocrine Connections (2018) **7**, 413–424

Introduction

Primary aldosteronism (PA) has been recognized as the most common cause of secondary hypertension (HT), with estimated prevalence of 5–12% of all hypertensive subjects (1, 2). In patients with refractory HT, PA may be present in up to 20% (3). Unilateral aldosterone-producing adenoma (APA) and bilateral idiopathic hyperplasia (IHA) are the main subtypes; unilateral hyperplasia, inherited familial forms of PA and adrenal carcinoma are rare (4). Recommended treatment is adrenalectomy in unilateral PA and medical treatment with mineralocorticoid receptor antagonists in bilateral PA.

Several studies have demonstrated that PA patients have increased cardiovascular and renal morbidity compared with patients with essential HT (5, 6, 7, 8).

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https://doi.org/10.1530/EC-18-0039

Indeed, a recent study also demonstrated increased cardiovascular mortality (9). Increased prevalence of diabetes mellitus type 2 and the metabolic syndrome may also be present (10). Aldosterone, often associated with cortisol excess, causes deleterious effects on the cardiovascular system and increases metabolic risk, with end-organ damage that is at least partly independent of aldosterone effect on blood pressure (BP) (11). Experimental rat studies first demonstrated that chronic aldosterone infusion in the setting of high-salt intake induced oxidative stress and caused myocardial inflammation, accumulation of fibrillary collagen and subsequent interstitial fibrosis independent of the BP elevation (12, 13, 14). The cardiac injury was reversed either with adrenalectomy or mineralocorticoid receptor antagonist treatment (15). Subsequent echocardiographic studies have demonstrated increased left ventricular (LV) mass in PA compared with essential HT with similar BP (16). Echocardiographic videodensitometric and ultrasonic backscatter signal analyses have shown alterations in myocardial texture in PA, suggestive of increased collagen deposition and fibrosis (17, 18, 19). PA patients have more cardiac arrhythmias than in essential HT, presumably caused by cardiac fibrosis and/or LV hypertrophy (5, 20).

Specific PA treatment decreases LV mass both after adrenalectomy and after medical treatment with mineralocorticoid receptor antagonists in the long term, while echocardiographic studies with shorter follow-up time only showed significant reduction in LV mass after adrenalectomy (21, 22). In a recent meta-analysis, no difference in reduction of LV mass was found between adrenalectomy and mineralocorticoid receptor antagonist treatment after an average follow-up of four years, despite a larger reduction in BP after adrenalectomy (23).

Cardiac magnetic resonance imaging (CMR) offers a highly accurate, non-invasive, 3-dimensional assessment of cardiac structure, function and tissue characteristics. Contrast enhancement techniques including late gadolinium enhancement are accurate and reproducible in evaluating the presence of irreversible focal myocardial fibrosis/scars caused by myocardial infarction or hypertrophic cardiomyopathy (24, 25). Contrast enhancement is associated with adverse outcomes in several cardiac conditions (26, 27). However, the most commonly used contrast enhancement technique, late gadolinium enhancement, requires a comparison between affected and unaffected myocardium and may not be sensitive to early, potentially reversible diffuse myocardial fibrosis affecting the whole myocardium (28).

Recently, T1 mapping techniques assessing T1 relaxation times have allowed quantitative assessment of tissue composition and detection of both focal and diffuse interstitial myocardial fibrosis. T1 mapping performed before and after administration of gadolinium-based contrast media allows quantification of the extracellular volume (ECV), a surrogate measure for the extracellular space, which is expanded in myocardial fibrosis due to interstitial accumulation of collagen type I and III (28, 29).

Two CMR studies applying contrast enhancement techniques have revealed results consistent with increased myocardial fibrosis in PA, but only one applied a quantification technique for enhancement assessment (30, 31). One CMR study addressed the effect of medical mineralocorticoid receptor antagonist treatment in PA on cardiac volume overload and LV hypertrophy, with mineralocorticoid receptor antagonist treatment causing significant decreases in both LV mass and cardiac volumes (32). Further, CMR with T1 mapping has been used to evaluate the effect of HT and subsequent treatment with spironolactone on interstitial myocardial fibrosis in mice (33). Thus, the extent of fibrosis induced by PA and its reversibility is still incompletely known. We therefore aimed to assess myocardial mass and fibrosis in PA, using both dynamic contrast enhancement and T1 mapping, before and after adrenalectomy or medical treatment with mineralocorticoid receptor antagonist.

Subjects and methods

Study participants and design

This study was performed between October 2013 and March 2016 at Haukeland University Hospital, Bergen. Fifteen patients with recently confirmed PA (PA1) and 24 age- and sex-matched normotensive healthy subjects (HS) were included for baseline CMR with dynamic contrast enhancement (CMR1-CE) between October 2013 and June 2014 (Fig. 1). After completion of this substudy, they were invited to a follow-up CMR with T1 mapping (CMR2-T1, June 2015–March 2016), performed at least one year after the start of specific PA treatment. One patient was excluded from follow-up due to deterioration of renal function and four HS declined follow-up. An additional 17 newly diagnosed patients (PA2) were recruited of whom 16 completed the protocol.

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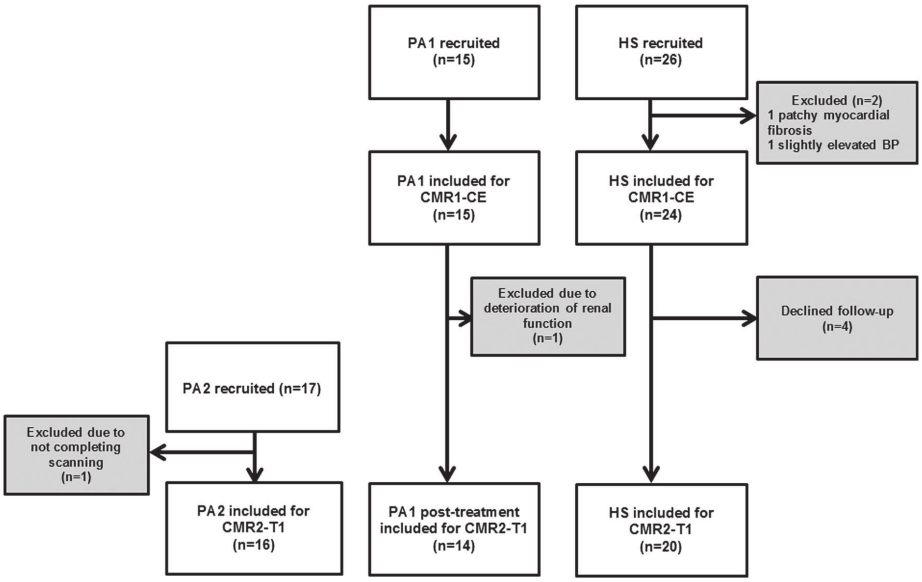

Figure 1
Study flow chart for the three study groups; PA1, HS and PA2. CMR1-CE, cardiac magnetic resonance imaging with dynamic contrast enhancement; CMR2-T1, cardiac magnetic resonance imaging with T1 mapping; HS, healthy subjects; PA1, primary aldosteronism group 1; PA2, primary aldosteronism group 2.

Diagnosis and clinical assessment

All PA patients had their diagnosis confirmed by saline infusion testing (SIT), and adrenal vein sampling (AVS) for subtype differentiation planned or performed at the time of study inclusion, according to current guidelines (4). Positive SIT was defined as a post-infusion plasma aldosterone level >140 pmol/L (34, 35). BP was measured in a standardized manner in all study groups, with three repetitive measurements in a seated position, after at least five minutes rest. Height and weight were recorded for all.

CMR performed with dynamic contrast enhancement (CMR1-CE)

PA1 and HS were examined with a 3T GE Signa Excite Scanner (Milwaukee, WI, USA). A phased-array cardiac coil with eight elements was used. All images were obtained during breath-hold with electrocardiography (ECG) triggering. For evaluation of LV mass and LV volumes, short-axis CINE views were obtained, using a Fiesta sequence. A pre-contrast mid-ventricular short-axis view and a 4-chamber view were then performed before a gadolinium-based contrast agent (Gadovist 1 mmol/mL, Bayer Pharma AG) 0.2 mL/kg body weight was administered as an intravenous bolus injection. An inversion recovery gradient echo sequence was applied. Contrast images in the same views as pre-contrast were then acquired at seven consecutive time points (2, 4, 6, 8, 10, 12 and 14 min) post-contrast.

CMR performed with T1 mapping (CMR2-T1)

The scanner used for the CMR1-CE was replaced in June 2014. The CMR2-T1 substudy was therefore performed with a 3T Siemens Magnetom Skyra (Erlangen, Germany). All images were obtained during breath-hold with ECG triggering. CINE views were performed using the same slice parameters as for the baseline CMR1-CE. The inversion recovery-based MOLLI sequence was used for T1 mapping (MyoMaps; Siemens, Erlangen) in accordance with the recommendations of the Society for Cardiovascular Magnetic Resonance (SMR) (36, 37). A native pre-contrast T1 mapping mid-ventricular short-axis view was then performed before the gadolinium-based contrast agent (Gadovist 1 mmol/mL, Bayer Pharma AG) 0.15 mL/kg body weight was administered as an intravenous bolus injection. Further T1 mapping mid-ventricular short-axis views were then performed 10, 15 and 20 min post-contrast.

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ECG, oxygen saturation and pulse were monitored continuously during all CMR procedures. BP was measured regularly during all image sequences.

Estimation of LV mass, volumes and function

For estimation of LV mass, LV volumes and function, the CMR images were analysed by using the software Segment, version 1.9 R3697 (http://segment.heiberg.se) (38). End-diastole and end-systole were defined and LV endo- and epicardial borders were manually delineated in all slices in end-diastole and end-systole (excluding papillary muscles). Values for LV mass, LV end-diastolic volume (LV-EDV), end-systolic volume (LV-ESV), stroke volume (LV-SV), cardiac output (CO) and ejection fraction (EF) were then calculated, and mass and volumes were indexed to body surface area.

Estimation of myocardial fibrosis from contrast enhancement images

For the contrast enhancement image acquisition, a protocol earlier published by Su and coworkers was adopted and slightly modified (31). From mid-ventricular short-axis views performed pre- and at the different time points post-contrast, three non-overlapping regions of interest (ROI) were placed both in the interventricular septum and within the LV lumen (Fig. 2A). The average signal intensities (SI) both from the interventricular septum and the lumen were calculated. The contrast enhancement value was defined as the following ratio adopted from (31):

$$\text{Contrast enhancement value} = \frac{\text{SI post-contrast myo} - \text{SI native myo}}{\text{SI post-contrast blood} - \text{SI native blood}}$$

SI post-contrast myo is the SI of myocardium after contrast, SI native myo is the SI of myocardium before contrast, SI post-contrast blood is the SI within the LV lumen after contrast and SI native blood is the SI within the lumen before contrast.

Estimation of myocardial fibrosis from T1 mapping

T1 mapping measurements were performed using syngo. via software (Siemens, Erlangen). One ROI covering most of the interventricular septum and one ROI within the LV lumen were drawn both from the native pre-contrast T1 mapping and the post-contrast T1 mapping short-axis views (Fig. 2B). The ECV was then calculated as a surrogate for the extracellular space using the following formula (28, 37):

$$\text{ECV (\%)} = \frac{\frac{1}{\text{T1 post-contrast myo}} - \frac{1}{\text{T1 native myo}}}{\frac{1}{\text{T1 post-contrast blood}} - \frac{1}{\text{T1 native blood}}} \times (100 - \text{haematocrit})$$

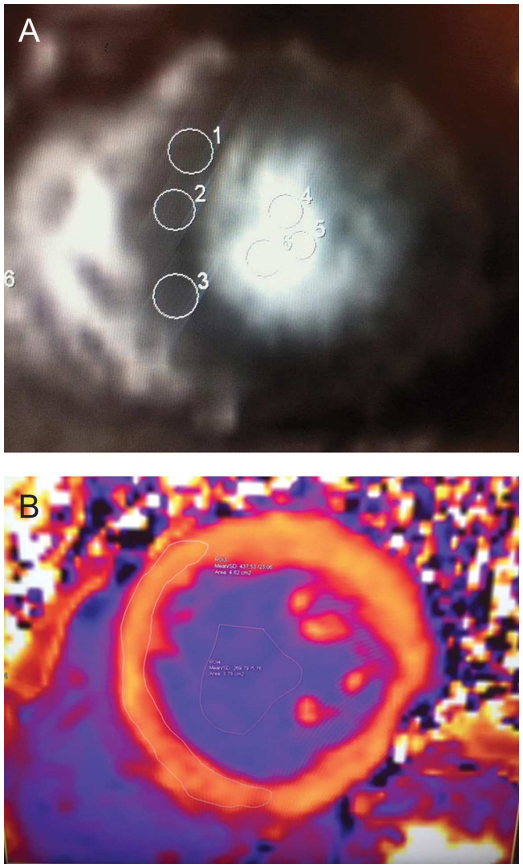

Figure 2
CMR imaging of the heart. (A) Post-contrast short-axis view of the heart with three ROIs drawn in the interventricular septum and lumen for calculation of contrast enhancement values (substudy CMR1-CE). (B) Post-contrast T1 mapping short-axis view of the heart with a ROI drawn in the interventricular septum and lumen for calculation of ECV (substudy CMR2-T1). CMR1-CE, cardiac magnetic resonance imaging with dynamic contrast enhancement; CMR2-T1, cardiac magnetic resonance imaging with T1 mapping; ECV, extracellular volume; ROI, region of interest.

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T1 post-contrast myo is the SI of myocardium after contrast, T1 native myo is the SI of myocardium before contrast, T1 post-contrast blood is the SI within the LV lumen after contrast and T1 native blood is the SI within the lumen before contrast.

All post-processing measurements for both CMR1-CE and CMR2-T1 were performed by two investigators (MG/KS or MG/TL), and the mean values of the measurements from the two investigators were used for statistical analyses.

Statistical analysis

All parameters are expressed as median and ranges. Student's *t*-test and Mann–Whitney *U* test were used to compare normally and not normally distributed continuous data between two groups, and Wilcoxon signed-rank test for within-patient not normally distributed data. One-way ANOVA was used to compare continuous data between more than two groups. For comparison of categorical variables, the chi-square test or Fisher's exact probability test was used. Bland–Altman plots were used for analysing the agreement between the measurements performed by the two investigators. A deviation of less than 10% between the values obtained by the two investigators was considered acceptable. *P* values less than 0.05 were considered statistically significant.

Written informed consent was obtained from all participants. The study was approved by the Regional Committee for Medical and Health Research Ethics (REC West/University of Bergen).

Results

Clinical characteristics: CMR with dynamic contrast enhancement (CMR1-CE)

The clinical characteristics of PA1 and HS at the time of the baseline CMR1-CE are shown in Table 1. PA1 and HS did not differ in terms of age or sex, but the PA1 patients had higher body mass index (BMI) and higher BP than the HS (median BMI 26 (range 22–34) vs 23 (18–27) kg/m^2; $P<0.001$ and BP 138/90 vs 118/75 mmHg; $P<0.001$ for both systolic and diastolic BP).

In PA1, AVS performed for subtype differentiation showed unilateral PA in 9/15 patients, the remaining had bilateral (5/15) or not representative (1/15) AVS. Three PA1 patients had been adrenalectomized within the past three months prior to inclusion for CMR1-CE. Five had started medical mineralocorticoid receptor antagonist treatment within the past 4–7 months, while the remaining had not yet started specific PA treatment.

One patient in PA1 had established coronary disease at baseline (paroxysmal atrial fibrillation). One had experienced several cerebral infarctions before the age of 50 years. Two had diabetes mellitus type 2, and two had chronic kidney disease, with estimated glomerular filtration rates of 47 and 54 (mL/min/1.73 m^2) at baseline, respectively. One PA patient without a history of coronary disease had sequelae from a small cardiac infarction found on his CMR. He was referred for cardiac evaluation.

Clinical characteristics: CMR with T1 mapping (CMR2-T1)

For PA1, the CMR2-T1 was performed at least one year after the start of specific treatment either with unilateral adrenalectomy or medical treatment with mineralocorticoid receptor antagonists (Table 1). Eight of 14 PA1 patients had been adrenalectomized, with median 18 (range 12–21) months since adrenalectomy. The remaining 6/14 were on medical mineralocorticoid receptor antagonist treatment, with median 21 (12–27) months since start of this medication. BP at follow-up was still significantly higher in the PA1 than in the HS group (median BP 134/81 vs 124/73 mmHg; systolic BP $P=0.02$/diastolic BP $P=0.012$), despite a median two antihypertensive drugs in the PA1 group. In PA1, only small non-significant BP changes appeared after treatment, with median systolic BP reduction 7 mmHg; $P=0.064$ and median diastolic BP reduction 5 mmHg; $P=0.148$ (not shown).

Among the 16 newly diagnosed patients in PA2 performing CMR2-T1, AVS showed unilateral disease in six patients and bilateral in 10 (Table 1). Only one of the newly diagnosed PA patients in PA2 had started specific PA treatment with spironolactone two months before the CMR2-T1; none in PA2 had yet been adrenalectomized. One of the PA2 patients had ischaemic coronary disease treated with percutaneous coronary intervention as comorbidity, two had atrial fibrillation (one paroxysmal and one radiofrequency ablated), and one had diabetes mellitus type 1. Median BP was 145/91 mmHg in PA2, with median three (0–6) antihypertensive drugs.

LV function and LV mass: CMR with dynamic contrast enhancement (CMR1-CE)

The results from LV function and LV mass measured with CMR1-CE are listed in Table 2. Baseline LV mass

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Table 1 Clinical and biochemical characteristics of PA1, HS and PA2 at substudy CMR1-CE and substudy CMR2-T1.*

	CMR1-CE		CMR2-T1		
	PA1	HS	PA1 post-treatment	HS	PA2
Participants in study group, *n*	15	24	14	20	16
Characteristics					
Males:females	12:3	17:7	11:3	13:7	12:4
Age (years)	58 (34–72)	53 (34–70)	59 (36–68)	53 (35–71)	57 (29–72)
Body mass index (kg/m^2)	26 (22–34)	23 (18–27)	26 (23–35)	22 (19–29)	29 (19–36)
Duration of hypertension (years)	15 (2–25)	0			10 (0–25)
Systolic blood pressure (mmHg)	138 (123–178)	118 (100–136)	134 (105–180)	125 (105–136)	145 (123–182)
Diastolic blood pressure (mmHg)	90 (60–108)	75 (61–88)	81 (69–105)	75 (60–94)	91 (75–115)
Number of antihypertensive drugs (*n*)	3 (0–5)	0	2 (0–6)	0	3 (0–6)
Biochemistry					
Hypokalaemia (*n*)**	14				7
Lowest s-potassium measured (mmol/L)	3.0 (2.3–3.7)				3.6 (2.5–4.0)
S-potassium (mmol/L)	3.3 (2.8–4.4)		4.3 (3.8–5.3)		3.7 (3.3–4.7)
P-aldosterone (pmol/L)	680 (384–2252)	***	412 (57–3420)	293 (116–855)	490 (277–2110)
P-renin activity (μg/L/h)	0.1 (0.1–3.0)	***	0.8 (0.2–26.7)	1.7 (0.3–4.0)	0.4 (0.1–0.8)
ARR (pmol/μg/h)****	6065 (168–22,520)		288 (21–2650)	224 (53–662)	1580 (610–3700)
Direct renin concentration (mIE/L)****					2.2 (2.0–8.2)
ARR (pmol/mIE)****					126 (46–1055)
Post-SIT aldosterone at diagnosis (pmol/L)	348 (169–1144)				297 (167–1460)
AVS results PA patients					
AVS lateralization (*n*)	9		8		6
AVS non-lateralized (*n*)	5		5		10
AVS not representative (*n*)	1		1		0
Treatment status PA patients					
Adrenalectomized (*n*)	3		8		0
Months since adrenalectomy	2.5 (0.5–3.0)		18 (12–21)		
MR antagonist treatment (*n*)	5		6		1
Spironolactone (daily dose in mg)	50 (25–100)		50 (25–75)		25
Eplerenone (daily dose in mg)			100 (*n* = 1)		
Months since start MR antagonist treatment	6 (4–7)		21 (12–27)		2
Not started specific treatment (*n*)	7		0		15

*Data shown as median (range) or as number; **hypokalaemia was defined as s-potassium <3.5 mmol/L at least once; ***p-aldosterone and p-renin activity in the healthy subjects (HS) were measured at the time of the CMR2-T1; ****ARR calculated with plasma renin activity: normal range <750 pmol/μg/h. The renin assay was in February 2016 changed from plasma renin activity (Gammacoat, Diasorin) to direct renin concentration (LIAISON Direct Renin, DiaSorin). 7/16 of the patients in the PA2 group therefore had their renin measured as direct renin concentration. ARR calculated with direct renin concentration: normal range <50 pmol/mIE with aldosterone <300 pmol/L, normal range <35 pmol/mIE with aldosterone >300 pmol/L.
ARR, aldosterone–renin ratio; AVS, adrenal vein sampling; CMR1-CE, cardiac magnetic resonance imaging with dynamic contrast enhancement; CMR2-T1, cardiac magnetic resonance imaging with T1 mapping; HS, healthy subjects; MR antagonist, mineralocorticoid receptor antagonist; PA1, primary aldosteronism group 1; PA2, primary aldosteronism group 2; SIT, saline infusion test.

indexed to body surface area differed significantly between the PA1 and HS group (LV mass index 69 (53–91) vs 51 (40–72) g/m^2; $P<0.001$). No differences between the two groups were found in cardiac function measured as LV-EDV index, LV-ESV index, LV-SV index, LV-EF, HR or CO index.

Dynamic contrast enhancement results (CMR1-CE)

Figure 3 illustrates the contrast enhancement values in PA1 and HS measured from 2 to 14 min post-contrast. No difference was found in contrast enhancement value between PA1 and HS at any time point. For both PA1 and HS, the contrast enhancement showed a trend towards reduced values from 2 to 14 min post-contrast.

LV function and LV mass-CMR with T1 mapping (CMR2-T1)

LV function and LV mass indexes for PA1, HS and PA2 measured with CMR2-T1 are shown in Table 2. At follow-up CMR2-T1, PA1 had a significantly reduced LV mass index (LV mass index 58 (40–86) g/m^2; $P<0.001$ vs baseline values). LV mass index in PA1 was still higher, but no longer significantly different to LV mass index in HS (LV mass index 58 (40–86) vs 57 (45–75) g/m^2; P=ns). When analysing the adrenalectomized (n=8)

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Table 2 CMR left ventricular parameters for PA1, HS and PA2 at substudy CMR1-CE and substudy CMR2-T1.*

	CMR1-CE		CMR2-T1			*P* value PA1 vs HS (CMR1-CE)**	*P* value PA1 post-treatment (CMR2-T1) vs PA1 baseline (CMR1-CE)**
	PA1 ($n=15$)	HS ($n=24$)	PA1 post-treatment ($n=14$)	HS ($n=20$)	PA2 ($n=16$)		
LV-EDV index (mL/m^2)	85 (72–109)	91 (62–120)	79 (61–104)	91 (76–121)	97 (69–116)	ns	ns
LV-ESV index (mL/m^2)	33 (20–52)	37 (24–51)	31 (20–47)	37 (23–54)	38 (19–51)	ns	ns
LV-SV index (mL/m^2)	53 (33–68)	53 (38–73)	48 (33–66)	53 (36–72)	57 (49–86)	ns	ns
LV-EF (%)	62 (45–74)	62 (52–67)	60 (48–73)	60 (42–71)	62 (51–74)	ns	ns
HR (beats/min)	64 (39–87)	66 (50–84)	63 (44–80)	53 (38–66)	54 (42–68)	ns	ns
CO index (L/min/m^2)	3.5 (2.2–4.4)	3.5 (2.3–5.0)	2.6 (2.0–4.5)	3.0 (1.9–4.0)	3.1 (2.0–4.8)	ns	ns
LV mass index (g/m^2)	69 (53–91)	51 (40–72)	58 (40–86)	57 (45–75)	70 (50–92)	<0.001	<0.001

*Data shown as median (range); **Student's *t*-test used.
CO, cardiac output; CMR, cardiac magnetic resonance imaging; CMR1-CE, cardiac magnetic resonance imaging with dynamic contrast enhancement; CMR2-T1, cardiac magnetic resonance imaging with T1 mapping; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; HS, healthy subjects; LV, left ventricular; PA1, primary aldosteronism group 1; PA2, primary aldosteronism group 2; SV, stroke volume.

and the medically treated ($n=6$) PA1 patients separately, the adrenalectomized patients had a larger and highly significant reduction of LV mass index, compared with the medically treated patients who showed a trend towards, but had no significant reduction in LV mass index (adrenalectomized PA1 median reduction of LV mass index −9 g/m^2; $P=0.003$ vs mineralocorticoid receptor antagonist treated PA1 reduction of LV mass index −5 g/m^2; $P=0.075$, not shown).

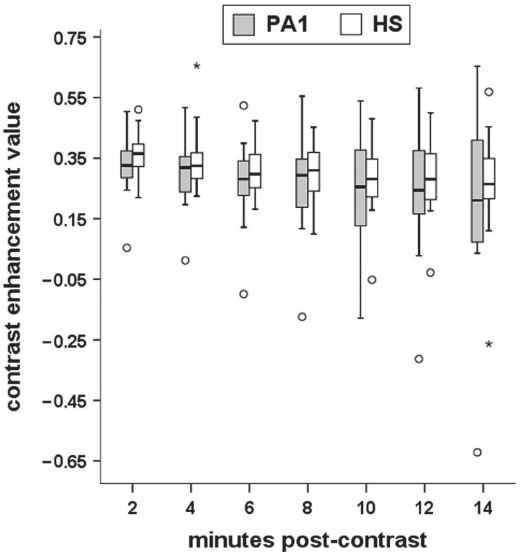

Figure 3
Box plots of dynamic contrast enhancement distribution at 2–14 min post-contrast for PA1 and HS (substudy CMR1-CE). The horizontal line indicates the median, the box represents the 25th–75th percentiles, and the whiskers represent the minimum and maximum value. Circles and asterisk indicate outliers. CMR1-CE, cardiac magnetic resonance imaging with dynamic contrast enhancement; HS, healthy subjects; PA1, primary aldosteronism group 1.

The newly diagnosed PA2 group had significantly higher LV mass index at CMR2-T1 than both the treated PA1 and HS (PA2 median LV mass index 70 (50–92); $P=0.019$ vs treated PA1; PA2 vs HS $P<0.001$).

For PA1, LV-EDV index showed a non-significant trend of reduction from baseline CMR1-CE to follow-up CMR2-T1 ($P=0.076$). In the HS group, LV-EDV remained unchanged (Table 2). When comparing the adrenalectomized vs medically mineralocorticoid receptor antagonist-treated PA patients, only the PA1 patients treated with mineralocorticoid receptor antagonists had a significant reduction in LV-EDV index (medically treated PA1 median change in LV-EDV index −14 mL/m^2; $P=0.03$, vs adrenalectomized PA1 −7 mL/m^2; $P=0.54$ (not shown)).

T1 mapping results (CMR2-T1)

The results from the T1 mapping performed with CMR2-T1 for both treated PA1, newly diagnosed PA2 and HS are shown in Fig. 4. No increase in ECV as a CMR marker of interstitial fibrosis was found in either PA1 or PA2 compared with HS. On the contrary, ECV was significantly lower in the untreated PA2 than in the HS 10 min post-contrast, and in both PA groups compared with the HS group 20 min post-contrast. No significant difference in ECV was found between PA1 and PA2 at any time-point post-contrast.

Discussion

In this study, we applied CMR to explore the effects of PA, before and after treatment, on myocardial morphology

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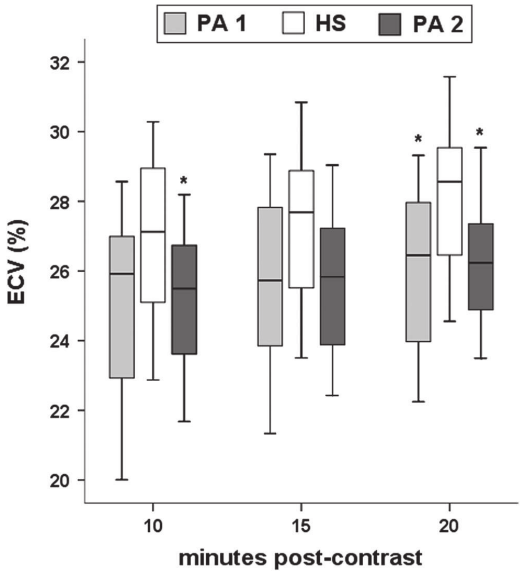

Figure 4
Box plots of the ECV distribution from T1 mapping at 10, 15 and 20 min post-contrast for PA1 (post-treatment), PA2 (newly diagnosed) and HS (substudy CMR2-T1). The horizontal line indicates the median, the box represents the 25th–75th percentiles, and the whiskers represent the minimum and maximum value. *$P<0.05$ vs HS. CMR2-T1, cardiac magnetic resonance imaging with T1 mapping; ECV, extracellular volume; HS, healthy subjects; PA1, primary aldosteronism group 1; PA2, primary aldosteronism group 2.

and function. The myocardial hypertrophy observed at diagnosis rapidly normalized with treatment, most pronounced after adrenalectomy. However, surprisingly neither dynamic contrast enhancement nor T1 mapping revealed myocardial fibrosis in newly diagnosed PA compared with HS.

The finding of lack of increased myocardial fibrosis in PA contrasts both earlier experimental studies and human ultrasonographic backscatter signal and videodensitometric studies (12, 13, 17, 18, 19). However, CMR is the recommended imaging modality for myocardial tissue characterization, and our findings also contrast two former 1.5 Tesla CMR studies using contrast enhancement techniques, which both showed higher contrast enhancement in PA than in essential hypertensive patients and healthy controls (30, 31). However, in one of these studies (30), contrast enhancement was measured as the presence of a non-infarct diffuse pattern of LGE, which is a less objective method to evaluate diffuse myocardial fibrosis (28). The other study used the same formula for dynamic contrast enhancement as we (31). However, there is still no universally accepted optimal dynamic contrast enhancement quantification technique, which may be reflected in the discrepancy of our results despite applying the same technique (28, 39). The PA patients in the two former studies were slightly younger than both our PA groups, but had a similar duration of HT. Possible differences in PA subtypes, severity of PA phenotype, gender distribution or severity of HT might also have influenced our discrepant results.

The recent developments of T1 mapping techniques enable quantitative assessments of changes in myocardial tissue composition and the presence of diffuse myocardial fibrosis, with estimation of the ECV percentage. T1 mapping thus facilitates a non-invasive alternative to myocardial biopsies and histochemical analyses (40). If myocardial fibrosis is present, expansion of the extracellular interstitial space should yield higher ECV. ECV has been shown to correlate well with histological fibrosis in several cardiac diseases (28), and this is the first time this technique is used to study PA. Similar to dynamic contrast enhancement, T1 mapping showed no evidence of increased myocardial fibrosis. In contrast, we found that both the newly diagnosed and treated PA group in our study had, at 20 min post-contrast, lower ECV than the healthy subjects. Thus, the left ventricular intracellular fraction is increased, which indicates more pronounced cardiomyocyte hypertrophy than ECV increase in both PA groups. Our findings clearly challenge the widely held conception that substantial myocardial fibrosis is typical in PA. Myocardial interstitial fibrosis caused by aldosterone and salt loading has been histologically proven in experimental animal studies (12, 41, 42). Human myocardial fibroblasts may differ from rodents when exposed to excess amounts of aldosterone, and the experimental levels of aldosterone and sodium in rodents may not reflect the exposure in humans with PA. To our knowledge, only one small human post-mortem study of five patients of high age with adrenal adenomas with suspected PA due to concomitant HT and low or low-normal s-potassium levels has been performed. That study showed histological fibrosis both in the myocardium as well as in the pancreas and lungs (43). However, ageing is associated with progressive fibrosis (44), and in the former study, the patients were approximately ten years older than in our study. Moreover, significant fibrosis may well be an end stage of long-standing and untreated PA. Furthermore, the fact that risks of cardiovascular complications in untreated PA are reduced to the same level as in essential HT after specific PA treatment, supports that irreversible fibrosis is not present (20). Essentially, this is the first study

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to apply CMR T1 mapping with quantitative assessment of ECV, which is the recommended gold standard parametric mapping technique for evaluation of diffuse myocardial fibrosis in a newly published consensus statement (37).

We also measured the effect of PA treatment on LV mass index and found a significant reduction after treatment. Regression of LV hypertrophy is associated with improved cardiovascular outcome in essential HT (45). When analysing the adrenalectomized and medically treated PA patients separately, reduction of LV mass index was highly significant only for the adrenalectomized group. Although not significant, we observed a strong trend towards reduction of LV mass index also in the medically treated group, with a median follow-up time of less than two years. These findings are in accordance with another CMR study, which showed significant regression of LV mass index in patients with suspected PA both after 3- and 6-month treatment with mineralocorticoid receptor antagonists (32). One echocardiographic study showed no significant change of LV mass index one year after start of mineralocorticoid receptor antagonist treatment, whereas another large study showed borderline significant reduction in LV mass index in medically treated PA after a median follow-up of 36 months (21, 22). The regression in LV mass after specific PA treatment occurred despite no significant reduction in BP. In the Framingham Offspring study, the aldosterone-to-renin ratio was identified as a key correlate of LV hypertrophy (46). Studies conducted in patients with essential HT have similarly shown a direct relationship between aldosterone levels and changes in LV geometry (47, 48). Thus, the reduction of LV mass after PA treatment is probably due to both reduced aldosterone levels and improved BP.

In the PA patients, we found a significant reduction in LV-EDV index only in the medically treated subgroup, in accordance with a former study (32). PA is associated with increased plasma brain natriuretic peptide and intracardiac volume overload, which is reversed by mineralocorticoid receptor antagonists or adrenalectomy. In the former study, the intracardiac volume overload was refractory to thiazides, while low doses of spironolactone showed a prominent diuretic effect. Small numbers may explain why no difference was found in LV-EDV index in the adrenalectomized patients in the current study.

A limitation of our study is that not all PA1 patients were completely treatment naïve at the time of the CMR1-CE; some had already undergone adrenalectomy and some had commenced treatment with mineralocorticoid receptor antagonists shortly before. Among the newly diagnosed PA2 patients participating in the CMR2-T1, only one patient had started specific PA treatment shortly before his CMR. PA patients with unilateral disease often have a more florid phenotype, and the relatively large proportion of patients with bilateral disease in PA2 may represent a less severe form of PA, which may have influenced the T1 mapping results.

The lack of an additional hypertensive control group is a limitation. If evidence of increased myocardial fibrosis in PA compared with in healthy subjects had been found, such a control group would have been necessary to determine whether these changes were caused by aldosterone excess or haemodynamic effects of hypertension itself. Furthermore, it could have shown whether the lower ECV in both treated and untreated PA post-contrast would be caused by aldosterone or haemodynamic effects of hypertension *per se*.

The replacement of the CMR scanner used at baseline with a new scanner for the follow-up CMR2-T1, may pose a further limitation. On the other hand, the use of two different techniques to study myocardial fibrosis with overlapping results certainly strengthens the study. A further asset is that all post-image analyses were performed by two different investigators. A limitation is that the dynamic contrast enhancement technique and T1 mapping were applied at different time points of the study, and we were therefore unable to examine the study participants with these two methods simultaneously, which would have given direct comparisons of the two methods. The PA1 patients had all received specific PA treatment for at least a year when examined with T1 mapping but were relatively treatment naïve when examined with dynamic contrast enhancement, which precludes direct comparisons of contrast enhancement and T1 mapping results in this group. In order to examine treatment-naïve PA patients with T1mapping, we therefore included also the newly diagnosed PA2 group for the CMR2-T1.

Habitual dietary sodium intake of the study participants was not recorded, and it is unknown whether the PA patients had been given specific dietary counselling to reduce their salt intake prior to or during the study period. In experimental animal studies, high levels of aldosterone infusion did not cause cardiovascular damage when sodium intake was very low (12). Similarly, in humans, extraordinarily high aldosterone concentrations in populations with chronic sodium deficiency was not associated with cardiovascular damage (49). A recent study demonstrated that dietary salt intake influenced the reduction of LV mass after PA treatment (50). Thus, dietary sodium intake may have influenced our results.

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In conclusion, specific PA treatment rapidly reduced LV mass. Both dynamic contrast enhancement and, notably, the recommended T1 mapping, showed no evidence of increased myocardial fibrosis in PA patients compared with healthy individuals. Thus, myocardial fibrosis does not seem to be a significant clinical problem in PA if diagnosed and treated within time frames commonly used in clinical practice.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This study was supported by the Regional Health Authorities in Western Norway.

Acknowledgements
The authors are very grateful to the participating patients and controls for their cooperation. They thank Jarle B Seter, Merete S Lemme, Anne Marie Kira, Jan A Monssen and Christel Jansen for their expert technical assistance.

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Received in final form 5 February 2018
Accepted 12 February 2018
Accepted Preprint published online 12 February 2018

Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

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ISBN: 978-82-308-3688-0