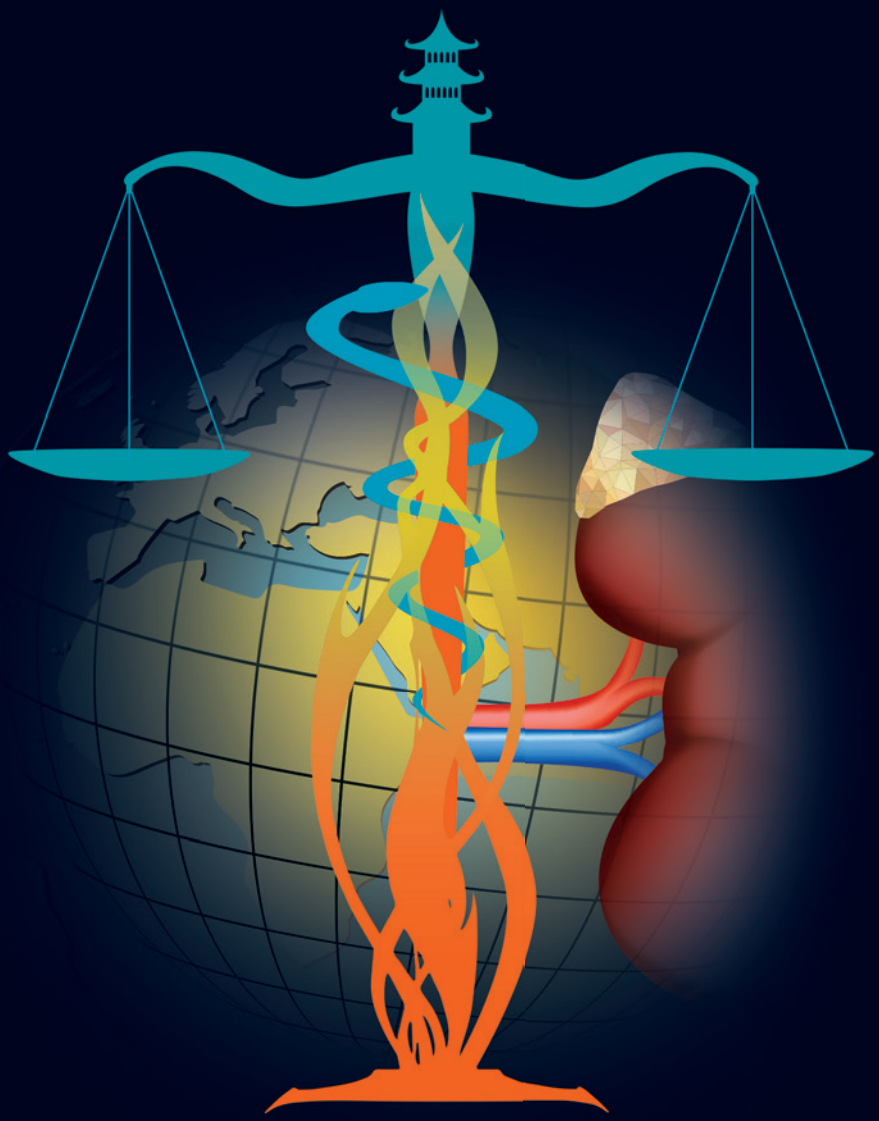
Primary Aldosteronism in General practice: Organ Damage, Epidemiology, and treatment

- PAGODE study -



Sabine Käyser

STELLINGEN

behorende bij het proefschrift:

Primary Aldosteronism in General practice: Organ Damage, Epidemiology, and treatment

1) Primair hyperaldosteronisme: wat niet weet, wat wél deert. *(dit proefschrift)*
2) De prevalentie van primair hyperaldosteronisme bij patiënten met nieuw ontdekte hypertensie in de huisartspraktijk is 1,4% tot 4,9%. *(dit proefschrift)*
3) Ook bij patiënten met hypertensie en een normaal kalium moet aan primair hyperaldosteronisme worden gedacht. *(dit proefschrift)*
4) Bij patiënten met nieuw ontdekte hypertensie op basis van primair hyperaldosteronisme komt linker ventrikel hypertrofie waarschijnlijk vaker voor dan bij patiënten met nieuw ontdekte hypertensie zonder primair hyperaldosteronisme. *(dit proefschrift)*
5) De aldosteron-renine ratio heeft geen toegevoegde waarde voor de keuze van het soort antihypertensivum bij de behandeling van essentiële hypertensie. *(dit proefschrift)*
6) Het verdient aanbeveling om het advies uit de richtlijn 'Primair hyperaldosteronisme' van de *Endocrine Society* (2016), namelijk om specifieke groepen patiënten te screenen op primair hyperaldosteronisme, op te nemen in de richtlijn 'Cardiovasculair risicomanagement' van Nederlandse huisartsen. *(dit proefschrift)*
7) Wat gij graag wilt dat u geschiedt, misgun dat ook een ander niet.
8) Het geven van passende leefstijladviezen is onmisbaar bij de behandeling van hypertensie: dit hoort een vast onderdeel te zijn van elk consult.
9) Natriumchloride is om te strooien, kaliumchloride is om te eten.
10) Het onterecht en ongedocumenteerd niet stellen van de diagnose hypertensie kan niet beschouwd worden als het beredeneerd afwijken van de richtlijn.
11) Vanuit het perspectief van de volksgezondheid is de publieke en politieke ophef over fipronil-eieren op zijn minst merkwaardig te noemen, in ogenschouw nemend dat de verkoop van sigaretten ongehinderd kan doorgaan.
12) Als je er middenin zit is het een drama, als je ernaar kijkt is het een klucht. *(bron: scheurkalender)*

Sabine Käyser
6 november 2018

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Primary Aldosteronism in General practice: Organ Damage, Epidemiology, and treatment

- PAGODE study -

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1

General introduction

This thesis addresses the question whether screening for primary aldosteronism (PA) should be implemented for all patients with newly diagnosed hypertension in primary care. As hypertension is the principle clinical clue to initiate a search for PA, this introductory chapter starts with a concise overview of hypertension in primary care. Subsequently, the background of PA is described, including the rationale and practice of diagnosing and treating PA. Special attention will be paid to the criteria that need to be fulfilled to accept screening for PA in the primary care setting. This information is put into the perspective of the main question of this thesis. Finally, the objectives and outline of this thesis are described.

HYPERTENSION

Persistently elevated blood pressure, also called hypertension, is worldwide the leading risk factor for disease burden in both men and women, and attributes to 9.4 million deaths per year.[1,2] Globally, the prevalence of hypertension in the general population is estimated to be 30% to 45%, with a higher prevalence with increasing age.[3,4] Although hypertension is a problem of both developed and developing countries, the prevalence differs between countries and ethnic groups within countries.[5,6] For example, the prevalence of hypertension in the Netherlands is 50.6% in Dutch-African men, and 32% in white-Dutch men. In woman the prevalence of hypertension is 41.9%, and 18.1%, respectively.[7]

In the majority of patients (approximately 85% to 90%) who are diagnosed with hypertension, hypertension is called primary or essential hypertension, and even after a comprehensive diagnostic analysis no specific cause of the high blood pressure can be established. In the other 10% to 15% of the patients an identifiable underlying cause of the elevated blood pressure can be detected, which is referred to as secondary hypertension. Examples of secondary hypertension are endocrine disease (PA, pheochromocytoma), renal disease, or substance abuse (licorice, cocaine). Secondary hypertension is thought to be relatively rare in the primary care setting, and is therefore usually not considered by general practitioners (GPs). However, given the potential for curative treatment in these patients, diagnosing underlying causes could be worthwhile. This may be particularly true for PA, for which a diagnosis is even more important since cardiovascular damage is disproportionally high when compared to essential hypertension (for further details see paragraph ‘Primary aldosteronism’ in this chapter).

Hypertension results in premature atherosclerosis of the arterial blood vessels and hypertrophy of the heart muscle. This ultimately becomes clinically manifest as cardiovascular disease such as stroke, cerebral hemorrhage, heart failure, acute coronary syndrome, (ruptured) aortic aneurysm, renal failure, and occlusive peripheral arterial disease. Moreover, hypertension is associated with other diseases such as diabetes and dementia.[8,9]

Most people are not aware that they have hypertension, as an elevated blood pressure is generally not associated with specific symptoms. Therefore, elevated blood pressure may be discovered during a (periodic) visit to a healthcare worker, a jobsite screening, or when hypertension has caused a complication (e.g. stroke). Rarely, hypertension is discovered when a patient presents with symptoms related to an underlying cause for secondary hypertension (e.g. central obesity in Cushing's syndrome).

Treatment of hypertension focuses on primary or secondary prevention of cardiovascular disease. The majority of patients has multiple cardiovascular risk factors in addition to hypertension, such as hypercholesterolemia and/or an unhealthy lifestyle (e.g. smoking). Because multiple risk factors potentiate each other, treatment is aimed at blood pressure reduction as well as management of other risk factors for cardiovascular disease.

In the Netherlands treatment of hypertension in the context of primary prevention of cardiovascular disease is usually started in primary care. In this setting an adapted SCORE (=Systematic COronary Risk Evaluation) table is used to guide therapy. The SCORE cardiovascular risk assessment model provides an estimated 10-year absolute risk for fatal and non-fatal cardiovascular disease, taking into account gender, age, smoking, systolic blood pressure, total cholesterol to high-density lipoprotein cholesterol ratio, and comorbidity.[10] According to the guidelines, all hypertensive patients should receive lifestyle advice, and periodic follow-up encounters should be planned to evaluate treatment. If blood pressure remains elevated, the cardiovascular risk score (SCORE) guides whether medical treatment should be started, or intensified, according to the guideline.[11]

Treatment of hypertension in the context of secondary prevention is usually started in referral centres where the patient is admitted for specialized investigations, or when a cardiovascular event has occurred. After return to primary care, the GP becomes in charge of monitoring antihypertensive treatment.

Diagnosis of hypertension in primary care

The Dutch primary care guideline concerning hypertension is called the 'Dutch guideline for Cardiovascular Risk Management' (Dutch CVRM guideline). According to this guideline hypertension is diagnosed when an elevated blood pressure is found at two or more different encounters. An elevated blood pressure is defined as repeated office systolic blood pressure levels of >140 mmHg (150-160 mmHg in patients ≥80 years), measured over a prolonged period of time.[11] For out-of-office blood pressure measurements different cut-offs for the diagnosis of hypertension should be used: when measured with an electronic home blood pressure device the cut-off for systolic blood pressure is >135 mmHg, and when using 24-hour ambulatory blood pressure monitoring (ABPM) the cut-off is >130 mmHg for the average 24-hour blood pressure. In this guideline the diastolic blood pressure is not taken into account, and the number of repeated measurements as well as the period over which the elevated office blood pressures should be measured are not specified.

In this thesis 'hypertension' is defined according to the guideline of the European Society of Hypertension: an elevated blood pressure is a systolic blood pressure of ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg, measured by an auscultatory or semiautomatic sphygmomanometer in the doctor's office.[3] For out-of office blood pressure measurements different cut-offs are used: ≥135 and/or 85 mmHg for an electronic home blood pressure measuring device, and ≥130 and/or 80 mmHg for the average 24-hour blood pressure using ABPM. It has to be noted that the definition of elevated blood pressure varies across international guidelines. Table 1, second column, shows examples of different definitions for hypertension.

Lower cut-offs for out-of-office blood pressures are used because the blood pressure in the doctor's office can be higher than normal due to awareness of the patient being at the doctor's office. If this 'awareness associated elevated blood pressure' is observed at multiple visits, and out-of-office blood pressure is normal, this phenomenon is referred to as 'white-coat' hypertension. White-coat hypertension is present in approximately 13% of the general population.[3] Possibly, patients with white-coat hypertension have an intermediate risk for cardiovascular disease, new-onset diabetes, and sustained hypertension compared to patients with normal blood pressure.[12-16] Conversely, a normal blood pressure in the doctor's office with an elevated blood pressure in the usual home environment is termed 'masked hypertension.' Masked hypertension is present in about 13% of all hypertensive patients. It has a similar or even higher incidence of cardiovascular events compared to patients with hypertension,[17] and an increased risk of diabetes compared to normotensive patients.[18-20] Despite the risk of not detecting white-coat hypertension or of missing masked hypertension, out-of-office blood pressure measurements are not required to confirm or refute the diagnosis of hypertension according to the current Dutch CVRM guideline.

PRIMARY ALDOSTERONISM

PA is a group of disorders in which aldosterone production has become autonomous, i.e. independent from the physiological regulation by the renin-angiotensin-aldosterone system (RAAS), and potassium. Under normal conditions the RAAS, plasma potassium, and adrenocorticotropic hormone (ACTH) are the major controllers of aldosterone secretion. In conditions of decreasing blood pressure and circulating volume, renin secretion by the renal juxtaglomerular cells stimulates the conversion of angiotensinogen to angiotensin I. Angiotensin converting enzyme (ACE) from vascular endothelium converts angiotensin I into angiotensin II. This latter compound is not only a strong vasoconstrictor, thus increasing blood pressure, but stimulates adrenal aldosterone secretion as well. The hormone aldosterone specifically interacts with renal tubular mineralocorticoid receptors. Through complex activation of several tubular ion channel transporters, the final effect is an increased

Table 1 Summary of guidelines concerning the definition of hypertension and diagnosing secondary hypertension

Guideline (country, year)	Criteria for the diagnosis of HT
Dutch guideline for Cardiovascular Risk Management (Netherlands, 2012)[11]	Office systolic BP >140mmHg (150-160 mmHg in patients ≥80 years). HBPM systolic >135mmHg. ABPM systolic >130mmHg.
NICE-guideline, Hypertension in adults: diagnosis and management (United Kingdom, 2016)[21]	If office BP is ≥140/90 mmHg offer ABPM to confirm the diagnosis of HT. If a person is unable to tolerate ABPM, HBPM is a suitable alternative to confirm the diagnosis of HT. Stage 1 HT: office BP ≥140/90mmHg and subsequent mean daytime ABPM or mean HBPM ≥135/85 mmHg. Stage 2 HT: office BP ≥160/100mmHg and subsequent mean daytime ABPM or mean HBPM ≥150/95 mmHg or higher. Severe hypertension: office systolic BP ≥180 mmHg or office diastolic blood pressure ≥110 mmHg.
American College of Cardiology/American Heart Association Task Force Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults (United States of America, 2017)[22]	Normal BP: <120/80 mmHg. Elevated BP: 120-129/<80 mmHg. Stage 1 HT: systolic 130-139 or diastolic 80-89 mmHg. Stage 2 HT: systolic ≥140 or diastolic ≥90 mmHg. Out-of-office BP measurements are recommended to confirm the diagnosis of HT and for evaluation of BP-lowering therapy.
The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline (United States of America, 2016)[23]	HT >140/90 mmHg

In which patients should screening for secondary hypertension be considered?	Patients in primary care specifically addressed?
- serum potassium ≤3.5 mmol/L - suspicion of chronic kidney damage - therapy resistant HT, defined as a systolic BP >140 mmHg despite the use of three different classes of antihypertensive agents in adequate dosage	Yes (primary care guideline): refer if one or more of the conditions in the previous column is met.
- HT in patients <40y - sudden worsening of HT - presentation of accelerated HT: 180/110 mmHg with signs of papilloedema and/or retinal haemorrhage - poor response to treatment - elevated creatinine or reduced eGFR - isolated hypokalemia	Yes: refer if one or more of the conditions in the previous column is met. Notably, the NICE-guideline applies to both general practitioners (GPs), and medical specialists.
In the presence of HT, and any of the following concurrent conditions: - age <30y - resistance to antihypertensive treatment - hypokalemia (spontaneous or diuretic induced) - muscle cramps or weakness - incidentally discovered adrenal mass - obstructive sleep apnea - family history of early-onset HT or stroke	No
Patients with: - sustained BP >150/100 mm Hg on each of three measurements obtained on different days - HT (BP >140/90 mm Hg) resistant to three conventional antihypertensive drugs (including a diuretic), or controlled BP (<140/90 mmHg) on ≥ four antihypertensive drugs - HT and spontaneous or diuretic-induced hypokalemia - HT and adrenal incidentaloma - HT and sleep apnea - HT and a family history of early onset HT or cerebrovascular accident at a young age (<40 years) - all hypertensive first-degree relatives of patients with PA	Yes: explicit recommendations for referral by primary care physicians of patients with suspected PA to specialized centres for further work-up.

Table 1 -Continued

Guideline (country, year)	Criteria for the diagnosis of HT
Hypertension Canada's 2017 Guidelines for diagnosis, risk, assessment, prevention, and treatment of hypertension in adults (Canada, 2017)[24]	Automated device: office BP ≥135/85 mmHg. Sphygmomanometer: office BP ≥140/90 mmHg. HBPM: mean ≥135/85 mmHg. ABPM daytime: mean ≥135/85 mmHg. ABPM: 24-hours ≥130/80 mmHg. ABPM or HPBM is preferred, but not obligate to diagnose HT.
Guideline for the diagnosis and management of hypertension in adults (Australia, 2016)[25]	Optimal BP: <120/80 mmHg. Normal BP: 120-129 and/or 80-84 mmHg. High normal: 130-139 and/or 85-89 mmHg. Grade 1 (mild) HT: 140-159 and/or 90-99 mmHg. Grade 2 (moderate) HT: 160-179 and/or 100-109 mmHg. Grade 3 (severe) HT: ≥180 and/or ≥110 mmHg. Isolated systolic HT: >140 and <90 mmHg.
The Japanese Society of Hypertension Guidelines for the Management of Hypertension (Japan, 2014)[26]	BP ≥140/90 mmHg ABPM or HPBM is preferred but not obligate to diagnose HT. HBPM is preferred over ABPM.

renal sodium reabsorption (with concomitant water reabsorption) and renal potassium excretion (Figure 1). The ensuing increase in circulating volume inhibits renin and aldosterone secretion by negative feedback. In patients with PA volume expansion and inhibition of aldosterone secretion are uncoupled, as evidenced by a suppressed renin level.[28] Therefore, PA is characterized by inappropriately high plasma aldosterone levels for sodium status, and by suppressed renin levels.

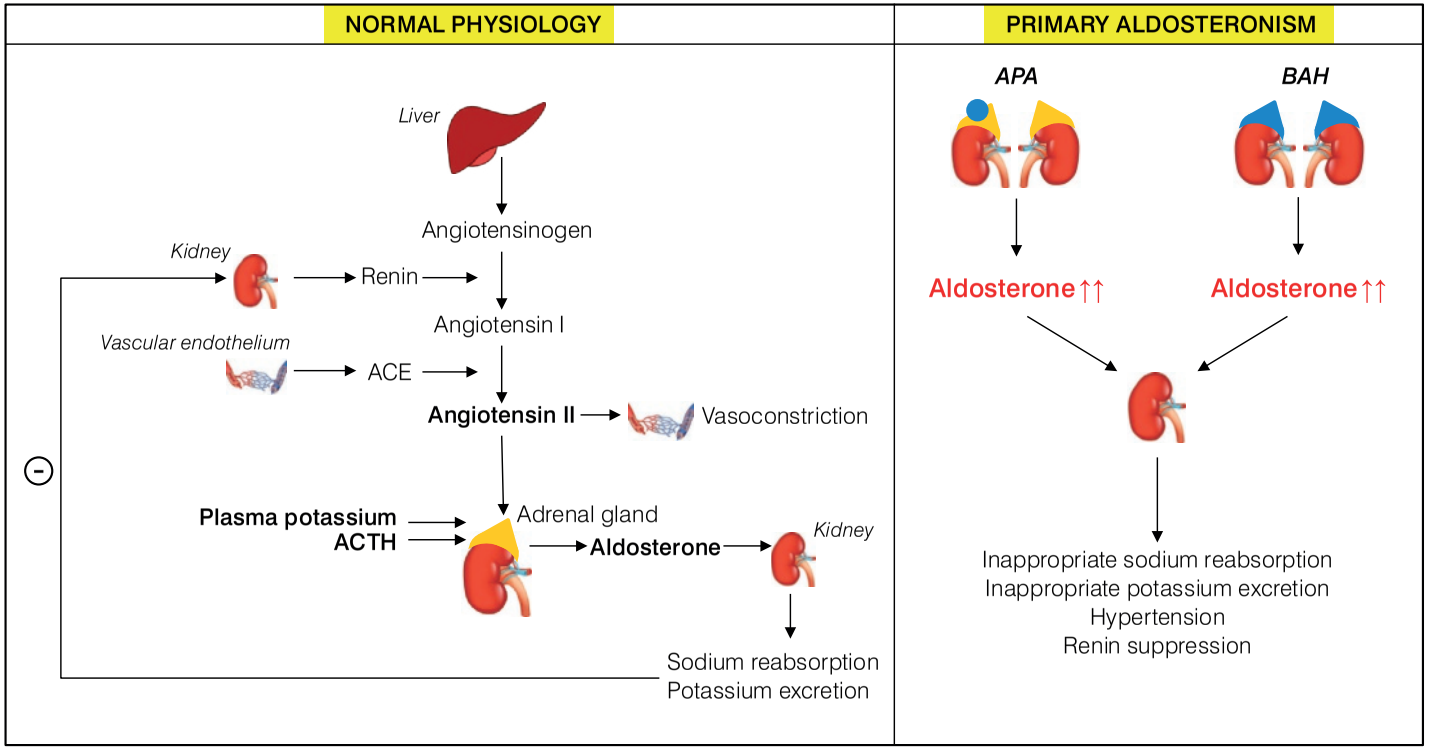
Previously, PA was thought to be a rare cause of hypertension (prevalence <1% of the hypertensive population). However, over the last 25 years it has become clear that PA is more frequent, affecting 1% to 23% of all hypertensive patients.[29,30] This wide range indicates uncertainty regarding the prevalence, and it might be explained by variability in diagnostic procedures, differences in patient selection (primary care, referral centres, severity of hypertension), and the current reappraisal of the normokalemic variant of PA.[31-34]

In which patients should screening for secondary hypertension be considered?	Patients in primary care specifically addressed?
Hypertensive patients with one of the following: - unexplained spontaneous hypokalemia (potassium <3.5 mmol/L) or marked diuretic-induced hypokalemia (potassium <3.0 mmol/L) - resistance to treatment with ≥3 drugs - an incidental adrenal adenoma	No
It should be considered in patients with HT, especially those with moderate-to-severe or treatment-resistant HT, and those with hypokalemia. Referral to a specialist for investigation is recommended when PA is suspected.	Yes
Selective screening of high-risk groups for PA such as those with resistant (refractory) HT, grade II-III HT, and hypokalemia.	No However, the Japan Endocrine Society has a PA-guideline which is especially developed for GPs (2009): '… recommends measurement of plasma renin activity and plasma aldosterone concentration in all patients initially diagnosed as hypertensive …' [27]

Signs suggestive of PA are hypokalemia (spontaneous or diuretic-induced), therapy resistant hypertension, a family history of PA, or an incidentaloma in the presence of hypertension. However, specific clinical features are often lacking, and this is part of the explanation why it has been reported that the diagnosis of PA may be delayed by a mean of eight years.[35]

There are two major subtypes of PA: the aldosterone-producing adenoma (30% to 50% of the cases, also known as Conn's disease), and bilateral adrenal hyperplasia (50% to 70% of the cases; Figure 1). Treatment of an aldosterone-producing adenoma differs from treatment of bilateral adrenal hyperplasia. An adenoma is preferably removed by adrenalectomy,[36-38] while bilateral adrenal hyperplasia is treated with a mineralocorticoid receptor antagonist (such as spironolactone or eplerenone).[39] Because of these different treatment strategies, subtype differentiation is pivotal for selecting those patients with PA who are suitable for surgical treatment.

Figure 1 Aldosterone secretion in normal physiology and in primary aldosteronism



ACE, angiotensin converting enzyme. ACTH, adrenocorticotropin hormone. APA, aldosterone-producing adenoma. BAH, bilateral adrenal hyperplasia.

Timely and correct detection of PA is relevant for four reasons:

1) Hypertension due to underlying autonomous aldosterone secretion causes more severe cardiovascular damage than the same blood pressure levels in patients with essential hypertension.[40-42]
2) PA requires specific treatment: not only the hypertension should be controlled, but the deleterious effects of the aldosterone excess should be counteracted as well.[23]
3) Outcomes of treatment are superior in patients with younger age, and a shorter duration of hypertension.[43-46]
4) Quality of life is significantly lower in patients with untreated PA compared to patients with essential hypertension, and it improves or even normalizes by specific therapy.[47,48]

Diagnostic work-up in primary aldosteronism

The diagnostic workup for PA comprises three steps: case detection, confirmation, and subtype differentiation.[23] For detection of PA the Endocrine Society guideline recommends to measure plasma aldosterone and renin levels in a venous blood sample with calculation of the aldosterone-to-renin ratio (ARR). This screening test is suitable for application in primary care. If the results of these tests suggest the presence of PA, referral to a specialist (internist or endocrinologist) is needed to confirm or refute the diagnosis (due to the need for specialized investigations, e.g. a confirmation test, computed tomography etc.). As both aldosterone and renin may be influenced by many factors, a blood sample must be drawn under prespecified conditions: mid-morning after the patient has been out of bed for two hours, and the patient has been sitting for 5-15 minutes. The use of antihypertensive medication should be stopped or adjusted, as some of these agents can interfere with the test results (e.g. beta blockers suppress renin).[23] An elevated ARR is suspect for PA, and therefore requires further testing.[49]

Cut-offs of the ARR for screening for PA vary widely in literature due to the use of different measurement units, which depend on the type of biochemical assay. The Endocrine Society guideline provides cut-off values of the ARR for the different measurement units, but leaves room for different cut-offs.[23] There is still no definite agreement on the optimal ARR cut-off value.

Because of its limited specificity, an elevated ARR by itself is not sufficient to definitely diagnose PA. A confirmation test is mandatory by demonstrating non-suppressible aldosterone secretion. Only patients who present with '*spontaneous hypokalemia, plasma renin below detection levels, plus plasma aldosterone concentration >20 ng/dL (550 pmol/L)*' may not need further confirmatory testing.[23] In all other cases the Endocrine Society guideline recommends to choose one of four confirmation tests: the oral sodium loading test, the intravenous sodium loading test, the fludrocortisone suppression test, or the captopril challenge test.[23] The work-up for subtype differentiation of PA is the last step to determine the most appropriate treatment. We will not discuss this, as this is beyond the context of this thesis.

Screening for primary aldosteronism in primary care

In the Netherlands the diagnosis and treatment of hypertension typically belong to the domain of the GP, who generally follows the Dutch CVRM guideline to diagnose and treat hypertension.[11] With regard to secondary hypertension the guideline advises to consider a underlying cause of hypertension if a patients presents with clinical clues for such underlying cause.[11] This guideline has no specific recommendations on how to diagnose PA. Regarding PA, the guideline advises to refer patients with hypertension and hypokalemia, or patients with therapy resistant hypertension for specialist consultation. However, as hypokalemia is absent in approximately 70% of all patients with PA, it is likely that the majority of PA patients will be missed if the current Dutch CVRM guideline is followed.[50] In addition, most GPs are unfamiliar with PA and its diagnostic process. Consequently, screening for PA is rarely performed in primary care. This results in a delay before the diagnosis of PA is made, and targeted treatment can be initiated. The diagnostic difficulties are illustrated by a typical patient as described in the following patient vignette (Box 1).

In this patient, the difficult to control blood pressure appeared to be caused by underlying PA. The duration between the diagnosis of hypertension and the diagnosis of PA in this clinical example was almost two years. This case is not unusual in clinical practice, not only in the Netherlands but worldwide. Although two years is too long to detect PA, the mean delay is even longer.[35,51] The current practice raises the question: is the current Dutch CVRM guideline really up to date to diagnose secondary causes of hypertension, in particular PA? For comparison of the recommended strategies of the most relevant international guidelines for screening for secondary hypertension, in particular specified for the primary care setting, see Table 1.

In this thesis we describe several studies that address the question whether screening for PA in all newly diagnosed hypertensive patients should be introduced as a standard procedure in primary care. For justification of screening for a disease we need to consider the ten suitability criteria (or principles) of Wilson and Jungner (World Health Organization, 1968) (Table 2, left column).[52] Table 2, right column, shows that when applied to the detection of PA, several criteria are not fully met.

Box 1 Patient vignette

Just a patient with difficult-to-control hypertension?

Mrs. P is a 58 year old woman who visits her GP in 2015 because of dizziness. She works as an employee at a bank and perceives a high level of job stress. Her office blood pressure is 164/99 mmHg. The GP considers her blood pressure to be related to the stress at work, to the menopause, or to both factors, and advises her to perform home blood pressure measurements.

Two months later Mrs. P hands in an average home blood pressure of 154/98 mmHg (normal <135/85 mmHg). Standard blood tests for hypertension are unremarkable, including a plasma potassium of 3.6 mmol/L (normal 3.5-4.5 mmol/L). Assessment of the cardiovascular risk profile according to the Dutch CVRM guideline[11] indicates no smoking and no family history of cardiovascular disease. Body mass index is 26 kg/m^2 and physical exercise is rarely performed. A total cholesterol to high-density lipoprotein cholesterol ratio of 5.8 indicates her 10-years risk for cardiovascular disease to be 11%. The GP initially recommends lifestyle changes: reduction of job-related stress and engaging in more regular physical exercise.

Four months later home blood pressure is 158/98 mmHg and therefore the GP starts the antihypertensive amlodipine (5 mg daily). Two months later our patient returns with a home blood pressure of 150/90 mmHg. Mrs. P admits she forgot her medication a few times and the GP also discusses with her the obstacles to change her lifestyle more extensively. She is absorbed by her work, so exercising and preparing dinner with fresh ingredients is still not a priority. She promises to intensify exercise together with a friend to two times a week. A new evaluation is scheduled in six months but then her home blood pressure has not substantially decreased: 156/92 mmHg. The GP prescribes additional lisinopril (10 mg daily) and two weeks later her blood pressure is 146/88 mmHg, plasma potassium is 3.6 mmol/L and the eGFR is unchanged within the normal range.

For the time being the GP is satisfied with this result, and a next visit is scheduled in four months. At that point it appears that Mrs. P home blood pressure has not improved at all: 154/92 mmHg. She insists she takes her medication every morning, and she feels tired and 'shaky'. The GP decides to add chlorthalidone 12,5 mg once daily. One month later plasma potassium has decreased to 3.3 mmol/L. The GP advises a potassium enriched diet, but despite eating a lot of beans, tomatoes, and bananas, potassium remains too low: 3.1 mmol/L after two weeks. While using now three antihypertensive agents, Mrs. P remains hypertensive: 150/90 mmHg. Therefore, the GP switches from chlortalidone to spironolactone 25mg. Three months later she returns being very tired, and although plasma potassium has increased to 3.7 mmol/L, her blood pressure is still uncontrolled: 148/92 mmHg. Because of the persistent difficulty to control blood pressure satisfactorily, the GP consults with her colleague internist in the hospital who advises referral because of the suspicion of PA. The internist advises to stop the spironolactone and lisinopril (as these interfere with biochemical testing in the diagnostic work-up for PA), and to start potassium suppletion. In the hospital the diagnosis of PA is confirmed as documented by an elevated plasma aldosterone, suppressed renin, elevated ARR, and a positive saline infusion test.

Table 2 Criteria of early disease detection by Wilson and Jungner applied to screening for primary aldosteronism in Dutch primary care

	Criteria for screening in general	Criteria applied for screening for primary aldosteronism
1.	The condition sought should be an important health problem.	PA is an important health problem in a considerable number of patients (assuming an incidence of 5%, there are approximately 5000 new patients with PA per year in the Netherlands). In addition, PA carries a high risk of cardiovascular complications, and untreated PA is associated with a poor quality of life.
2.	There should be an accepted treatment for patients with recognized disease.	Treatment depends on the subtype of PA: an adenoma is preferably treated by adrenalectomy, while bilateral hyperplasia should be treated with mineralocorticoid receptor antagonists. Both treatments are highly effective.
3.	Facilities for diagnosis and treatment should be available.	In the Netherlands healthcare facilities for diagnosis and treatment of PA are widely available.
4.	There should be a recognizable latent or early symptomatic stage.	Early symptoms consist of hypertension, and in some patients of hypokalemia.
5.	There should be a suitable test or examination.	A suitable test is available for screening: a venous blood sample for measurement of aldosterone and renin.
6.	The test should be acceptable to the population.	The screening test can be performed in primary care by adding the screening to the standard laboratory tests, which are performed when hypertension is diagnosed.
7.	The natural history of the condition, including development from latent to declared disease, should be adequately understood.	The pathogenetic mechanism of PA, and development from latent to declared disease, are reasonably well understood.
8.	There should be an agreed policy on whom to treat as patients.	After screening a confirmation test will confirm or refute the diagnosis of PA, thus guiding which patients will benefit from PA specific treatment.
9.	The cost of case finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.	An early diagnosis of PA might be cost-effective, but it is unknown if this is valid for the Dutch population.
10.	Case-finding should be a continuing process and not a “once and for all” project.	Screening for PA can be easily incorporated in standard hypertension care.

PA, primary aldosteronism.

In conclusion, there are several criteria for early screening for PA in primary care that are not yet met. Several aspects, such as the prevalence of PA in primary care,[53,54] and cost-effectiveness[55,56] are insufficiently known. In addition, it is also uncertain whether PA has already caused cardiovascular damage at the stage when the hypertension is detected for the first time, which would give additional weight to arguments for early screening.

However, there are also well-founded reasons to consider detection of PA at an early stage in the primary care setting:

1) Hypertension is almost invariably diagnosed in primary care.
2) Due to interference of antihypertensive medication with the ARR, screening for PA is best done when the patient takes no antihypertensive medication. This is most easily done in newly diagnosed cases of hypertension. In addition, a wash-out period of antihypertensive medication in case of later screening is not without risk.[57]
3) Although the exact prevalence of PA in primary care is still a matter of debate, even in case of a low proportional prevalence, the absolute number of patients with PA is high.
4) PA-induced cardiovascular damage may be reversible by proper early treatment of PA.[58,59]
5) Using the ARR for early detection of PA may have an additional advantage. Even if the ARR does not indicate the possibility of PA, it reflects the state of the renin-angiotensin-aldosterone system, especially the renin component, and thus may predict the presence or absence of an antihypertensive response to ACE-inhibitors, angiotensin receptor blockers, calcium channel blockers, and diuretics. If this is proven to be true, it would mean that measurement of the ARR in hypertensive patients might be even a more cost-effective tool, since it both excludes PA and directs antihypertensive therapy.

OBJECTIVES AND OUTLINE OF THE THESIS

The major inspiration for the studies described in this thesis came from this question: should screening for PA be introduced in primary care? To address this question we first want to address the following specific objectives in the studies reported in this thesis:

1) To assess the prevalence of PA in previous studies carried out in both primary care and referral centres, we describe a meta-analysis of studies that were performed to assess the prevalence of PA in *Chapter 2*.
2) To assess the proportion of patients with PA in patients with newly diagnosed hypertension in Dutch general practice. In *Chapter 3* the prevalence of PA in Dutch primary care centres is estimated in patients with newly diagnosed hypertension.

3) To assess the presence of cardiovascular and renal damage in patients with newly diagnosed hypertension and PA at the time of diagnosing hypertension in primary care. In *Chapter 4* we explore cardiovascular damage in patients with newly diagnosed hypertension with and without PA.
4) To study whether the blood pressure response to antihypertensive treatment in patients with newly diagnosed hypertension in general practice can be predicted by the level of the ARR. In *Chapter 5* the association between the status of the RAAS (expressed as ARR) and the blood pressure response to antihypertensive treatment within one year of treatment is assessed.

In *Chapter 6* we summarize the main findings of this thesis, and finish with a discussion of the clinical implications, recommendations for future research, and key messages.

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2

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

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ABSTRACT

Context

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

Objective

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

Data sources

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

Study selection

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

Data extraction

Dual extraction and quality assessment were the forms of data extraction.

Data synthesis

Thirty-nine studies provided data on 42510 patients (nine studies, 5896 patients from primary care). Prevalence estimates varied from 3.2% to 12.7% in primary care and from 1% to 29.8% in referral centers. Heterogeneity was too high to establish point estimates (I^2 = 57.6% in primary care; 97.1% in referral centers). Meta-regression analysis showed higher prevalences in studies 1) published after 2000, 2) from Australia, 3) aimed at assessing prevalence of secondary hypertension, 4) that were retrospective, 5) that selected consecutive patients, and 6) not using a screening test. All studies had minor or major flaws.

Conclusions

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

INTRODUCTION

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

Identifying PA as the underlying cause of (therapy resistant) hypertension is considered important for two reasons. First, PA is associated with an increased rate of cardiovascular complications.[1-3] Second, specific treatment by mineralocorticoid receptor antagonists or adrenalectomy is effective in reducing these cardiovascular complications[4-6] and health costs.[7] Therefore, an early diagnosis and treatment of PA are key for increasing the chance of improvement and even cure of hypertension, and for preventing cardiovascular complications.[8-10]

In primary care centers, reported prevalences vary from 6% to 13%; in secondary care centers, prevalences of 23% to almost 30% have been reported.[11-13]

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

METHODS

Data sources and searches

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

We conducted a systematic search on four electronic databases: PubMed, EMBASE, Web of Science, and the Cochrane Library were searched for English, German, French, Spanish, and Dutch articles on the prevalence of PA published between January 1, 1990, and January 31, 2015. We used the following search terms: ((“Hyperaldosteronism” [Mesh]) OR (hyperaldosteronism [Title/Abstract]) OR (aldosteronism [Title/Abstract]) OR (Conn syndrome [Title/Abstract]) OR (Conns syndrome [Title/Abstract]) OR (Conn’s syndrome [Title/Abstract]) OR (hyperaldosteronism [Other Term]) OR (aldosteronism [Other Term]) OR (Conn syndrome [Other Term]) OR (Conn’s syndrome [Other Term]))
AND

((“Prevalence” [Mesh]) OR (prevalence [Title/Abstract]) OR (prevalences [Title/Abstract]) OR (occurrence [Title /Abstract]) OR (occurrences [Title/Abstract]) OR (“Incidence” [Mesh]) OR (incidence [Title/Abstract]) OR (incidences [Title/Abstract]) OR (“Epidemiology” [Mesh]) OR (“epidemiology” [Subheading]) OR (epidemiology [Title/Abstract]) OR (epidemiologic [Title/Abstract]) OR (epidemiological [Title/Abstract]) OR (prevalence [Other Term]) OR (prevalences [Other Term]) OR (incidence [Other Term]) OR (incidences [Other Term]) OR (occurrence [Other Term]) OR (occurrences [Other Term]) OR (epidemiology [Other Term]) OR (epidemiologic [Other Term]) OR (epidemiological [Other Term])) (see Supplementary File 1).

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

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

Study selection

Two researchers (SK and TD) independently assessed eligibility of retrieved articles on title and abstract. Full-text articles were retrieved if necessary.

Studies were considered eligible for inclusion if they met the following criteria:

1) Data presented as an original study, short report, or letter on the prevalence of PA;
2) Prospective, retrospective, or cross-sectional study design;
3) Study population of adult patients (≥18 years of age) with hypertension;
4) Use of a confirmation test (intravenous sodium loading test (IV SLT), oral SLT, captopril suppression test, or fludrocortisone suppression test) to verify the diagnosis of PA (performed in at least 50% of the patients with positive screening test).[13]

Studies were excluded if:

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

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

Data extraction and quality assessment

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

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

After the final inclusion, SK and TD rated the methodological quality and risk of bias in individual studies using the "Methodological evaluation of Observational REsearch (MORE) – Observational Studies of Incidence or Prevalence of Chronic Diseases" protocol.[15] This protocol comprises the following items:

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

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

Data synthesis and analysis

To estimate the prevalence of PA, we computed random effect pooled proportions for primary care and referral centers separately.[16] Logit transformation was used to get quantities from prevalence.

To explore sources of heterogeneity, we performed random effects logistic regression analysis with prevalence of PA as dependent variable.[17,18] We based the choice of variables on controversies discussed in the Endocrine Society guideline[13] and on our expectations of explanatory factors for bias in prevalence studies. We distinguished three categories of potential predictors of prevalence estimates:

1) Time: studies published in different periods (two categories: 1990 till 2000, and after 2000);
2) Geographic region where studies were performed: Asia, Australia, Europe, Latin America, and United States of America;

3) Factors concerning study design:
   a) Data collection (prospective or retrospective);
   b) Study objective (to assess the prevalence of PA, to assess the prevalence of secondary hypertension, other);
   c) Method of patient selection (consecutive, convenience, self selection). We defined convenience as arbitrarily selected individuals from the target population other than general such that each individual had uncontrolled probability of selection;[19]
   d) Limited to therapy resistant hypertension or not;
   e) Plasma potassium level at inclusion (normokalemia or hypokalemia (serum potassium ≤3.5 mmol/L));
   f) Medication regimen (medication adjusted according to the Endocrine Society guideline, medication adjusted otherwise, only mineralocorticoid receptor antagonists discontinued or medication unchanged);[13]
   g) Potassium level at confirmation testing (corrected hypokalemia or normokalemia);
   h) Type of screening test (ARR-based test, no screening test, other screening test);
   i) Number of screening tests (one test or multiple tests);
   j) Patient position during screening tests (supine or not supine);
   k) Cut-off levels used for screening tests (unrestrictive or restrictive). We included only studies using ARR-based tests. Unrestrictive was arbitrarily defined as an ARR cut-off value of 20-60 (aldosterone in ng/dL and renin in ng/mL/h); restrictive was defined as an ARR cut-off level of more than 60 or an ARR cut-off level of 20-60 with a plasma aldosterone level of more than 15 ng/dL and/or a suppressed renin level.
   l) Percentage of patients with positive screening who underwent a confirmation test (100% or ≥80% or 50-80%);
   m) Type of confirmation test (IV SLT, oral SLT, captopril suppression test, fludrocortisone suppression test[13]);
   n) Cut-off levels used for the IV SLT confirmation test (unrestrictive or restrictive). Unrestrictive was defined if the used cut-off level of plasma aldosterone after saline was at least 8 ng/dL, and restrictive if that cut-off level was lower than 8 ng/dL. The number of studies concerning other confirmation tests were too low for analysis of the effects of different cut-off levels.

We explored the association of each of these factors with the estimate of the prevalence of PA individually in a univariate analysis. To correct for correlations between factors among studies, we built a model with the set of explanatory factors that remained significant in a multivariable model. We set the entry level of potentially valid predictors for the model at $p=0.10$. Because of the relatively low number of studies in primary care, we could only develop a model for referral centers.

Because gender is not considered a factor in the diagnosis of PA and studies were unselective with respect to gender, we did not take gender into account in the statistical analysis.

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

Statistical analysis

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

RESULTS

Search results and study selection

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

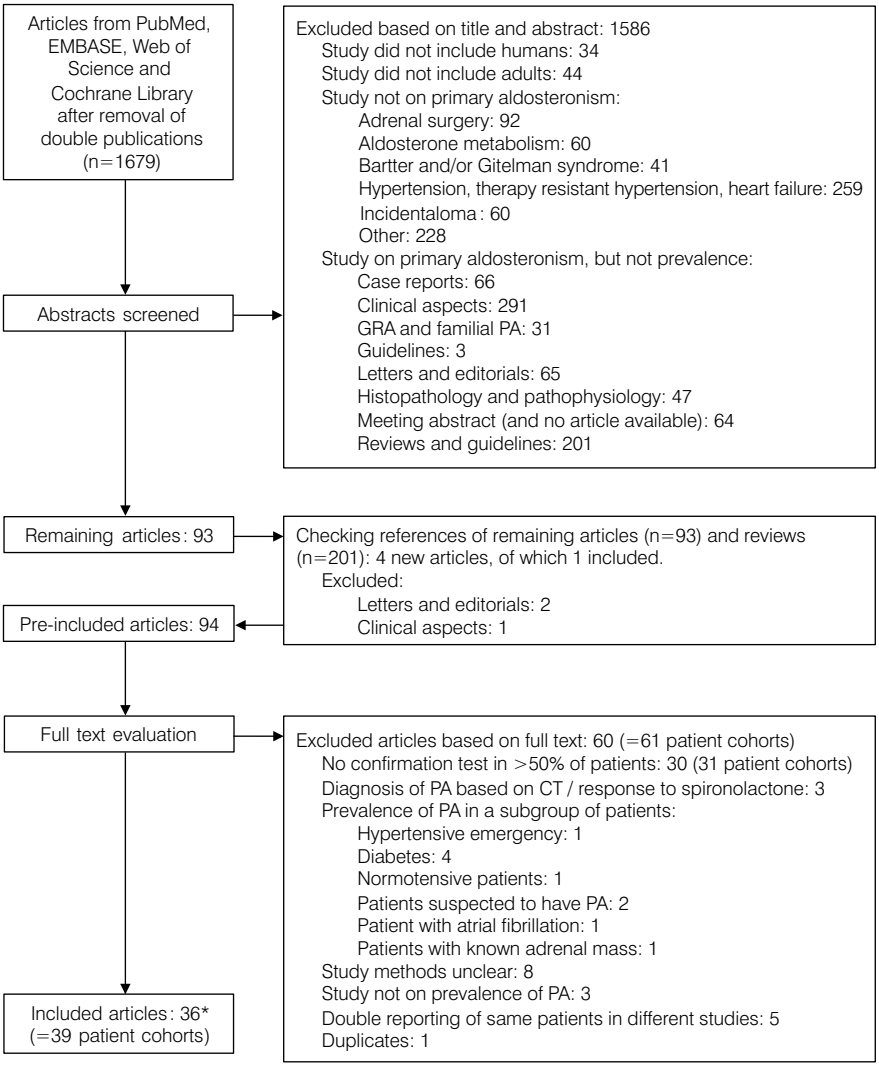
Prevalence of primary aldosteronism in primary care

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

Prevalence of primary aldosteronism in referral centers

Thirty studies were conducted in hypertension referral centers, providing data for 36614 patients (Table 1; Supplementary Table 2).[1,20-56] The number of included patients varied from 50 to 7343 (median 322.5). PA prevalence ranged from 1.0% to 29.8%.

Figure 1 Flow diagram of studies considered for systematic review



*The 36 articles contain 39 studies (=patient cohorts). Mulatero[20] and Rossi[21] report five and three cohorts, respectively, of which four and one, respectively, were included. The reason for exclusion of the cohorts are explained in Supplementary Table 1. As a result 36 (included articles) + 60 (excluded articles) = 94. GRA, glucocorticoid remediable aldosteronism. PA, primary aldosteronism.

Table 1 Summary of studies on prevalence of primary aldosteronism in primary care and referral centers

Author Year	Country	Setting	Design	Period	n	Population	Screening test	Confirmation test	Prevalence (%)
Gordon[22,a] 1993	Australia	PC	Prosp	NR	52	HT	ARR	FST	11.5
Loh[23,a] 2000	Singapore	PC	Prosp	1998	350	HT	ARR and PAC	IV SLT	4.6
Mosso[24,a] 2003	Chile	PC	Retro, prosp[b]	1998-2002	609	HT	ARR	FST	6.1
Omura[25,a] 2004	Japan	PC	Prosp	1995-1999	1020	New HT	PAC and PRA	Captopril test	6.0
Schwartz[26,a] 2005	United States	PC	Prosp	2000-2002	118	HT	No screening	Oral SLT	12.7
Westerdahl[27] 2006	Sweden	PC	Cross	NR	200	HT	ARR	FST	8.5
Williams[28,a] 2006	United States	PC	Cross	1996-2005	347	HT	ARR and PAC	Oral SLT	3.2
Fogari[29] 2007	Italy	PC	Prosp	1999-2002	3000	HT	ARR	IV SLT	5.9
Westerdahl[30] 2011	Sweden	PC	Cross	NR	200	New HT	ARR	FST	5.5
Anderson[31] 1994	United States	RC	Prosp	1976-1991	4429	HT	IV SLT	Oral SLT	1.4
Gordon[32,a] 1994	Australia	RC	Retro	1992-1993	199	HT	ARR	FST	8.5
Abdelhamid[33] 1996	Germany	RC	Prosp	NR	3900	HT	Urinary aldo	IV SLT	6.6

Table 1 -Continued

Author Year	Country	Setting	Design	Period	n	Population	Screening test	Confirmation test	Prevalence (%)
Brown[34] 1996	Australia	RC	Prosp	1988-1992	74	HT	ARR	IV SLT and FST	2.7
Rossi[21] 1998	Italy	RC	Prosp	NR	320	HT	ARR	IV SLT	5.9
Lim[35,c] 2000	UK	RC	Prosp	1995-1997	465	HT	ARR	FST	8.8
Rossi[36] 2002	Italy	RC	Prosp	1997-1999	1046	HT	ARR post-captopril	IV SLT	6.3
Trenkel[37] 2002	Germany	RC	Prosp	1997-1999	146	HT	ARR	IV SLT	1.4
Martell[38,a] 2003	Spain	RC	Prosp	2000-2002	50	RHT	No screening	IV SLT	15.9
Stowasser[39,a] 2003	Australia	RC	Prosp	2000-2002	300	HT	ARR	FST	18.0
Strauch[40,a] 2003	Czech Republic	RC	Retro	1997-2001	402	HT	ARR	IV SLT	19.2
Calhoun[41] 2004	United States	RC	Prosp	2000-2002	114	RHT	Urinary aldo and PRA	Oral SLT	29.8
Mulatero[20,d] 2004	Italy	RC	Retro	1994-2002	7343	HT	ARR and PAC	IV SLT	8.0
	United States	RC	Retro	1999	1112	HT	ARR and PAC	Oral SLT	10.8
	Singapore	RC	Retro	1995-2001	3850	HT	ARR and PAC	IV SLT	4.6
	Chile	RC	Retro	2000-2002	914	HT	ARR	FST	7.2
Milliez[1,a] 2005	France	RC	Prosp	1997-1999	5438	HT	ARR and PAC	Captopril test	2.3
Nishizaka[42] 2005	United States	RC	Prosp	2000-2004	265	RHT	Urinary aldo	Oral SLT	21.9

Rossi[43] 2006	Italy	RC	Prosp	2001-2004	1125	New HT	ARR[e]	Captopril test[f]	11.2
Douma[44,a] 2008	Greece	RC	Retro	1988-2008	1616	RHT	ARR and SAC	IV SLT and FST	11.3
Morillas[45] 2008	Spain	RC	Prosp	2005-2006	183	HT	ARR and PAC	IV SLT	6.0
Ribeiro[46] 2009	Brazil	RC	Prosp	2007	105	HT	ARR	IV SLT	1.0
Di Murro[47,a] 2010	Italy	RC	Retro	2007-2008	325	New HT	ARR and PAC	IV SLT	13.2
Matrozova[48,a,g] 2010	Bulgaria	RC	Prosp	2005-2008	376	HT	ARR and PAC	Captopril test	6.9
Pedrosa[49] 2011	Brazil	RC	Cross	2008-2010	125	RHT	ARR	IV SLT	5.6
Rios[50,a] 2011	Argentina	RC	Prosp	2006-2009	123	HT	ARR	IV SLT	6.5
Sigurjonsdottir[51,a,h] 2012	Sweden	RC	Prosp	2000-2003	122	HT	ARR and SA	Oral SLT	13.9
Yin[52,a] 2012	China	RC	Prosp	2007-2010	313	HT	ARR	Captopril and IV SLT	12.5
Sang & Jiang[53,a] 2013	China	RC	Cross[c]	2010-2011	1656	RHT	ARR	IV SLT	7.1
Jansen[54,a] 2014	The Netherlands	RC	Prosp	2006-2011	178	RHT	No screening	IV SLT	15.2

[a]Additional data received from author. [b]Study design: partly retrospective. 305 patients from a previous study were included,[55] the other patients were prospectively included. [c]In this review, only patients who were assessed by our predefined inclusion criteria were included in the analysis (prevalence is 41/464 = 8.8%). However, usually when cited, prevalence of 9.2% is reported.[56] [d]Because of missing number of included patients, the study from Australia (Brisbane) is excluded. [e]ARR ≥40 and/or post-captopril ARR ≥30 and/or LDF (logistic discriminant function) score ≥0.50. [f]ARR ≥40 plus post-captopril ARR ≥30 and/or LDF (logistic discriminant function) score ≥0.50. [g]Patient who were analyzed because of an incidentaloma were excluded. [h]Patients studied in primary care were excluded because of a <50% confirmation test.

Aldo, aldosterone. ARR, aldosterone-to-renin ratio. Cross, cross-sectional. FST, fludrocortisone suppression test. HT, hypertension (defined as blood pressure >140/90 with or without medication). IV SLT, intravenous sodium loading test. NR, not reported. Oral SLT, oral sodium loading test. PAC, plasma aldosterone concentration. PC, primary care. PRA, plasma renin activity. Prosp, prospective. RC, referral centre. Retro, retrospective. RHT, resistant hypertension. SAC, serum aldosterone concentration.

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

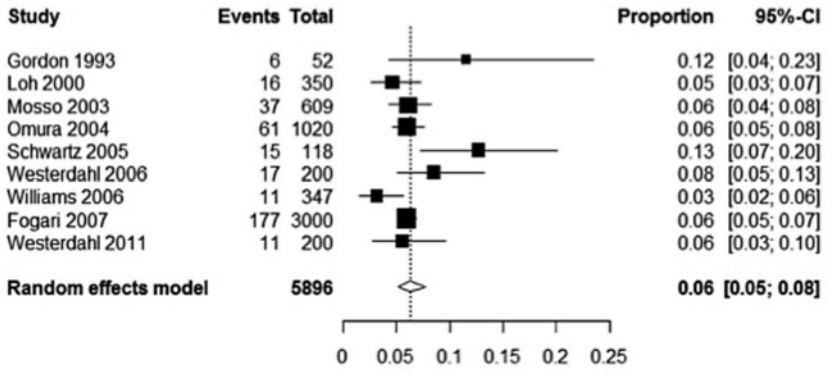
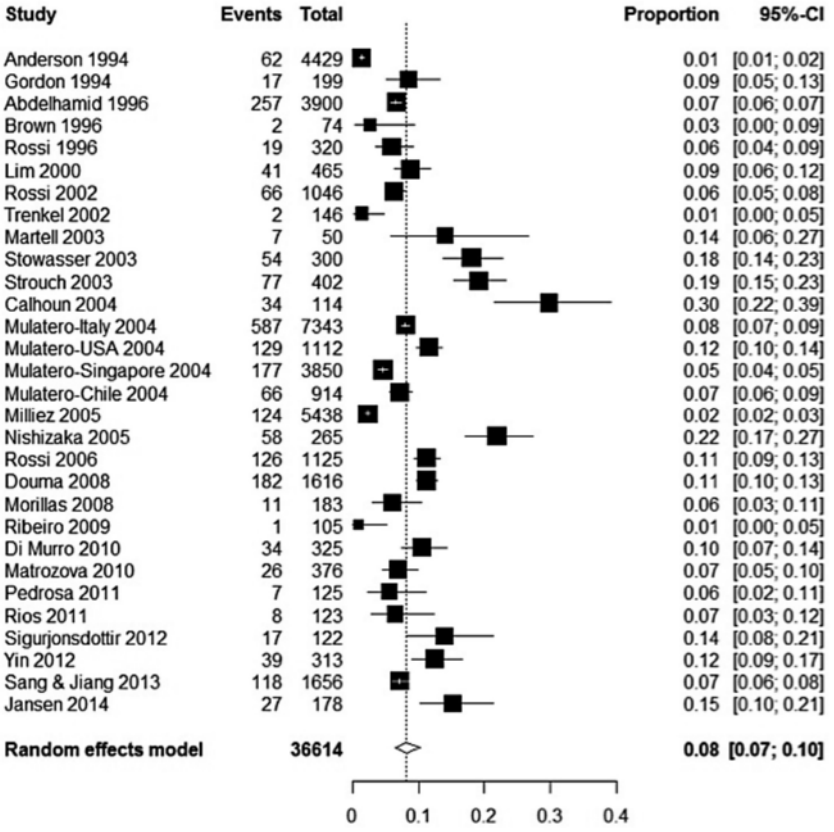


Figure 3 Forest plot for the prevalence of primary aldosteronism in referral centres



Differences across studies in the reported prevalence of primary aldosteronism

Forest plots show the weighted mean and the confidence intervals for the prevalence of PA (Figure 2, and Figure 3; Supplementary Figure 1). Heterogeneity (I^2) was large: in primary care, $I^2 = 57.6\%$ (0% to 78%); in referral centers, $I^2 = 97.1\%$ (96.7% to 97.5%). Therefore, we used meta-regression analysis to explore possible sources of heterogeneity (see page 40).

Prevalence of hypokalemia in patients with primary aldosteronism

Twenty-eight of the 39 studies reported the number of PA patients with hypokalemia. In primary care studies, hypokalemia was present in 0% to 37.5% of the patients with confirmed PA (n=6). In referral centers, hypokalemia ranged from 0% to 67% among patients with confirmed PA (n=22). Five studies (two primary care studies,[26,28] and three studies from referral centers[32,34,38]) restricted inclusion to normokalemic patients (Supplementary Table 3).

Prevalence of primary aldosteronism in patients with varying severity of hypertension

Seven studies provided data on patients with resistant hypertension and five studies reported on the relation between prevalence of PA and severity of hypertension. The weighted man PA prevalence was 5.5%, 4.2%, 10.2%, and 16.4% for high-normal blood pressure, stage 1, stage 2, and stage 3 hypertension, respectively.[20,24,43,48,50]

Differences in diagnostic methods

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

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

Quality assessment

The results of the quality assessment using the MORE protocol showed that all studies had minor flaws including assessment of sampling bias and type of outcome. More importantly, five studies were classified as having a major flaw because of a patient exclusion rate of more than 10%. For individual quality assessments, see Supplementary Table 4, and Supplementary Figure 2. Some descriptive items or

items concerning internal and external validity were neither reported nor addressed in many studies such as role of funding, precision and reliability of estimates, and consideration of sampling bias.

Meta-regression analysis

In primary care, univariate analysis showed a significant association between PA prevalence and five factors: year of publication ($p<0.001$), region ($p<0.001$), study objective ($p<0.001$), medication regimen ($p=0.04$), and type of screening test ($p<0.001$) (Supplementary Table 5). The highest prevalence estimates were found when the publication year was before 2000, when the study was performed in Australia, when the primary study objective was other than to assess the prevalence of PA, when medication regimen was unchanged, and when no screening test was performed.

Univariate analysis in referral centers showed a significant association between PA prevalence and five variables: year of publication ($p=0.04$), study objective ($p=0.02$), method of patient selection ($p<0.001$), type of hypertension ($p=0.01$), and type of screening test ($p<0.001$). The highest prevalence estimates were found when the year of publication was after 2000, when the primary study objective was other than to assess the prevalence of PA, when patient inclusion was consecutive, when the study population comprised patients with therapy resistant hypertension, and when no screening test was performed.

Multivariate analysis

By combining the possible explanatory variables in a single model (only possible for referral centers), we found a set of six variables to independently affect the prevalence of PA: year of publication ($p<0.001$), region ($p=0.002$), study design ($p=0.004$), study objective ($p=0.044$), method of patient selection ($p<0.001$), and type of screening test ($p=0.02$) (Table 2). This model for referral centers showed the highest prevalence in studies that were performed after 2000, when the study was performed in Australia, when the study was retrospective, when the study objective was to assess the prevalence of secondary hypertension, when patient inclusion was consecutive, and in studies in which no screening test was performed.

To clarify the prediction of the random effect logistic regression model, we provide a table with examples how variation of the six explanatory variables affects the predicted prevalence (Supplementary Table 6).

Table 2 Solutions for the fixed effects of the random effect logistic regression model in referral centers

Variable	Description OR	OR (95% CI)	Overall *P*-value
Publication year	2000-current vs 1990-2000	9.29 (3.17-27.16)	<0.001
Region	USA vs Europe	4.88 (2.07-11.57)	0.002
	Latin America vs Europe	0.53 (0.28-1.01)	
	Asia vs Europe	1.50 (0.71-3.17)	
	Australia vs Europe	5.57 (1.94-15.99)	
Study design	Retrospective vs Prospective	2.31 (1.39-3.84)	0.004
Study objective	Prevalence PA vs Other	1.71 (0.81-3.62)	0.044
	Prevalence secondary HT vs Other	2.83 (1.12-7.17)	
	Prevalence PA vs Prevalence secondary HT	0.60 (0.40-0.91)	
Patient selection method	Consecutive vs Convenience	4.95 (1.82-13.48)	<0.001
	Self selection vs Convenience	3.40 (0.90-12.89)	
	Consecutive vs Self selection	1.46 (0.88-2.42)	
Screening test	No screening vs Other	3.25 (1.51-7.01)	0.02
	ARR vs Other	0.75 (0.39-1.43)	
	No screening vs ARR	4.36 (1.52-12.54)	

The model estimates the prevalence of PA as a function of the six above mentioned variables. The resulting ORs (according to the model) represent the ratios of the odds for PA of two groups. ARR, aldosterone-to-renin ratio. HT, hypertension. OR, odds ratio. PA, primary aldosteronism.

DISCUSSION

In this systematically performed review and meta-regression analysis we confirm the previously reported wide variations in prevalences, both in studies performed in the primary care setting (3.2% to 12.7%) and in those performed in referral centers (1.0% to 29.8%). Although previous reviews and meta-analysis studies[57-59] reported mean prevalences, our study shows that it is pointless to provide point estimates in the absence of reporting contextual key factors. We established several factors that, at least partially, are responsible for the gross heterogeneity among studies on prevalence of primary aldosteronism.

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

The very first studies that investigated the prevalence of PA were performed in centers in Australia in self-selected patients or on the basis of retrospective data.[22,32]

This might partially explain why studies from Australia have a more than 5.5-fold higher odds than those that were carried out in Europe. An alternative explanation is that the prevalence of PA is indeed higher in Australia. Studies performed in the Unites States also showed nearly 5-fold higher odds. Whether this is due to the same reasons as may apply to Australian studies cannot be ascertained.

It is plausible that prospective studies are more appropriate to estimate prevalences. Our finding that retrospective studies report higher prevalences than prospective ones suggest that the current "epidemic" of PA is partly explained by reliance on retrospective studies.[60]

It is difficult to explain why studies that had the objective to assess the prevalence of secondary hypertension showed a nearly 3-fold higher prevalence of PA than studies that had other objectives, including studies that had the objective to assess specifically the prevalence of PA. However, the latter category was small and this may be a fortuitous finding.

The higher yield in the diagnosis of PA when testing consecutive patients than using other methods of patient selection is to be expected since less patients will be missed.

As a screening test, most studies (n=20) used the ARR. The reliability of the ARR is disputed because of its susceptibility to disturbances by external factors, variable cut-off levels and its mediocre sensitivity and specificity.[26,54,61,62] This might explain why studies that did not use any screening test showed the highest prevalences. One can speculate that when using the ARR, some patients may be missed and this would argue for performing directly a confirmation test when attempting to detect PA.

Variation in diagnostic strategies

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

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

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

Importance of proper prevalence estimates for case identification

As recently noted by Funder, considerably less than 1% of the hypertensive patients are screened for PA each year, not to mention diagnosed and properly treated.[65] While the prevalence of PA remains under debate, undiagnosed and untreated PA has important medical implications, such as the detrimental effect on the cardiovascular and renal systems due to aldosterone.[1-4,66-74] Proper treatment of PA, both surgically or with medication, appears to reduce the risk of both cardiovascular and renal complications.[71,75] It is therefore self-evident that identifying PA in hypertensive patients has important benefits. To design a strategy for identification of PA or to allocate health care resources to PA, it is important to know the prevalence of PA among hypertensive patients. Although our study shows that this knowledge is currently insufficient, it also provides us with clues as to what factors cause under- or overestimation of the prevalence of PA. Based on that, we would urge to perform a multicontinental prospective study in which consecutive hypertensive patients are screened for PA by a standardized confirmation test.

Limitations

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

CONCLUSIONS

This study of 5896 patients in primary care and 36614 patients in referral centers demonstrates that the wide range in reported prevalences of primary aldosteronism is associated with year of publication, study region, study objective, modes of data collection, patient selection, and use of screening test. The heterogeneity of studies precludes a reliable estimate of the prevalence of PA. Because of the significant impact of a diagnosis of primary aldosteronism on health care resources and the necessary facilities, our findings urge for better designed prospective prevalence studies. Prerequisites for such a study are international or even intercontinental agreement on a uniform screening and a confirmation test. Next, a survey by screening and, if screening is positive, a confirmation test for PA in all hypertensive patients should be performed, in both primary care and referral centers, with all untested patients being accounted for.

Disclosure statement

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SUPPLEMENTARY FILES

Supplementary File 1. Search

PubMed

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

Filters: **Dutch; English; French; German; Spanish**

Items found: 624

EMBASE

1. exp hyperaldosteronism/
2. (hyperaldosteronism or aldosteronism).ti,ab.
3. (Conn syndrome or Conns syndrome or Conn’s syndrome).ti,ab.
4. 1 or 2 or 3
5. exp prevalence/
6. exp incidence/
7. (incidence or incidences).ti,ab.
8. 6 or 7
9. exp epidemiology/
10. (epidemiology or epidemiologic or epidemiological).ti,ab.
11. 9 or 10
12. (prevalence or prevalences or occurrence or occurrences).ti,ab.
13. 5 or 12
14. 8 or 11 or 13
15. 4 and 14
16. limit 15 to ((dutch or english or french or german or spanish) and yr=”1990 -Current”)
17. limit 16 to ((dutch or english or french or german or spanish) and yr=”1990 -Current”)

Items found: 1239

Web of Science

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

Items found: 743

Cochrane Library

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

Publication Year from 1990 to 2015

Items found: 8

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

Total items found: 2614

Suppplementary File 2. References in supplementary files

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Supplementary Table 1 Excluded studies based on full text reading

First author	Year	Country	Setting	Remark
No confirmation test in >50% of patients				
Cortes[1]	2000	Chile	RC	
Daimon[2]	2014	Japan	PC	
Denolle[3]	2000	France	RC	
Ducher[4]	2012	France	RC	
Eide[5]	2004	Norway	RC	
Gallay[6]	2001	USA	RC	
Gallego[7]	2007	Spain	RC	
Garcia[8]	2011	USA	NR	
Gombet[9]	2007	France	RC	
Gonzaga[10]	2010	USA	RC	
Gregori[11]	2014	Italy	RC	
Hannemann[12]	2012	Germany	NR	
Ito[13]	2011	Japan	PC	
Jefic[14]	2006	USA	RC	
Lim[15]	1999	UK	PC	
Mosso[16]	1999	Chile	PC	
Mysliwiec[17]	2010	Poland	RC	
Olivieri[18]	2004	Italy	PC	
Pardes[19]	2010	Argentina	RC	
Rayner[20]	2000	South Africa	RC	
Rayner[21]	2001	South Africa	PC	
Rosenbaum[22]	2012	France	PC	
Rossi[23]	1998	Italy	RC	Article comprising three studies: 2 studies without >50% confirmation test were excluded from analysis, 1 study was included.
Sabio[24]	2005	Spain	RC	
Schmiemann[25]	2012	Germany	PC	
Schwartz[26]	2002	USA	PC	
Sharma[27]	1994	India	RC	
Takayanagi[28]	2000	Japan	RC	
Volpe[29]	2012	Sweden	PC	
Williams[30]	2006	UK	RC	
Diagnosis of PA based on computed tomography or reaction to spironolactone				
Hood[31]	2005	UK	PC	
Niizuma[32]	2008	Japan	RC	
Nogueira[33]	2008	Brasil	RC	

Supplementary Table 1 -Continued

First author	Year	Country	Setting	Remark
Prevalence of PA in a subgroup of patients				
Hypertensive emergency				
Börgel[34]	2010	Germany	RC	
Diabetes				
Li[35]	2013	China	NR	
Mukherjee[36]	2010	Singapore	RC	
Murase[37]	2013	Japan	RC	
Umpierrez[38]	2007	USA	NR	
Normotensive patients				
Markou[39]	2013	Greece	RC	
Patients suspected to have PA				
Solar[40]	2012	Czech rep.	RC	
Ye[41]	2012	China	RC	
Patients with atrial fibrillation				
Rossi[42]	2013	Italy	RC	
Patients with known adrenal mass				
Godula[43]	2013	Portugal	RC	
Study methods unclear				
Benchetrit[44]	2002	Israel	NR	
Gouli[45]	2011	Greece	RC	
Mulatero[46]	2004	Italy	RC	Article comprising five studies: 1 study was excluded (Australia)
Mysliwiec[47]	2012	Poland	RC	
Papanastasiou[48]	2014	Greece	RC	Is the same as Gouli (2011)
Sy[49]	2012	China	PC	
Trifanescu[50]	2013	Romania	RC	
Wu[51]	2014	Taiwan	RC	
Study not on prevalence of PA				
Adlin[52]	2013	USA	NR	Study on aldosterone
Kao[53]	2013	Taiwan	NR	Clinical aspects
Sakthiswary[54]	2012	Malasia	NR	Study on aldosterone
Double reporting of same patients in different studies				
Calhoun[55]	2002	USA	NR	= Nishizaka 2005
Fardella[56]	2000	Chile	NR	= Mosso 2003
Nishikawa[57]	2000	Japan	NR	= Omura 2004
Rossi[58]	2007	Italy	NR	= Rossi 2006
Rossi[59]	2010	Italy	NR	= Rossi 2006
Duplicates				
Gordon[60]	1993	Australia	RC	Summary of two previously reported studies

NR, not reported. PA, primary aldosteronism. PC, primary care. RC, referral center. rep, republic.

Supplementary Table 2 Studies on prevalence of primary aldosteronism in primary care and referral centers

PRIMARY CARE					
Author Year	**Country**	**Design**	**Period**	**n**	**Population % male Age: mean (SD)**
Gordon[22] 1993	Australia	Prosp[c]	NR	52	HT 65% male Age: 56y (7y)[d]
Loh[23] 2000	Singapore	Prosp[c]	1998	350	HT 39% male Age: 55y (9y)
Mosso[24] 2003	Chile	Retro and prosp[c,e]	1998-1999[c] 2001-2002[c]	609	HT 36% male Age: 54y (11y)
Omura[25] 2004	Japan	Prosp	1995-1999	1020	HT newly diagnosed[c] 55% male Age: 52y[c]
Schwartz[26] 2005	USA	Prosp[c]	2000-2002	118	HT normoK$^+$ 62% male Age: 29-63y[f]
Westerdahl[27] 2006	Sweden	Cross	NR	200	HT % male NR Age: ≤75y[f]
Williams[28] 2006	USA	Cross[c]	1996-2005[c]	347	HT normoK$^+$ 54% male Age: 49y (7y)[d]
Fogari[29] 2007	Italy	Prosp	1999-2002	3000	HT 48% male Age: 51y (6y)
Westerdahl[30] 2011	Sweden	Cross	NR	200	HT newly diagnosed 43% male Age: 24-75y[f]
Total number				**5896**	
REFERRAL CENTERS					
Anderson[31] 1994	USA	Prosp	1976-1991	4429	HT[g] % male NR Age: NR
Gordon[32] 1994	Australia	Retro[c]	1992-1993[c]	199	HT normoK$^+$ 50% male[c] Age: 54y (16y)
Abdelhamid[33] 1996	Germany	Prosp	NR	3900	HT % male NA[c] Age: NA[c]
Brown[34,h] 1996	Australia	Prosp	1988-1992	74	HT normoK$^+$ % male NA[c] Age: NA[c]

Medication[a]	Screening test[b]	Screening positive	Confirmation test cut-off	Prevalence
MRA withdrawn 3 weeks, rest unchanged	ARR >30	6 (11.5%)	FST (in 6/6) Cut-off NR	6 (11.5%)
Unchanged (patients using MRA were excluded)	ARR >20 and PAC >15 ng/dL	63 (18%)	IV SLT (in 56/63) PAC >10 ng/dL	16 (4.6%)
Withdrawn ≥15 days, CCB allowed	ARR >25	63 (10.3%)	FST (in 62/63) PAC ≥5 ng/dL	37 (6.1%)
No medication (unless budralazin)	PAC >12 ng/dL and PRA <1 ng/mL/h	134 (13.1%)	Captopril test (in 83/134[c]) ARR >20	61 (6.0%)
All withdrawn 2 weeks	No screening	NAp	Oral SLT Urinary aldo ≥12 μg/24h and PRA ≤1 ng/mL/h	15 (12.7%)
Withdrawn 2 weeks, CCB allowed	ARR >100 pmol/L per ng/L	50 (25%)	FST (in 26/50) PAC >160 pmol/L	17 (8.5%)
Standard	ARR >25 and PAC >8 ng/dL	26 (7.5%)	Oral SLT (in 26/26[c]) Urinary aldo >17 μg/24h	11 (3.2%)
Standard + all medication withdrawn 1 week	ARR >25	684 (22.8%)	IV SLT (in 650/684) PAC >7.5 ng/dL	177 (5.9%)
Standard	ARR >65 pmol/L per mU/L	36 (18%)	FST (in 27/36) PAC >225 pmol/L (day 4) or PAC >305 nmol/L (day 3)	11 (5.5%)
				351
Withdrawn 1 week (when possible)	IV SLT (afternoon) Aldosterone >8.5 ng/dL	NR	Oral SLT 3 days (saline + fludrocortisone or deoxycorticosterone) (in NR/NR) Urinary aldo <8 μg/24h	62 (1.4%)
Unchanged	ARR >30	22 (11.1%)	FST (in 17/22) Cut-off NA[c]	17 (8.5%)
Standard	Urinary aldosterone >50 nmol/24h or 18-OH-B >20 nmol/24h	NA[c]	IV SLT (in 257/257) Cut-off NR	257 (6.6%)
Withdrawn 3 days	ARR >2000 pmol/L per pmol A1/mL/h (PRC)[c] ARR >525 pmol/L per pmol A1/mL/h (PRA) [c]	6 (8.1%) 4 (5.4%)	IV SLT and FST (in 6/6[i]) PAC >140 pmol/L	2 (2.7%)

Supplementary Table 2 -Continued

Author Year	Country	Design	Period	n	Population % male Age: mean (SD)
Rossi[21] 1998	Italy	Prosp	NR	320	HT
Lim[35] 2000	UK	Prosp	1995-1997	465	HT % male NR Age: NR
Rossi[36] 2002	Italy	Prosp	1997-1999	1046	HT 51% male Age: 50y (12y)
Trenkel[37,h] 2002	Germany	Prosp	1997-1999	146	HT % male NA[c] Age: NA[c]
Martell[38] 2003	Spain	Prosp[c]	2000-2002[c]	50	RHT normoK$^+$ 52% male Age: 52y (9y)
Stowasser[39] 2003	Australia	Prosp[c]	2000-2002	300	HT % male NA[c] Age: NA[c]
Strauch[40] 2003	Czech Republic	Retro[c]	1997-2001	402	HT 43% male Age: 51y (12y)
Calhoun[41] 2004	USA	Prosp	2000-2002	114	RHT 37% male Age: 57y (11y)
Mulatero[20,k] 2004	Italy	Retro	1994-2002	7343	HT >160/100mm Hg % male NA[c] Age: NA[c]
	USA	Retro	1999	1112	HT % male NA[c] Age: NA[c]
	Singapore	Retro	1995-2001	3850	HT % male NA[c] Age: NA[c]
	Chile	Retro	2000-2002	914	HT % male NA[c] Age: NA[c]

Medication[a]	Screening test[b]	Screening positive	Confirmation test cut-off	Prevalence
Standard	ARR >30	NR	IV SLT (in all) PAC >208 pmol/L	19 (5.9%)
Withdrawn 7-10days (60%), 'no MRA or α-blocker allowed'	ARR ≥750 pmol/L per ng/mL/h	77 (16.6%)	FST (in 45/77) PAC ≥7.5 ng/dL	41[j] (8.8%)
Standard	ARR post-captopril ≥35	134 (12.8%)	IV SLT (in 134/134) PAC ≥7.5 ng/dL	66 (6.3%)
Unchanged	ARR ≥25 (pg/mL)/ (pg/mL)	27 (18.4%)	IV SLT (in 14/27) PAC >100 pg/mL	2 (1.4%)
Withdrawn 7-10 days	No screening	NAp	IV SLT (in 44/50; 6 excluded due to white coat HT) <50% suppression of aldosterone compared to baseline value	7 (15.9%)
BB withdrawn 2 weeks, MRA 4 weeks, ARB or ACE-I allowed	ARR >30 or ARR >20 when RHT	59 (19.7%)	FST (in 59/59) PAC ≥6 ng/dL	54 (18%)
Withdrawn 2 weeks, α-blocker allowed	ARR ≥50	87 (21.6%)	IV SLT (in 87/87[c]) PAC >85 ng/L	77 (19.2%)
MRA withdrawn ≥6 weeks, rest unchanged	Urinary aldo >12 μg/24h and PRA <1.0 ng/mL/h	NR	Oral SLT (NA[c]) Urinary aldo >12 μg/24h and PRA <1.0 ng/mL/h with urinary sodium >200 mEq/24h	34 (29.8%)
Standard[c]	ARR >40 and PAC >15 ng/dL	905[c]	IV SLT (in 905/905[c]) PAC >5 ng/dL	587 (8.0%)
Standard, although ACE-I and ARB have not been withdrawn when the ratio was positive under treatment[c]	ARR>20 and PAC >15 ng/dL	NA[c]	Oral SLT (in all[c]) Urinary aldo >12 μg/d	120 (10.8%)
NA[c]	ARR >20 and PAC >15 ng/dL	NA[c]	IV SLT (in all[c]) PAC >10 ng/dL	177 (4.6%)
Interfering drugs such as diuretics, ACE-I, ARB and BB were stopped for ≥15 days[c]	ARR >25	NA[c]	FST (in all[c]) PAC >5 ng/dL and PRA <1 ng/mL/h	66 (7.2%)

Supplementary Table 2 -Continued

Author Year	Country	Design	Period	n	Population % male Age: mean (SD)
Milliez[1] 2005	France	Prosp[c]	1997-1999	5438[c]	HT % male NA[c] Age: NA[c]
Nishizaka[42] 2005	USA	Prosp	2000-2004	265	RHT 44% male Age: 56y (12y)
Rossi[43] 2006	Italy	Prosp	2001-2004	1125	HT newly diagnosed 56% male Age: 46y (12y)
Douma[44] 2008	Greece	Retro	1988-2008[c]	1616	RHT 51% male Age: 56y (13y)
Morillas[45] 2008	Spain	Prosp	2005-2006	183	HT 61% male Age: 58y (13y)
Ribeiro[46] 2009	Brazil	Prosp	2007	105	HT (90%borderline or stage 1) 25% male Age: 55y (11y)
Di Murro[47] 2010	Italy	Retro[c]	2007-2008	325	HT newly diagnosed 61% male[c] Age: 51y (10y[c])
Matrozova[48,m] 2010	Bulgaria	Prosp[c]	2005-2008[c]	376[n]	HT 34% male[c] Age:48y (14y)[c]
Pedrosa[49] 2011	Brazil	Cross	2008-2010	125	RHT 43% male Age:52y (10y)
Rios[50] 2011	Argentina	Prosp[c]	2006-2009	123	HT 39% male Age:43y (11y)
Sigurjonsdottir[51,o] 2012	Sweden	Prosp	2000-2003[c]	122[p]	HT 61% male[c] Age: 56y (12y)[c]
Yin[52] 2012	China	Prosp[c]	2007-2010	313	HT 46% male Age: 46y (13y[c])

Medication[a]	Screening test[b]	Screening positive	Confirmation test cut-off	Prevalence
Standard	ARR >23 and PAC >178 pg/mL and PRA ≤5 pg/mL and urinary aldosterone >23 μg/24h[c]	NA[c]	Captopril test (in all[c]) <70% suppression of aldosterone compared to baseline PAC[c]	124 (2.3%)
MRA withdrawn ≥6 weeks, rest unchanged	Urinary aldo >12 μg/24h	58	Oral SLT (in 58/58[l]) Urinary aldo >12μg/24h and PRA <1.0 ng/mL/h with urinary sodium >200 mEq/24h	58 (21.9%)
Standard	ARR ≥40 and/or post-captopril ARR ≥30 and/or LDH-score ≥0.50	230 (20.4%)	ARR ≥40 plus post-captopril ARR ≥30 and/or LDH-score ≥ 0.50 (in 230/230)	126 (11.2%)
Standard	ARR >65.16 pmol/L per pmol/L/min and SAC >416 pmol/L	338 (20.9%)	IV SLT and FST (in 338/338[c,i]) IV SLT: SAC ≥222 pmol/L FST: SAC >139 pmol/L	182 (11.3%)
Unchanged	ARR >30 and PAC >20 ng/dL	NR	IV SLT (in NR/NR) PAC >10 ng/dL	11 (6.0%)
Use of MRA and BB were excluded: unchanged	ARR >25	9 (8.6%)	IV SLT (in 8/9) PAC >5 ng/dL	1 (1.0%)
Standard	ARR >40 and PAC >15 ng/dL with suppressed PRA	almost 17%[c]	IV SLT (in all[c]) PAC ≥5 ng/dL	43 (13.2%)
MRA withdrawn 45 days, rest stopped 7-10days. CCB or α-blocker allowed	ARR >750 pmol/L per ng/mL/h and PAC >416 pmol/L	94[c,n]	Captopril test (in 87/94[c]): ARR >970 (pmol/L)/(ng/mL/h)	26[n] (6.9%)
MRA withdrawn 3 weeks, rest unchanged	ARR >20	14 (11.2%)	IV SLT (in 14/14) PAC >10 ng/dL	7 (5.6%)
Standard	ARR >25	20 (16.3%)	IV SLT (18/20) PAC >5 ng/dL	8 (6.5%)
Standard	ARR >1.28 and SA >0.43 nmol/l	28 (22.8%)	Oral SLT (in 25/28[c]) Urinary aldo >28 nmol/24h	17[q] (13.9%)
Standard	ARR >25	72 (23%)	Captopril test (in 72/72) ARR >13 ng/dL IV SLT (in 2/72[r]) SAC >6.75 ng/dL	39 (12.5%)

Supplementary Table 2 -Continued

Author Year	Country	Design	Period	n	Population % male Age: mean (SD)
Sang & Jiang[53] 2013	China	Cross[c]	2010-2011	1656	RHT 57% male Age: 18-65y[f]
Jansen[54] 2014	The Netherlands	Prosp	2006-2011[c]	178	RHT 53 % male Age: 49y (9y)
Total number				**36614**	

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

A1, Angiotensin 1. ACE-I, angiotensin-converting-enzyme inhibitor(s). aldo, aldosterone. ARB, angiotensin receptor blocker. ARR, aldosterone-to-renin ratio. BB, beta blocker. CCB, calcium channel blocker. Cross, cross-sectional. FST, fludrocortisone suppression test. HT, hypertension (defined as blood pressure >140/90 with or without medication. IV SLT, intravenous sodium loading test. LDH, logistic discriminant function. MRA, mineralocorticoid receptor antagonist. n, number of patients. NA, non available (data untraceble due to elapsed time). NAp, not applicable. normoK^+, normokalemia. NR, not reported. Oral SLT, oral sodium loading test. PAC, plasma aldosterone concentration. PC, primary care. PRA, plasma renin activity. Prosp, prospective. RC, referral center. Retro, retrospective. RHT, resistant hypertension. SAC, serum aldosterone concentration. y, year(s).

[a] Standard (=according to the Endocrine Society guideline[13]): MRAs stopped for at least 4 weeks, all other anti-hypertensive drugs stopped for at least 2 weeks, except for calciumantagonists and α-blockers.
[b] ARR calculated with PAC in ng/dL and PRA in ng/mL/h, unless stated otherwise.
[c] Data received from author.
[d] Standard error of the mean converted to standard deviation.
[e] Study design: partly retrospective. 305 patients from a previous study were included,[55] the other patients were prospectively included.
[f] Mean age and standard deviation not reported.
[g] The study population consisted of poorly controlled hypertensive patients.
[h] In this analysis only the hypertensive study population is included.
[i] Hypertensive patients with elevated ARR performed an intravenous sodium loading test as well as a fludrocortisone suppression test.
[j] In the original article a prevalence of 43/465 is reported. However, 2/465 have not been identified by screening and confirmation testing: one patient with a negative FST had a right adrenal adenoma, which was detected on CT scanning (histological examination after adrenalectomy confirmed a Conn's adenoma), and one patient had already been diagnosed with PA. In this review, only patients who were assessed by our pre-defined inclusion criteria were included in the analysis (prevalence is 41/464 = 8.8%). However, usually when cited, a prevalence of 9.2% is reported.[56]
[k] Due to missing number of included patients, the study from Australia (Brisbane) is excluded.
[l] If urinary aldosterone was elevated (>12 μg/24h), but urinary sodium was low (<200 mEq/24h), the 24h urinary assessments were repeated after 3 days of dietary salt supplementation. However, if urinary aldosterone and urinary sodium exceeds cutoff values during normal diet (routine high sodium diet), additional sodium loading was omitted (because of risks and little additional value). So, the confirmatory test is the 24h urine under high sodium diet.
[m] Patients who were analyzed because of an incidentaloma were excluded.
[n] In this number incidentalomes are excluded (n=376+96=472).

Medication[a]	Screening test[b]	Screening positive	Confirmation test cut-off	Prevalence
MRA withdrawn 4 weeks, rest unchanged	ARR >20	494 (29.8%)	IV SLT (in 494/494) PAC >8 ng/dL	118 (7.1%)
MRA and BB withdrawn 4 weeks: rest unchanged	Screening not used for prevalence analysis	NAp	IV SLT (in 178/178) PAC >235 pmol/L	27 (15.2%)
				2375

[o] Patients studied in primary care were excluded due to <50% confirmation test (6/18 = 33%, data confirmed by author).
[p] Information from author by email: the original paper states that the number of patients is 123.
[q] Including dropouts in analysis.
[r] All of the patients with elevated ARR underwent the captopril test, and two of the patients underwent an intravenous sodium loading test because of the confused results of the captopril test (data received from author).

Supplementary Table 3 Studies in patients with primary aldosteronism reporting the number of patients with hypokalemia

PRIMARY CARE		
Author (year)	**Number of PA patients assessed**	**Number of patients with hypokalemia (%)**
Gordon[61] (1993)	6	0 (0%)
Loh[62] (2000)	16	6 (37.5%)
Mosso[63] (2003)	37	1 (2.7%)
Omura[64] (2004)	61	15 (24.6%)
Fogari[65] (2007)	177	44 (24.8%)
Westerdahl[66] (2011)	11	3 (27.3%)†
Total	**308**	**69**
REFERRAL CENTERS		
Anderson[67] (1994)	62	19 (30%)
Lim[68] (2000)	41	2 (4.4%)
Rossi[69] (2002)	66	26 (39.4%)
Stowasser[70] (2003)	54	7 (13%)
Strauch[71] (2003)	77	15 (19%)*
Mulatero[46] (2004)		
Italy	587	146 (24.9%)
USA	120	44 (36.7%)
Singapore	177	66 (37.3%)
Chile	66	6 (9.1%)
Milliez[72] (2005)	124	121 (98%)
Nishizaka[73] (2005)	58	23 (39.7%)
Rossi[74] (2006)	126	12 (9.6%)
Douma[75] (2008)	182	83 (45.6%)
Ribeiro[76] (2009)	1	0 (0%)
Di Murro[77] (2010)	43	18 (42%)*
Matrozova[78] (2010)	38	21 (55.3%)‡
Pedrosa[79] (2011)	7	0 (0%)*
Rios[80] (2011)	8	4 (50%)
Sigurjonsdottir[81] (2012)	17	5 (29%)*
Yin[82] (2012)	39	26*(67%)
Sang & Jiang[83] (2013)	118	62 (52.5%)
Jansen[84] (2014)	27	13 (48.1)
Total	**2038**	**719**

*Data obtained from the authors. †Estimated from box plot. ‡Including 12 patients who were diagnosed with PA after analysis for incidentaloma. Five studies included only patients with normokalemia.[85-89] Six studies did not report the number of patients who had hypokalemia.[23, 90-94] PA, primary aldosteronism.

Supplementary Table 4A Quality assessment according to the MORE protocol, part A

Author (year)	DESCRIPTIVE							EXTERNAL VALIDITY							INTERNAL VALIDITY					
								Sampling of subjects				Subject flow				Measurements of outcome		Reporting of outcome		
	Descriptive information	Funding	Role of funding	Conflict of interest	Ethical approval	Aim	Study design	Sampling method	Sampling frame	Sampling bias	Response rate (%)	Eligibility fract (%)	Enroll fract (%)	Recruit fract (%)	Source of measure	Validation study method	Reliability estimates	Type of outcome	Precision of estimate	Estimate in total sample
Abdelhamid[90] (1996)	C	-	-	-	Y	PA/O	Pro	-	HC	-	> 60	100	100	100	OM	C-ES	-	PP	-	-
Anderson[67] (1994)	C	-	-	-	-	O	Pro	-	HC	Disc	> 60	100	100	100	OM	C-ES	-	PP	-	-
Brown[86] (1996)	C	G	-	-	Y	PA	Pro	Self	DB	-	40-60	-	32	-	OM	C-ES	-	PP	-	-
Calhoun[92] (2004)	C	G	-	-	Y	PA	Pro	Cs	HC	-	> 60	100	100	100	OM	C-ES	-	PP	-	-
Di Murro[77] (2010)	C	-	-	-	-	PA	-	Cs	HC	-	> 60	100	100	100	OM	C-ES	-	PP	-	-
Douma[75] (2008)	C	NF	NA	No	Y	PA	Ret	Cs	HC	Disc	> 60	70	100	70	MR	C-ES	-	PP	-	-
Fogari[65] (2007)	C	G	-	-	Y	PA	Pro	Cv	HC	-	> 60	100	100	100	OM	C-ES	Pub	PP	-	-
Gordon[61] (1993)	C	-	-	-	-	-	-	Self	-	Asse	> 60	-	100	-	OM	C-ES	-	PP	-	-
Gordon[85] (1994)	C	-	-	-	-	PA	-	Cs	HC	Disc	> 60	100	100	100	OM	C-ES	-	PP	-	-
Jansen[84] (2014)	C	G/I	No	No	Y	O	Pro	Cs	HC	-	> 60	97	99	96	OM	C-ES	Pub	PP	-	-
Lim[68] (2000)	C	NF	NA	No	-	PA	-	Cs	HC	-	> 60	94	100	94	OM	C-ES	Discus	PP	-	-
Loh[62] (2000)	C	G	-	-	Y	PA	-	Cs	HC	-	> 60	96	92	89	OM	C-ES	Discus	PP	-	-
Martell[88] (2003)	C	-	-	-	Y	O	-	Cs	HC	Disc	> 60	88	100	88	OM	C-ES	-	PP	-	-
Matrozova[78] (2010)	C	G	-	-	Y	PA	-	Cs	HC	Disc	> 60	81	91	74	OM	C-ES	Pub	PP	-	-
Milliez[72] (2005)	C	-	-	-	-	O	Ret	Cs	MR	Disc	> 60	100	100	100	MR	C-ES	-	PP	-	-

Supplementary Table 4A -Continued

Author (year)	DESCRIPTIVE							EXTERNAL VALIDITY							INTERNAL VALIDITY					
								Sampling of subjects				Subject flow				Measurements of outcome		Reporting of outcome		
	Descriptive information	Funding	Role of funding	Conflict of interest	Ethical approval	Aim	Study design	Sampling method	Sampling frame	Sampling bias	Response rate (%)	Eligibility fract (%)	Enroll fract (%)	Recruit fract (%)	Source of measure	Validation study method	Reliability estimates	Type of outcome	Precision of estimate	Estimate in total sample
Morillas[94] (2008)	C	-	-	-	-	PA	Pro	-	HC	-	> 60	100	100	100	OM	C-ES	-	PP	-	-
Mosso[63] (2003)	C	G	-	-	Y	PA	Cro	Cs	MR	Disc	> 60	100	100	100	MR	C-ES	Pub	PP	-	-
Mulatero[46] (2004)																				
Italy	C	-	-	-	-	PA	Ret	Cv	HC	Disc	> 60	-	100	-	OM	C-ES	Discus	PP	-	-
USA	C	-	-	-	-	PA	Ret	Cv	HC	Disc	> 60	-	100	-	OM	C-ES	Discus	PP	-	-
Singapore	C	-	-	-	-	PA	Ret	Cv	HC	Disc	> 60	-	100	-	OM	C-ES	Discus	PP	-	-
Chile	C	-	-	-	-	PA	Ret	Cs	HC	Disc	> 60	100	100	100	OM	C-ES	Discus	PP	-	-
Nishizaka[73] (2005)	C	G	-	-	Y	O	Pro	Cs	HC	-	> 60	100	100	100	OM	C-ES	Pub	PP	-	-
Omura[64] (2004)	C	-	-	-	Y	O	Pro	-	HC	-	> 60	-	100	-	OM	C-ES	-	PP	-	-
Pedrosa[79] (2011)	C	G/I	-	No	Y	O	Cro	Cs	HC	Disc	> 60	82	82	67	OM	C-ES	-	PP	-	-
Ribeiro[76] (2009)	C	NF	NA	No	Y	PA	-	Cv	HC	Disc	> 60	100	98	98	OM	C-ES	-	PP	-	-
Rios[80] (2011)	C	-	-	No	-	PA	Cro	-	-	-	> 60	97	83	90	OM	C-ES	Pub	PP	-	-
Rossi[23] (1998)	C	-	-	-	-	O	-	-	HC	Disc	> 60	100	100	100	OM	C-ES	-	PP	-	-
Rossi[69] (2002)	C	-	-	-	Y	PA/O	Pro	Cs	HC	Disc	> 60	98	100	98	OM	C-ES /Val	Pub	PP	-	-
Rossi[74] (2006)	C	G	-	-	Y	PA	Pro	Cs	HC	Disc	> 60	99	95	94	OM	C-ES	Pub	PP	-	-
Sang & Jiang[83] (2013)	C	G	-	No	Y	PA	Cro	Cs	HC	-	> 60	84	100	84	-	-	Pub	PP	-	-
Schwartz[87] (2005)	C	G	-	-	Y	O	-	Self	O	Disc	> 60	50	84	42	OM	C-ES	-	PP	-	-
Sigurjonsdottir[81] (2012)	C	-	-	No	Y	O	Pro	Cs	HC	-	> 60	-	90	-	OM	C-ES	-	PP	-	-
Stowasser[70] (2003)	C	G	-	-	-	O	-	-	HC	Disc	> 60	-	100	-	OM	C-ES	-	PP	-	-

Strauch[71] (2003)	C	O	-	-	-	PA	-	Cs	HC	Disc	> 60	100	100	100	OM	C-ES	-	PP	-	-
Trenkel[91] (2002)	C	-	-	-	-	O	Pro	Cs	HC	-	> 60	-	100	-	OM	C-ES	-	PP	-	-
Westerdahl[93] (2006)	C	-	-	-	Y	PA/O	Cro	Self	DB	Disc	40-60	84	49	41	OM	C-ES	-	PP	-	-
Westerdahl[66] (2011)	C	O	-	No	Y	PA/O	Cro	Cs	-	-	> 60	-	100	-	OM	C-ES	-	PP	-	-
Williams[89] (2006)	C	G	-	-	Y	PA	-	Self	O	-	> 60	-	100	-	OM	Pub	Pub	PP	-	-
Yin[82] (2012)	C	O	-	No	Y	O	-	-	HC	-	> 60	88	100	88	OM	C-ES	-	PP	-	-

Data that were not reported are indicated by '-'. Abbreviations: Asse, Assessed. C, complete. C-ES, conformation test according to the Endocrine Society guideline.[95] Cs, consecutive. Cv, convenience. Cro, cross-sectional. DB, database. Disc, discussed. Discus, discussion. Eligibility fract, eligibility fraction (eligible/screened). Enroll fract, enrollment fraction (enrolled/eligible). G, grant. HC, health care. I, industry. MR, medical records. NA, not available. NF, no funding. O, other; OM, objectively measured. PA, aim to assess prevalence of primary aldosteronism. PP, point prevalence. Pro, prospective. Pub, published. Recruit fract, recruitment fraction (enrolled/screened). Ret, retrospective. Self, self selection. Val, validated. Y, yes.

Supplementary Table 4B Quality assessment according to the MORE protocol, part B

Author (year)	DESCRIPTIVE	EXTERNAL VALIDITY		INTERNAL VALIDITY
	Minor flaw	Major flaw	Minor flaw	Minor flaw
Abdelhamid[90] (1996)	-	-	-	PP-OCE
Anderson[67] (1994)	-	-	-	PP-OCE
Brown[86] (1996)	-	Exclusion rate from analysis >10%	Sampling method: self selection; Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Calhoun[92] (2004)	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Di Murro[77] (2010)	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Douma[75] (2008)	-	Sampling frame: medical records	-	PP-OCE
Fogari[65] (2007)	-	-	Sampling method: convenience; Sampling bias not addressed in analysis/discussed	PP-OCE
Gordon[61] (1993)	-	-	Subject flow not reported	PP-OCE
Gordon[85] (1994)	Target population not defined	-	-	PP-OCE

Supplementary Table 4B -Continued

Author (year)	DESCRIPTIVE	EXTERNAL VALIDITY		INTERNAL VALIDITY
	Minor flaw	Major flaw	Minor flaw	Minor flaw
Jansen[84] (2014)	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Lim[68] (2000)	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Loh[62] (2000)	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Martell[88] (2003)	-	-	-	PP-OCE
Matrozova[78] (2010)	-	-	-	PP-OCE
Milliez[72] (2005)	-	Sampling frame: medical records	-	PP-OCE
Morillas[94] (2008)	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Mosso[63] (2003)	-	Sampling frame: medical records	-	PP-OCE
Mulatero[46] (2004)				
Italy	-	-	Sampling method: convenience; Subject flow not reported	PP-OCE
USA	-	-	Sampling method: convenience; Subject flow not reported	PP-OCE
Singapore	-	-	Sampling method: convenience; Subject flow not reported	PP-OCE
Chile	-	-	-	PP-OCE
Nishizaka[73] (2005)	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Omura[64] (2004)	-	-	Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Pedrosa[79] (2011)	-	Exclusion rate from analysis >10%	-	PP-OCE
Ribeiro[76] (2009)	-	-	Sampling method: convenience	PP-OCE
Rios[80] (2011)	-	Exclusion rate from analysis >10%	Sampling bias not addressed in analysis/discussed; Sampling method not reported	PP-OCE
Rossi[23] (1998)	-	-	-	PP-OCE
Rossi[69] (2002)	-	-	-	PP-OCE
Rossi[74] (2006)	-	-	-	PP-OCE

Sang & Jiang[83] (2013)	-	-	Sampling bias not addressed in analysis/discussed	PP-OCE
Schwartz[87] (2005)	-	Exclusion rate from analysis >10%	Sampling method: self selection	PP-OCE
Sigurjonsdottir[81] (2012)	-	-	Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Stowasser[70] (2003)	-	-	Subject flow not reported	PP-OCE
Strauch[71] (2003)	-	-	-	PP-OCE
Trenkel[91] (2002)	-	-	Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Westerdahl[93] (2006)	-	Exclusion rate from analysis >10%	Sampling method: self selection; Sampling frame: database	PP-OCE
Westerdahl[66] (2011)	-	-	Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Williams[89] (2006)	-	-	Sampling method: self selection; Sampling bias not addressed in analysis/discussed; Subject flow not reported	PP-OCE
Yin[82] (2012)	-	-	Sampling bias not addressed in analysis/discussed; Sampling method not reported.	PP-OCE

Data that were not reported are indicated by '-'. PP-OCE, point prevalence, only crude estimates.

Supplementary Table 5 Univariate analysis

Variable	Setting	Comparison	OR (95% CI)	Overall *P*-value
Publication year	PC	2000-current vs 1990-2000	0.49 (0.38 - 0.64)	<0.001
	RC	2000-current vs 1990-2000	2.18 (1.04 - 4.58)	0.04
Region	PC	USA vs Europe	0.99 (0.22 - 4.44)	<0.001
	PC	Latin America vs Europe	0.93 (0.68 - 1.27)	
	PC	Asia vs Europe	0.81 (0.53 - 1.22)	
	PC	Australia vs Europe	1.87 (1.38 - 2.56)	
	RC	USA vs Europe	1.32 (0.33 - 5.29)	0.52
	RC	Latin America vs Europe	0.56 (0.28 - 1.15)	
	RC	Asia vs Europe	0.89 (0.48 - 1.67)	
	RC	Australia vs Europe	1.08 (0.41 - 2.80)	
Study design	PC	Retrospective vs Prospective	NA	NA
	RC	Retrospective vs Prospective	1.33 (0.80 - 2.22)	0.26
Study objective	PC	Prevalence PA vs Other	0.42 (0.34 - 0.52)	<0.001
	PC	Prevalence secondary HT vs Other	NA	NA
	PC	Prevalence PA vs Prevalence secondary HT	0.96 (0.77 - 1.18)	
	RC	Prevalence PA vs Other	0.88 (1.63 - 1.95)	0.02
	RC	Prevalence secondary HT vs Other	0.63 (0.33 - 1.18)	
	RC	Prevalence PA vs Prevalence secondary HT	1.40 (1.07 - 1.82)	
Patient selection method	PC	Consecutive vs Convenience	0.73 (0.35 - 1.53)	0.35
	PC	Self selection vs Convenience	NA	
	PC	Consecutive vs Self selection	NA	
	RC	Consecutive vs Convenience	1.82 (0.86 - 3.85)	<0.001
	RC	Self selection vs Convenience	0.46 (0.23 - 0.91)	
	RC	Consecutive vs Self selection	3.95 (2.87 - 5.45)	
Type of HT	PC	Therapy resistant HT vs HT	NA	NA
	RC	Therapy resistant HT vs HT	2.13 (1.19 - 3.83)	0.01
Patient selection on potassium	PC	No selection vs Only normokalemic patients	0.98 (0.28 - 3.46)	0.97
	RC	No selection vs Only normokalemic patients	1.06 (0.47 - 2.39)	0.88
Patient selection on medication	PC	Endocrine Society guideline vs Unchanged	0.43 (0.33 - 0.56)	0.04
	PC	Changed vs Unchanged	0.68 (0.42 - 1.12)	
	PC	MRA stop vs Unchanged	NA	
	PC	Endocrine Society guideline vs Changed	0.63 (0.36 - 1.10)	
	PC	Endocrine Society guideline vs MRA stop	1.17 (0.89 - 1.53]	
	PC	Changed vs MRA stop	1.86 (1.13 - 3.05)	

Supplementary Table 5 -Continued

Variable	Setting	Comparison	OR (95% CI)	Overall *P*-value
Patient selection on medication	RC	Endocrine Society guideline vs Unchanged	1.40 (0.58 - 3.38)	0.58
	RC	Changed vs Unchanged	1.51 (0.57 - 4.04)	
	RC	MRA stop vs Unchanged	2.33 (0.68 - 8.08)	
	RC	Endocrine Society guideline vs Changed	0.93 (0.48 - 1.78)	
	RC	Endocrine Society guideline vs MRA stop	0.60 (0.22 - 1.64)	
	RC	Changed vs MRA stop	0.65 (0.22 - 1.93)	
Potassium levels corrected	PC	Hypokalemia corrected vs Normokalemia	0.98 (0.28 - 3.46)	0.97
	RC	Hypokalemia corrected vs Normokalemia	1.06 (0.47 - 2.39)	0.88
Screening test	PC	No screening vs Other	2.81 (1.97 - 4.02)	<0.001
	PC	ARR vs Other	1.32 (0.89 - 1.95)	
	PC	No screening vs ARR	2.14 (1.81 - 2.52)	
	RC	No screening vs Other	1.88 (1.23 - 2.88)	<0.001
	RC	ARR vs Other	0.79 (0.43 - 1.46)	
	RC	No screening vs ARR	2.38 (1.51 - 3.77)	
Number of screening measurements	PC	One measurement vs Multiple measurements	0.85 (0.49 - 1.47)	0.49
	RC	One measurement vs Multiple measurements	0.75 (0.39 - 1.46)	0.38
Patient position during screening	PC	Supine vs Not supine	0.81 (0.50 - 1.31)	0.32
	RC	Supine vs Not supine	0.53 (0.22 - 1.24)	0.13
Cut-off screening test with ARR	PC	All unrestrictive	NA	NA
	RC	All unrestrictive	NA	NA
Percentage of patients with positive screening test who underwent confirmation test	PC	100% vs <80%	1.15 (0.39 - 3.40)	0.40
	PC	>80% vs <80%	0.84 (0.61 - 1.16)	
	PC	100% vs >80%	1.37 (0.47 - 3.96)	
	RC	100% vs <80%	1.88 (0.73 - 4.81)	0.24
	RC	>80% vs <80%	1.12 (0.34 - 3.62)	
	RC	100% vs >80%	1.68 (0.71 - 3.98)	
Type of confirmation test	PC	IV SLT vs Fludrocortisone	0.88 (0.69 - 1.11)	0.33
	PC	Oral SLT vs Fludrocortisone	1.09 (0.28 - 4.21)	
	PC	Captopril vs Fludrocortisone	1.24 (0.86 - 1.76)	
	PC	IV SLT vs Oral SLT	0.81 (0.20 - 3.18)	
	PC	IV SLT vs Captopril	0.71 (0.46 - 1.09)	
	PC	Oral SLT vs Captopril	0.88 (0.22 - 3.57)	

Supplementary Table 5 -Continued

Variable	Setting	Comparison	OR (95% CI)	Overall *P*-value
Type of confirmation test	RC	IV SLT vs Fludrocortisone	1.30 (0.53 - 3.23)	0.38
	RC	Oral SLT vs Fludrocortisone	2.08 (0.52 - 8.36)	
	RC	Captopril vs Fludrocortisone	1.86 (0.72 - 4.79)	
	RC	IV SLT vs Oral SLT	0.63 (0.20 - 1.96)	
	RC	IV SLT vs Captopril	0.70 (0.41 - 1.18)	
	RC	Oral SLT vs Captopril	1.12 (0.35 - 3.63)	
Cut-off IV SLT	PC	Restrictive vs Unrestrictive	NA	NA
	RC	Restrictive vs Unrestrictive	0.85 (0.43 - 1.73)	0.64

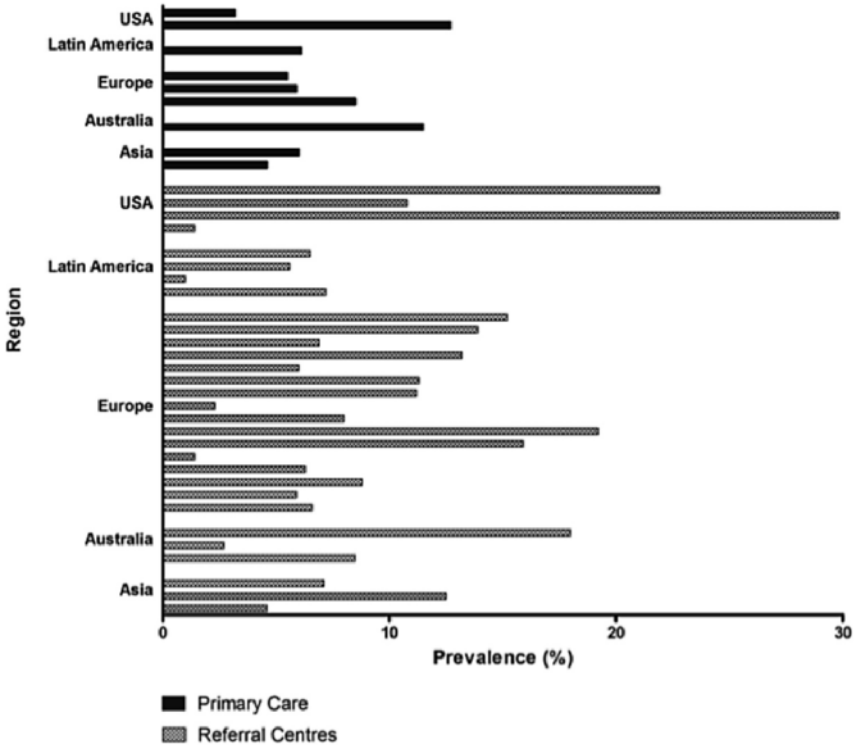
ARR, aldosterone-to-renin ratio. IV SLT, intravenous sodium loading test. HT, hypertension. NA, not applicable. OR, odds ratio. Oral SLT, oral sodium loading test. PA, primary aldosteronism. PC, primary care. RC, referral center.

Supplementary Table 6 Predicted prevalences according to the model

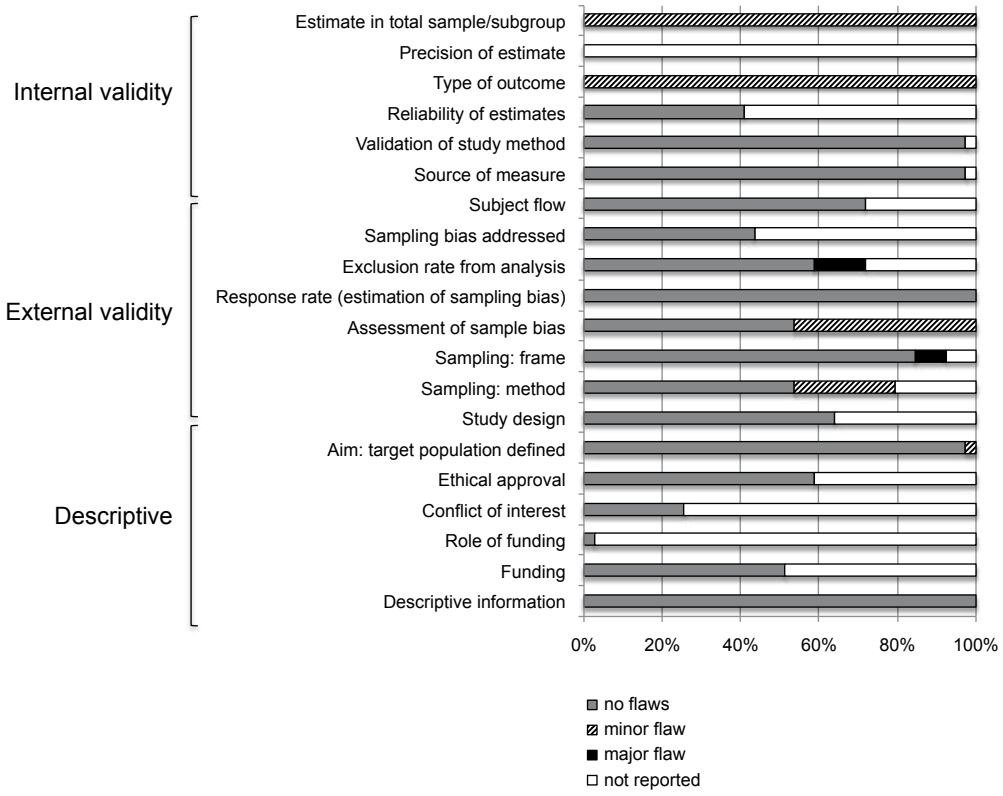
Changing variable	Publication year	Region	Study design	Study purpose	Patient selection method	Screening test	Prevalence (95% CI)
Publication year	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 (0.06345 - 0.17958)**
	1999-2000	Europe	Retrospective	Other	Consecutive	Other	0.01295 (0.00344 - 0.04749)
Region	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 (0.06345 - 0.17958)**
	2000-current	USA	Retrospective	Other	Consecutive	Other	0.37317 (0.24056 - 0.52804)
	2000-current	LatinAmerica	Retrospective	Other	Consecutive	Other	0.06088 (0.03073 - 0.11704)
	2000-current	Asia	Retrospective	Other	Consecutive	Other	0.15451 (0.07984 - 0.27792)
	2000-current	Australia	Retrospective	Other	Consecutive	Other	0.40424 (0.23498 - 0.59983)
Study design	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 (0.06345 - 0.17958)**
	2000-current	Europe	Prospective	Other	Consecutive	Other	0.05010 (0.02570 - 0.09542)
Study purpose	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 (0.06345 - 0.17958)**
	2000-current	Europe	Retrospective	Prevalence PA	Consecutive	Other	0.17246 (0.11326 - 0.25376)
	2000-current	Europe	Retrospective	Prevalence secondary HT	Consecutive	Other	0.25620 (0.13712 - 0.42748)
Patient selection method	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 (0.06345 - 0.17958)**
	2000-current	Europe	Retrospective	Other	Convenience	Other	0.02400 (0.00746 - 0.07443)
	2000-current	Europe	Retrospective	Other	Self Selection	Other	0.07716 (0.03636 - 0.15631)
Screening test	**2000-current**	**Europe**	**Retrospective**	**Other**	**Consecutive**	**Other**	**0.10856 (0.06345 - 0.17958)**
	2000-current	Europe	Retrospective	Other	Consecutive	No screening	0.28402 (0.19936 - 0.38724)
	2000-current	Europe	Retrospective	Other	Consecutive	ARR	0.08337 (0.03770 - 0.17436)
Combination for lowest prevalence	1999-2000	Latin America	Prospective	Other	Convenience	ARR	0.00046 (0.00004 - 0.00527)
Combination for highest prevalence	2000-Current	Australia	Retrospective	Prevalence secondary HT	Consecutive	No screening	0.40238 (0.18153 - 0.67148)

Predicted prevalences according to the model as a function of the six variables. The study of Di Murro[77] (2010) is chosen as reference study (bold). HT, hypertension.

Supplementary Figure 1 Bar plot for the prevalence of primary aldosteronism



Supplementary Figure 2 Quality assessment of the 39 included studies using the MORE criteria



MORE, Methodological evaluation of Observational REsearch (MORE).



3

Prevalence of primary aldosteronism in primary care: a cross-sectional study

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ABSTRACT

Background

Primary aldosteronism is the most frequent cause of secondary hypertension. Reported prevalences of primary aldosteronism vary considerably because of a large heterogeneity in study methodology.

Aim

To examine the proportion of patients with primary aldosteronism among patients with newly diagnosed, never treated hypertension.

Design and setting

A cross-sectional study set in primary care.

Methods

General practitioners measured aldosterone and renin in adult patients with newly diagnosed, never treated hypertension. Patients with elevated aldosterone-to-renin ratio and increased plasma aldosterone concentration underwent a saline infusion test to confirm or exclude primary aldosteronism. The source population was meticulously assessed to detect possible selection bias.

Results

Of 3748 patients with newly diagnosed hypertension, 343 patients were screened for primary aldosteronism. In 9 of 74 patients with an elevated aldosterone-to-renin ratio and increased plasma aldosterone concentration the diagnosis of primary aldosteronism was confirmed by a saline infusion test, resulting in a prevalence of 2.6% (95% confidence interval 1.4 to 4.9). All patients with primary aldosteronism were normokalemic and 8 out of 9 patients had sustained blood pressure >150/100 mmHg. Screened patients were younger ($p<0.001$) or showed higher blood pressure ($p<0.001$) than non-screened patients.

Conclusion

In this study a prevalence of primary aldosteronism of 2.6% in a primary care setting was established, which is lower than estimates reported from other primary care studies so far. This study supports the screening strategy as recommended by the Endocrine Society Clinical Practice Guideline. The low proportion of screened patients (9.2%), of the large cohort of eligible patients, reflects the difficulty of conducting prevalence studies in primary care clinical practice.

INTRODUCTION

Primary aldosteronism (PA) is the most frequent cause of secondary hypertension. Large variations of its prevalence have been reported, ranging from <1% to 30%.[1-5] This variance can be explained by the heterogeneity of studies owing to differences in patient selection, variability in diagnostic procedures, healthcare setting, and region of the world.[6]

Three aspects determine the clinical relevance of a diagnosis of PA. First, PA carries a high cardiovascular complication rate, independently of the level of blood pressure.[7-9] Second, PA requires specific treatment, depending on the underlying subtype: adrenal surgery for an aldosterone-producing adenoma, and a mineralocorticoid receptor antagonist in bilateral adrenal hyperplasia.[1] Third, quality of life is adversely affected by PA, and may improve after specific therapy such as an adrenalectomy.[10-12] Together with the long delay of eight years[13] in diagnosing PA in hypertensive patients, screening for PA in all patients with newly diagnosed hypertension might be beneficial. However, the Endocrine Society Clinical Practice Guideline does not advocate early screening for PA in patients with new hypertension apart from specific subgroups, such as patients with sustained blood pressure >150/100 mmHg on each of three measurements, and cases of hypertension and spontaneous hypokalemia.[1] The National Institute for Health and Care Excellence guideline advises '*simply to be aware of signs and symptoms and refer on the basis of a high index of suspicion*', for example, young onset hypertension (aged <40 years).[14,15] In the Netherlands, the primary care guideline for hypertension recommends that only patients with hypertension and hypokalemia and those with therapy resistant hypertension should be referred on suspicion of secondary hypertension.[16]

The primary objective of this study was to assess the proportion of patients with PA among patients with newly diagnosed, never treated hypertension presenting at Dutch primary care centres. Our secondary objective was to study selection bias in general practitioners' referral of patients for screening for PA.[17]

METHODS

Study setting and design

In this cross-sectional study patients from 55 primary care centres, from the Nijmegen region in the Netherlands, were recruited from 1 August 2013 to 31 December 2015 (Supplementary File 1).

The screening consisted of two phases. In the first biochemical screening phase, the plasma aldosterone-to-renin ratio (ARR) and plasma aldosterone concentration were determined in patients with newly diagnosed hypertension prior to starting antihypertensive treatment. In the second confirmatory phase, patients with an elevated ARR and elevated plasma aldosterone concentration underwent a saline

infusion test (SIT) to verify autonomous aldosterone secretion. The SIT is one of the four confirmation tests that is recommended by the Endocrine Society to confirm or exclude the diagnosis of PA.[1] A definite diagnosis of PA was made if saline loading failed to suppress the plasma aldosterone level.

Reporting of this study is in concordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.[18,19]

This study was approved by the Ethics Committee of the Radboud university medical center and all patients gave informed consent.

Participants and recruitment

Eligible patients had newly diagnosed, untreated hypertension and were aged ≥18 years. Hypertension was diagnosed according to the guideline of the European Society of Hypertension.[20] In brief, hypertension was diagnosed when: 1) office blood pressure was ≥140/90 mmHg on two or more different encounters within six months; 2) home blood pressure measurement (electronic device) was ≥135/85 mmHg; 3) 24-hour ambulatory blood pressure monitoring (ABPM) was ≥130/80 mmHg; or 4) daytime ABPM was ≥135/85 mmHg.

Every participating general practitioner (GP) was asked to draw a blood sample in eligible patients for measurement of plasma aldosterone and renin. This blood sample was obtained in the morning after the patients had been sitting for five minutes. Exclusion criteria were: (prior) use of antihypertensive medication, hypertensive crisis, heart failure class II-IV (according to the New York Heart Association[21]), estimated glomerular filtration rate of <45 ml/min/1.73m^2, pregnancy, breast feeding, diabetes mellitus, and presence of severe comorbidity (defined as seriously interfering with diagnostics or possible therapy). Patients who required immediate antihypertensive treatment (according to the GP) received specific medication with minimal effects on renin and aldosterone levels.[1]

Procedures

From 1 August 2013 to 14 December 2014, plasma aldosterone was measured using the Coat-A-Count aldosterone radioimmunoassay (RIA) from Siemens Medical Solutions Diagnostics (United States of America). From 15 December 2014 to 31 December 2015, plasma aldosterone was measured by the Active Aldosterone RIA kit from Beckman Coulter (Czech Republic). Plasma renin concentration was measured using the DSL-25100 active renin immunoradiometric assay (IRMA) from Diagnostic Systems Laboratories (United States of America).

The cut-off level of the ARR was >40 pmol/mU in combination with a plasma aldosterone of >400 pmol/L. The SIT consisted of intravenous infusion of two litres of sodium chloride 0.9% over four hours with the patient in the semi-recumbent position. After four hours blood was sampled for measurement of aldosterone. Usually, the aldosterone level will decrease after infusion of saline. In case of autonomous aldosterone secretion the negative feedback system is insufficient and aldosterone

levels remain too high. A plasma aldosterone concentration of >280 pmol/L was considered as definite PA, while an aldosterone level of <140 pmol/L excluded PA. In case of indeterminate values of 140 to 280 pmol/L the SIT was repeated. If still indeterminate, a diagnosis was reached by consensus after deliberation among clinical experts of the Department of Internal Medicine.

Data collection and processing

Data of all patients with newly diagnosed hypertension were extracted from the Electronic Health Records (EHRs) of the 55 participating centres. The dataset included demographics, clinical characteristics, biochemical test results, prescribed medication, and diagnoses coded according to the International Classification of Primary Care (ICPC).[22]

Inclusion criteria were applied to select all patients with a new diagnosis of hypertension: ICPC code hypertension (K86 or K87) between 1 August 2013 and 31 December 2015, or, when an ICPC code was not available, elevated blood pressure measurements were included according to the criteria described in the section 'Participants and recruitment' (above). For further details of inclusion criteria when an ICPC code was unavailable see Supplementary File 2.

Statistical analysis

Based on a population of nearly 200000 subjects (from 55 primary care centres), an incidence of hypertension of 0.6% yearly,[23,24] and a participation rate of 40%, this study aimed to enrol approximately 1100 patients. Anticipating a prevalence of PA of 5%, at least 931 patients were required to be included to estimate the prevalence of PA with an accuracy of 1.4% and a confidence level of 95%.

The statistical package SPSS Statistics version 22.0.0.1 was used to analyse the data. The proportion of patients with PA among patients with newly diagnosed hypertension was calculated by dividing the number of patients with confirmed PA by the number of patients with newly diagnosed hypertension who were screened for PA by using the ARR, plasma aldosterone, and the SIT. The total group of patients with newly diagnosed hypertension was extracted from the EHRs. To study selection bias between the screened and non-screened group, and taking into account the clustering of patients within practices, multilevel analyses[25] to calculate *P*-values were used. To assess if known patient characteristics influenced the referral for biochemical screening of PA, a multivariate multilevel logistic regression analysis with age, blood pressure, and comorbidity was entered into the model. Practice variation for biochemical screening was assessed by the intraclass correlation coefficient (ICC).

Independent samples t-tests for all screened patients were used to compare means between the PA and non-PA group, and a bootstrap test was used to corroborate the results.[26] Analysis of variance (ANOVA) was used to assess differences in means in case of three groups. Chi-square tests were used to

determine associations between categorical variables. Fisher's exact test was used in case of small sample size. As renin values showed a skewed distribution median and interquartile ranges (IQR) were calculated, using Mann-Whitney and Kruskal-Wallis tests to compare groups. Patients who did not complete the screening (n=18) were excluded from analyses.

RESULTS

Characteristics of the total study population

Out of 7205 patients with newly diagnosed hypertension that were identified, 3748 were found to be eligible after application of exclusion criteria (Figure 1). Of these patients, 361 patients (9.6%) were tested by measuring ARR and plasma aldosterone (Figure 1). Eighteen patients did not undergo a SIT for various reasons (Supplementary File 3). Therefore, 343 completed screening for PA (9.2%). Baseline characteristics between the group that proceeded to a SIT and the group that did not were comparable (Supplementary Table 1).

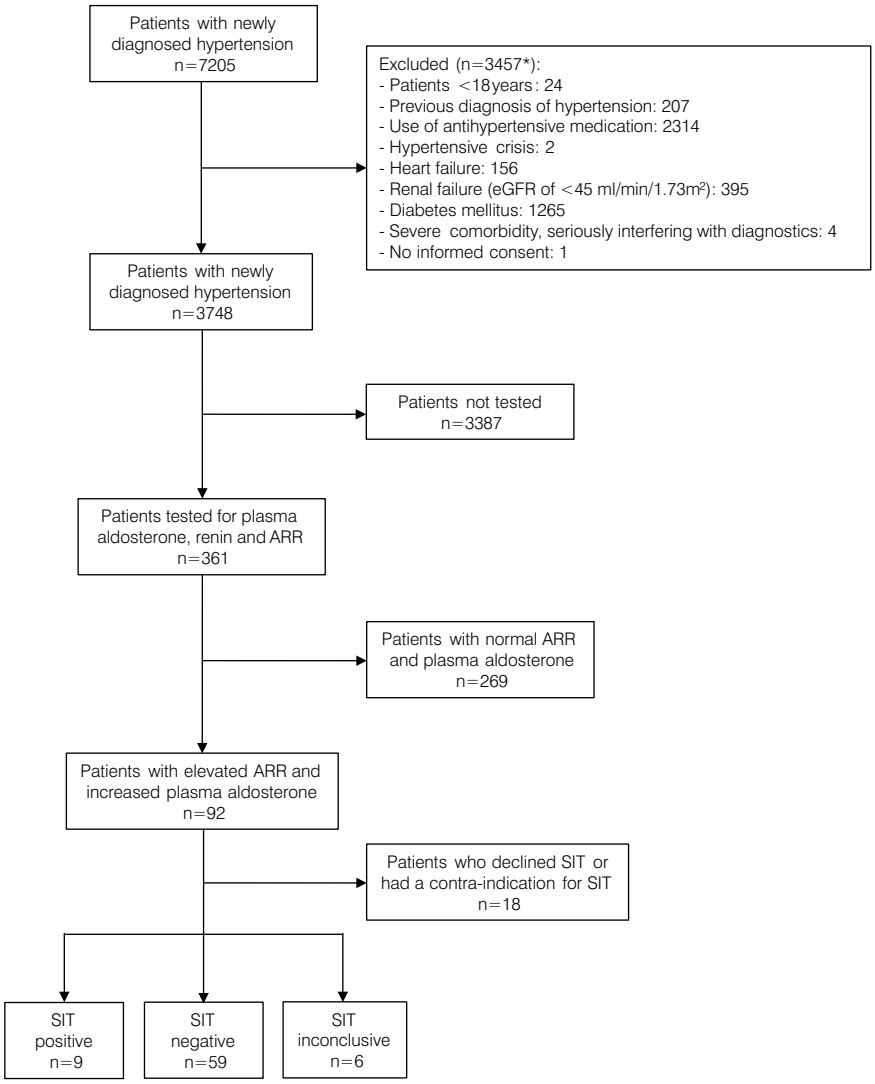
Prevalence of primary aldosteronism

Of all 361 biochemically screened patients, 92 (25.5%) showed an elevated ARR in combination with increased plasma aldosterone. Nine of 74 patients who underwent SIT showed insufficient aldosterone suppression, confirming the diagnosis of PA, and 18 patients declined SIT or had a contraindication for SIT (Figure 1). Hence, the prevalence of PA in patients with newly diagnosed hypertension was 9 out of 343 or 2.6% (95% CI 1.4 to 4.9). All nine patients with PA were normokalemic, and eight of them had sustained blood pressure >150/100 mmHg. As expected, the nine patients with PA had lower values of serum potassium and renin, and higher values of plasma aldosterone than patients without PA (Table 1).

Six patients had inconclusive test results after the first SIT and refused further diagnostic tests. Based on contextual information discussed at the expert meeting, the diagnosis of PA remained inconclusive. Hypertension of these six patients was treated with a mineralocorticoid receptor antagonist. Of the 74 patients who underwent SIT, 12 patients started immediate antihypertensive treatment after the diagnosis of hypertension (according to the Endocrine Society guideline).[1] None of these patients had a positive confirmation test.

When comparing the SIT positive group to the SIT negative group, serum potassium and renin levels were significantly lower in the SIT positive group than in the SIT negative group (Table 2).

Figure 1 Study flowchart



*Multiple patients were excluded by two or more exclusion criteria. ARR, aldosterone-to-renin ratio. eGFR, estimated glomerular filtration rate. SIT, saline infusion test.

Table 1 Baseline characteristics of all screened patients with newly diagnosed hypertension

Variable	Patients with available data n/n (PA-/PA+)	PA - (n=334)	PA + (n=9)	*P*-value
Demographics				
Male, n (%)	334/9	173 (51.8)	4 (44.4)	0.74
Age (years)	334/9	53.4 ± 11.2	54.3 ± 9.2	0.76
BMI (kg/m^2), n (%)	213/9			0.50
≤25		47 (22.1)	1 (11.1)	
>25 - ≤30		96 (45.1)	6 (66.7)	
>30		70 (32.9)	2 (22.2)	0.40
Smoking status, n (%)	163/9			
Current		36 (22.1)	2 (22.2)	
Former		55 (33.7)	1 (11.1)	
Never		72 (44.2)	6 (66.7)	
Blood pressure				
Systolic BP (mmHg)	332/9	163.8 ± 13.4	158.6 ± 10.2	0.17
Diastolic BP (mmHg)	329/9	96.3 ± 9.9	95.3 ± 9.1	0.75
ABPM (mmHg)	47/2	154.2 ± 15.7	166.5 ± 9.2	0.28
ABPMday (mmHg)	42/1	159.3 ± 15.6	161.0 ± NA	NA
Home BP (mmHg)	2/0	160.0 ± 0	NA	NA
Heart rate (beats/min)	192/7	75.2 ± 12.6	68.6 ± 12.8	0.22
Biochemical parameters				
Potassium (mmol/L)	332/9	4.43 ± 0.33	4.11 ± 0.26	0.006
Sodium (mmol/L)	332/9	141.7 ± 2.0	141.7 ± 2.6	0.95
Creatinine (μmol/L)	332/9	78.8 ± 14.6	75.8 ± 13.1	0.51
Aldosterone (pmol/L)	334/9	360.9 ± 220.5	668.4 ± 118.1	<0.001
Renin* (pmol/L)	334/9	0.78 (0.58-1.33)	0.48 (0.34-0.68)	0.003
ARR (pmol/mU)	334/9	29.8 ± 22.8	105.3 ± 48.6	0.002
Glucose (mmol/L)	301/9	5.4 ± 1.0	5.4 ± 0.5	0.75
Cardiovascular morbidity				
OSAS, n (%)	334/9	5 (1.5)	0 (0)	1.00
Atrial fibrillation, n (%)	334/9	2 (0.6)	0 (0)	1.00
Stroke, n (%)	334/9	2 (0.6)	0 (0)	1.00
Myocardial infarction, n (%)	334/9	2 (0.6)	0 (0)	1.00

Data are presented as mean ± standard deviation unless stated otherwise. *median (interquartile range). ABPM, systolic ambulatory blood pressure monitoring. ABPMday, daytime systolic ambulatory blood pressure monitoring. ARR, aldosterone-to-renin ratio. BMI, body mass index. Diastolic BP, office diastolic blood pressure. Home BP, home systolic blood pressure. min, minute. NA, not available. OSAS, obstructive sleep apnea syndrome. PA, primary aldosteronism. Systolic BP, office systolic blood pressure.

Table 2 Study characteristics of all patients who underwent a saline infusion test

Variable	Patients with available data n/n/n (SIT+/SIT-/SITi)	SIT positive (n=9)	SIT negative (n=59)	SIT inconclusive (n=6)	*P*-value[a]	*P*-value[b]
Demographics						
Male, n (%)	9/59/6	4 (44.4)	17 (28.8)	3 (50.0)	0.44	0.38
Age (years)	9/59/6	54.3 ± 9.2	51.4 ± 11.2	52.8 ± 4.6	0.40	0.72
BMI (kg/m^2), n (%)	9/59/6				0.65	0.17
≤25		1 (11.1)	15 (25.4)	0		
>25 - ≤30		6 (66.7)	27 (45.8)	6 (100)		
>30		2 (22.2)	17 (28.8)	0		
Smoking status, n (%)	9/58/6				0.53	0.60
Current		2 (22.2)	14 (24.1)	1 (16.7)		
Former		1 (11.1)	17 (29.3)	3 (50.0)		
Never		6 (66.7)	27 (46.6)	2 (33.3)		
Blood pressure						
Systolic BP (mmHg)	9/59/6	158.6 ± 10.2	163.9 ± 13.7	167.3 ± 12.5	0.20	0.42
Diastolic BP (mmHg)	9/59/6	95.3 ± 9.1	97.0 ± 9.8	101.5 ± 10.3	0.62	0.46
ABPM (mmHg)	2/13/1	166.5 ± 9.2	159.8 ± 18.6	171.0 ± NA	0.49	0.77
ABPMday (mmHg)	1/12/1	161.0 ± NA	162.8 ± 19.8	175.0 ± NA	NA	0.83
Home BP (mmHg)	0/1/0	NA	160.0 ± NA	NA	NA	NA
Heart rate (beats/min)	7/35/5	68.6 ± 12.8	76.5 ± 13.4	71.4 ± 13.6	0.17	0.31
Biochemical parameters						
Potassium (mmol/L)	9/58/6	4.11 ± 0.26	4.38 ± 0.39	4.3 ± 0.2	0.02	0.12
Sodium (mmol/L)	9/58/6	141.7 ± 2.6	142.0 ± 2.1	141.3 ± 2.1	0.74	0.75

Table 2 -Continued

Variable	Patients with available data n/n/n (SIT+/SIT-/SITi)	SIT positive (n=9)	SIT negative (n=59)	SIT inconclusive (n=6)	*P*-value[a]	*P*-value[b]
Biochemical parameters						
Creatinine (μmol/L)	9/58/6	75.8 ± 13.1	79.4 ± 13.5	88.2 ± 14.3	0.45	0.22
Aldosterone (pmol/L)	9/59/6	668.4 ± 118.1	592.3 ± 244.2	494.0 ± 94.5	0.15	0.35
Renin* (pmol/L)	9/59/6	0.48 (0.34-0.68)	0.67 (0.55-0.78)	0.55 (0.38-0.62)	0.04	0.02
ARR (pmol/mU)	9/59/6	105.3 ± 48.6	61.1 ± 19.9	75.9 ± 41.3	0.03	<0.001
Glucose (mmol/L)	9/56/5	5.4 ± 0.5	5.1 ± 0.8	5.2 ± 0.5	0.27	0.67
Cardiovascular morbidity						
OSAS, n (%)	9/59/6	0	0	0	NA	NA
Atrial fibrillation, n (%)	9/59/6	0	0	0	NA	NA
Stroke, n (%)	9/59/6	0	1 (1.7)	0	1.00	NA
Myocardial infarction, n (%)	9/59/6	0	0	0	NA	NA

Data are presented as mean ± standard deviation unless stated otherwise. [a]*P*-value: comparison of SIT positive and SIT negative. [b]*P*-value: comparison of SIT positive and SIT negative and SIT inconclusive. *median (interquartile range). ABPM, systolic ambulatory blood pressure monitoring. ABPMday, daytime systolic ambulatory blood pressure monitoring. ARR, aldosterone-to-renin ratio. BMI, body mass index. Diastolic BP, office diastolic blood pressure. Home BP, home systolic blood pressure. min, minute. NA, not available. OSAS, obstructive sleep apnea syndrome. PA, primary aldosteronism. SIT, saline infusion test. SITi, saline infusion test with inconclusive result. Systolic BP, office systolic blood pressure.

Screened versus non-screened patients

Screened patients were younger, had higher blood pressure, and had higher serum potassium levels than non-screened patients. More patients in the non-screened group suffered from stroke (Table 3). Multivariate multilevel logistic regression analysis showed an independent effect on biochemical screening of age, systolic blood pressure, and previous stroke ($p<0.001$, $p<0.001$, $p=0.016$, respectively). Referral for biochemical screening was more likely in younger patients (odds ratio (OR) 0.96, 95% CI 0.95 to 0.97), and in patients with higher blood pressure (OR 1.06, 95% CI 1.05 to 1.07). Patients who suffered from stroke had a lower chance to be referred for screening (OR 0.17, 95% CI 0.04 to 0.72) (Supplementary Table 2). Intraclass correlation coefficient was 0.22, which indicates a considerable variation in referral for screening among centres.

DISCUSSION

Summary

In this primary care study the prevalence for primary aldosteronism in patients with newly diagnosed hypertension is 2.6%. This number is lower than reported prevalences from other primary care studies so far. The low number of screened patients (9.2%) of the large cohort of eligible patients reflects the difficulty in studying prevalence of primary aldosteronism in primary care clinical practice, when the daily routine of GPs collides with a study protocol.

Strengths and limitations

This study set out to screen for PA in patients with newly diagnosed hypertension in a primary care setting. In many countries, the initial diagnosis of hypertension is predominantly made by GPs in primary care centres where there is no referral bias as is the case in prevalence studies from referral centres. A strength of this research was that specific subgroups in which PA may be more or less prevalent were excluded, such as patients with diabetes mellitus[27-29] and patients with a hypertensive crisis.[30] Another strong aspect of this study was that all patients were not treated with antihypertensive drugs at the time of inclusion or any time before. This minimises any confounding effects of these drugs on plasma aldosterone and renin.[1] Finally, a strong feature of the research was that the total source population was examined for incomplete screening and possible selection bias by digital scrutiny of the EHRs.[31]

Concerning the reliability and validity the ARR is generally considered the best first-line screening test for hypertensive patients in whom there is clinical suspicion of PA.[32,33] Yet, the ARR as an exploratory screening test has its limitations as it is influenced by many factors.[34,35] Selection bias was assessed by the use of ICPC codes from EHR data. These ICPC codes depend on the quality of recording. Because GPs may not always assign an ICPC code for hypertension,[36] the patients

Table 3 Baseline characteristics of all patients with newly diagnosed hypertension

Variable	Patients with available data n/n (screened/ non-screened)	Screened (n=343)	Non-screened (n=3387)	*P*-value*
Demographics				
Male, n (%)	343/3387	177 (51.6)	1586 (46.8)	0.15
Age (years)	343/3387	53.4 ± 11.1	58.5 ± 13.4	<0.001
BMI (kg/m^2), n (%)	222/1377			0.13
≤25		48 (21.6)	383 (27.8)	
>25 - ≤30		102 (45.9)	576 (41.8)	
>30		72 (32.4)	418 (30.4)	
Smoking status, n (%)	172/597			0.84
Current		38 (22.1)	135 (22.6)	
Former		56 (32.6)	218 (36.5)	
Never		78 (45.3)	244 (40.9)	
Blood pressure				
Systolic BP (mmHg)	341/2880	163.6 ± 13.3	156.3 ± 11.8	<0.001
Diastolic BP (mmHg)	338/2880	96.3 ± 9.9	89.8 ± 9.5	<0.001
ABPM (mmHg)	49/229	154.7 ± 15.6	147.0 ± 12.9	0.001
ABPMday (mmHg)	43/204	159.4 ± 15.4	152.1 ± 12.8	0.003
Home BP (mmHg)	2/12	160.0 ± 0	144.0 ± 5.2	NA
Heart rate (beats/min)	199/1659	75.0 ± 12.6	74.5 ± 12.1	0.74
Biochemical parameters				
Potassium (mmol/L)	341/1785	4.42 ± 0.34	4.37 ± 0.37	0.04
Sodium (mmol/L)	341/1685	141.7 ± 2.0	141.9 ± 2.1	0.08
Creatinine (μmol/L)	341/1996	78.8 ± 14.6	78.9 ± 15.2	0.87
Aldosterone (pmol/L)	343/0	369.0 ± 223.8	NA	NA
Renin# (pmol/L)	343/0	0.77 (0.58-1.31)	NA	NA
ARR (pmol/mU)	343/0	31.8 ± 26.6	NA	NA
Glucose (mmol/L)	310/1786	5.43 ± 1.01	5.45 ± 0.83	0.55
Cardiovascular morbidity				
OSAS, n (%)	343/3387	5 (1.5)	41 (1.2)	0.71
Atrial fibrillation, n (%)	343/3387	2 (0.6)	52 (1.5)	0.17
Stroke, n (%)	343/3387	2 (0.6)	156 (4.6)	0.003
Myocardial infarction, n (%)	343/3387	2 (0.6)	32 (0.9)	0.60

Data are presented as mean ± standard deviation unless stated otherwise. **P*-value calculated by univariate multilevel analyses. #median (interquartile range). ABPM, systolic ambulatory blood pressure monitoring. ABPMday, daytime systolic ambulatory blood pressure monitoring. ARR, aldosterone-to-renin ratio. BMI, body mass index. Diastolic BP, office diastolic blood pressure. Home BP, home systolic blood pressure. min, minute. NA, not available. OSAS, obstructive sleep apnea syndrome. PA, primary aldosteronism. Systolic BP, office systolic blood pressure.

with elevated blood pressure measurements without an ICPC code were also included. This improved case finding, but may have been too sensitive as the number of patients with newly diagnosed hypertension (n=3748) was higher than expected (Supplementary Table 3).

Comparison with existing literature

In this primary care study the proportion of patients with PA in patients with newly diagnosed hypertension was 2.6%. This is lower than reported in previous primary care studies that performed a confirmation test in at least half of biochemically screened patients, ranging from 3.2% to 11.5%.[37-46] The two studies that restricted their study population to patients with newly diagnosed hypertension found a prevalence of 5.5% and 6.0%.[40,45] Two other studies included only normokalemic patients with hypertension and established prevalences of 3.2% and 12.7%.[41,43]

Several explanations for the low prevalence in this research have to be considered. First, studies vary considerably in their methods and screening cut-offs. In this research a relatively low cut-off value for the ARR of >40 pmol/mU was deliberately used with the aim to miss as few PA patients as possible.[47,48] To prevent too many false-positive test results due to low renin hypertension, the criterion of a minimum plasma aldosterone level of 400 pmol/L was added.[49] However, the cut-off for the SIT in this study was quite strict.

In 26 patients an elevated ARR was found without an increased plasma aldosterone. These patients did not undergo a SIT and this might have also contributed to our low prevalence seen in this study. In addition, 18 eligible patients did not undergo the SIT and six other patients had inconclusive test results (Figure 1). Under the theoretical assumption that these 24 patients might have PA, the virtual prevalence would be maximally 9.1% (33/361). However, this possibility is unlikely.

Screened patients were younger and had higher blood pressures as compared to non-screened patients. This indicates that GPs intuitively followed the screening recommendation of the Endocrine Society guideline,[1] which recommends screening of patients with blood pressure >150/100 mmHg. In addition, for unknown reasons, GPs were less likely to perform biochemical screening in patients with newly diagnosed hypertension who suffered from a stroke. Apparently, GPs screened patients with a higher a priori chance of having PA and may have missed patients with a lower chance of having PA. It is therefore conceivable that this selective screening has contributed to an underestimation of the real PA prevalence. In addition, GPs might consider stroke as 'severe comorbidity', which was an exclusion criteria for participation in this research.

The present study illustrates that, despite a straightforward clinical protocol, an unbiased selection of patients for screening for PA is very hard to achieve in a primary care setting. The protocol in this research, intentionally designed to minimise bias, is apparently not compatible with routine daily practice in primary care.[17,50] This is reflected in the discrepancy between the a priori calculated sample size and the

number of included patients in this research. Although the planned number of screened patients was not reached, nonetheless a prevalence estimate was achieved with a narrow confidence margin (95% CI 1.4% to 4.9%).

Because the definition of hypertension may differ between countries, for example, the UK and the Netherlands[15,16] and population characteristics may vary, the denominator in the prevalence estimate may also differ between countries. Moreover, the authors' experience in the current research raises the question of whether similar potential selection bias might have confounded previously published primary care studies on the prevalence of PA. To assess the prevalence of PA more precisely and to circumvent selection bias, rigorous screening of all patients with newly diagnosed hypertension is required. Employing a computerised algorithm without involvement of the GP in the selection process might be a better screening strategy, for example, a pop-up in the screen when an elevated blood pressure is added for the second time or when an ICPC code for hypertension is entered.

Implications for research and practice

As previous studies have shown that hypokalemia is only present in a minority of PA patients, it should be noted that all PA patients in the current study were normokalemic. This re-emphasises that the absence of hypokalemia as a reliable clinical marker to exclude PA should be considered obsolete.[51]

A diagnosis of PA has enormous consequences, both on patients' well-being and on healthcare logistics and costs. Early screening followed by adequate treatment may not only improve quality of life, it may also be cost-saving.[52] Although the low prevalence, as found in this study, does not support indiscriminate screening of all new hypertensive patients for PA, all patients in this research project would have been missed following the current primary care guideline. In contrast, if the recommendations of the Endocrine Society guideline had been used, eight of the nine PA patients would have been picked up as they had a sustained blood pressure of >150/100 mmHg. It might be, therefore, reasonable to adopt the recommendations of the Endocrine Society guideline also in the field of primary care, so that the detection rate of PA may be improved.[1]

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Declaration

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Competing interests. The authors have declared no competing interests.

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SUPPLEMENTARY FILES

Supplementary File 1. Recruitment of primary care centres

We telephoned general practitioners to explain the need and purpose of this study. If they were willing to participate, we visited them for explanation and instructions. We built a website with information for patients as well as professionals. For daily consultation professionals could call a special phone number. During the study all involved professionals received periodic newsletters. We aimed to maximise protocol compliance by adapted laboratory forms, and repeated reminders.

Supplementary File 2. Selection of the source population

We selected our source population of patients with newly diagnosed hypertension by applying the following inclusion: 1) ICPC code hypertension (K86 or K87) between August 1st 2013 and December 31st 2015, or 2) when an ICPC code was not available, we included elevated blood pressure measurements according to the criteria described in 'Participants and recruitment'. Office blood pressure measurements had to be noted from February 1st 2013 to December 31st 2015, at least one measurement had to be performed on or after August 1st 2013. Elevated home blood pressure measurements and (daytime) ABPMs were included from August 1st 2013 to December 31st 2015. If patients fulfilled the second inclusion criterion, we checked if an ICPC code hypertension had been created before August 1st 2013. If so, these patients were subsequently excluded because their diagnosis of hypertension could not be new.

Supplementary File 3. Reasons why patients had no saline infusion test

Seven patients declined because it was too bothersome for them, two patients declined because of the costs of additional testing, two patients required referral to a cardiologist (this was noticed during the telephonic contact between researcher and GP prior to SIT), two patients needed to take care for an ill relative, two patients suffered from travel anxiety, one patient did not want to be notified of the screening results ('right of not knowing', in which patients could consent to perform an additional blood sample, while refusing to be noticed of the test result), and for two patients the reason for drop-out was missing.

GP, general practitioner. SIT, saline infusion test.

Supplementary Table 1 Baseline characteristics of patients with elevated aldosterone-to-renin ratio and increased plasma aldosterone who had a saline infusion test and those who had not

Variable	Patients with available data n/n (SIT/not SIT)	SIT (n=74)	No SIT (n=18)	*P*-value*
Demographics				
Male, n (%)	74/ 18	24 (32.4)	4 (22.2)	0.37
Age (years)	74/ 18	51.8 ± 10.5	55.7 ± 10.7	0.22
BMI (kg/m^2), n (%)	74/ 13			0.68
≤25		16 (21.6)	2 (15.4)	
>25 - ≤30		39 (52.7)	6 (46.2)	
>30		19 (25.7)	5 (38.5)	
Smoking status, n (%)	73/4			0.41
Current		17 (23.2)	0 (0)	
Former		21 (28.8)	3 (75.0)	
Never		35 (47.9)	1 (25.0)	
Blood pressure				
Systolic BP (mmHg)	74/ 18	163.5 ± 13.2	162.0 ± 11.2	0.74
Diastolic BP (mmHg)	74/ 18	97.1 ± 9.7	95.1 ± 7.4	0.35
ABPM (mmHg)	16/ 0	161.3 ± 17.2	NA	NA
ABPMday (mmHg)	16/ 0	163.5 ± 18.5	NA	NA
Home BP (mmHg)	1/ 0	160.0 ± NA	NA	NA
Heart rate (beats/min)	47/ 10	74.7 ± 13.4	75.9 ± 11.4	0.61
Biochemical parameters				
Potassium (mmol/L)	73/ 18	4.34 ± 0.37	4.41 ± 0.43	0.53
Sodium (mmol/L)	73/ 18	141.9 ± 2.2	141.6 ± 1.9	0.70
Creatinine (μmol/L)	73/ 18	79.7 ± 13.6	74.6 ± 13.0	0.28
Aldosterone (pmol/L)	74/ 18	593.6 ± 225.9	585.7 ± 179.7	0.99
Renin# (pmol/L)	74/ 18	0.64 (0.54-0.76)	0.68 (0.52-0.79)	0.93
ARR (pmol/mU)	74/ 18	67.7 ± 30.1	64.6 ± 21.7	0.72
Glucose (mmol/L)	70/ 18	5.16 ± 0.72	5.36 ± 0.69	0.58
Cardiovascular morbidity				
OSAS, n (%)	74/ 18	0 (0)	0 (0)	NA
Atrial fibrillation, n (%)	74/ 18	0 (0)	0 (0)	NA
Stroke, n (%)	74/ 18	1 (1.4)	0 (0)	NA
Myocardial infarction, n (%)	74/ 18	0 (0)	0 (0)	NA

Data are presented as mean ± standard deviation unless stated otherwise. **P*-value calculated by univariate multilevel analyses. #median (interquartile range). ABPM, systolic ambulatory blood pressure monitoring. ABPMday, daytime systolic ambulatory blood pressure monitoring. ARR, aldosterone-to-renin ratio. BMI, body mass index. Diastolic BP, office diastolic blood pressure. Home BP, home systolic blood pressure. Min, minute. NA, not available. OSAS, obstructive sleep apnea syndrome. PA, primary aldosteronism. Systolic BP, office systolic blood pressure. SIT, saline infusion test.

Supplementary Table 2 Multivariate multilevel logistic regression analysis of patients characteristics that may influence referral for screening for primary aldosteronism

Variable	OR (95% CI)	*P*-value
Demographics		
Gender (male)	0.89 (0.70-1.14)	0.36
Age (years)	0.96 (0.95-0.97)	<0.001
Blood pressure		
Systolic BP (mmHg)	1.06 (1.05-1.07)	<0.001
Comorbidity		
OSAS	1.04 (0.37-2.94)	0.95
Atrial fibrillation	0.64 (0.14-2.84)	0.55
Stroke	0.17 (0.04-0.72)	0.016
Myocardial infarction	1.67 (0.35-8.07)	0.52

CI, confidence interval. OR, odds ratio. OSAS, obstructive sleep apnea syndrome. Systolic BP, office systolic blood pressure.

Supplementary Table 3 Baseline characteristics of patients with newly diagnosed hypertension according to ICPC code versus patients without an ICPC code

Variable	Patients with available data n/n (ICPC/no ICPC)	ICPC K86/K87 (n=2042)	No ICPC K86/K87 (n=1706)	*P*-value*
Demographics				
Male, n (%)	2042/1706	964 (47.2)	803 (47.1)	0.84
Age (years)	2042/1706	57.4 ± 13.1	58.9 ± 13.5	0.03
BMI (kg/m^2), n (%)	823/789			0.036
≤25		202 (24.5)	231 (29.3)	
>25 - ≤30		339 (41.2)	345 (43.7)	
>30		282 (34.3)	213 (27.0)	
Smoking status, n (%)	407/366			0.56
Current		95 (23.3)	78 (21.3)	
Former		141 (34.6)	136 (37.2)	
Never		171 (42.0)	152 (41.5)	
Blood pressure				
Systolic BP (mmHg)	1546/1693	160.6 ± 13.4	153.8 ± 10.0	<0.01
Diastolic BP (mmHg)	1543/1693	93.2 ± 10.0	88.0 ± 8.8	<0.01
ABPM (mmHg)	165/113	151.9 ± 14.0	143.2 ± 11.6	<0.01
ABPMday (mmHg)	152/96	156.6 ± 13.4	148.40 ± 12.2	<0.01
Home BP (mmHg)	6/8	145.3 ± 5.4	147.0 ± 9.1	0.35
Heart rate (beats/min)	929/939	74.9 ± 11.9	74.2 ± 12.3	0.31
Biochemical parameters				
Potassium (mmol/L)	1238/906	4.35 ± 0.38	4.41 ± 0.34	0.01
Sodium (mmol/L)	1177/867	141.7 ± 2.2	142.1 ± 2.1	0.001
Creatinine (μmol/L)	1335/1020	78.5 ± 15.1	79.2 ± 15.1	0.77
Aldosterone (pmol/L)	242/119	403.2 ± 234.1	332.1 ± 203.1	0.005
Renin# (pmol/L)	242/119	0.76 (0.56-1.33)	0.76 (0.57-1.21)	0.08
ARR (pmol/mU)	242/119	35.5 ± 29.3	29.3 ± 22.1	0.07
Glucose (mmol/L)	1195/919	5.45 ± 0.88	5.44 ± 0.83	0.93
Cardiovascular morbidity				
OSAS, n (%)	2042/1706	28 (1.4)	18 (1.1)	0.48
Atrial fibrillation, n (%)	2042/1706	22 (1.1)	32 (1.9)	0.06
Stroke, n (%)	2042/1706	77 (3.8)	81 (4.7)	0.049
Myocardial infarction, n (%)	2042/1706	19 (0.9)	15 (0.9)	0.86

Data are presented as mean ± standard deviation unless stated otherwise. **P*-value calculated by univariate multilevel analyses. #median (interquartile range). ABPM, systolic ambulatory blood pressure monitoring. ABPMday, daytime systolic ambulatory blood pressure monitoring. ARR, aldosterone-to-renin ratio. BMI, body mass index. Diastolic BP, office diastolic blood pressure. Home BP, home systolic blood pressure. ICPC, International Classification of Primary Care. min, minute. K86, ICPC code for 'hypertension without organ damage'. K87, ICPC code for 'hypertension with organ damage'. NA, not available. OSAS, obstructive sleep apnea syndrome. PA, primary aldosteronism. Systolic BP, office systolic blood pressure. SIT, saline infusion test.



4

Do patients with primary aldosteronism have cardiovascular damage at time of diagnosing hypertension in primary care?

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ABSTRACT

Introduction

Patients with primary aldosteronism (PA) have a higher risk of cardiovascular complications compared to patients with essential hypertension (EHT) and similar blood pressure levels. It is unclear whether cardiovascular damage is already present at the time of diagnosing hypertension. The aim of this exploratory study was to assess cardiovascular organ damage in patients who were screened for PA at the time of diagnosing hypertension in primary care.

Methods

We prospectively assessed cardiovascular damage in six patients with newly diagnosed PA and 24 matched patients with EHT at the time when hypertension was diagnosed in the primary care. We performed detailed cardiovascular assessment, including ankle-brachial index, echocardiography, flow-mediated vasodilation, carotid ultrasonography, central aortic blood pressure, pulse wave velocity, and urinary albumin-to-creatinine ratio measurement.

Results

Two of the six patients with PA versus none of the patients with EHT ($p=0.04$) fulfilled the criteria of concentric LVH (>115 g/m^2 in men and >95 g/m^2 in women). After adjustment for gender, age and blood pressure, left ventricular mass index was higher in the patients with PA than in the patients with EHT. We did not observe differences in the other outcome measures between the patient groups.

Conclusion

At the time of the diagnosis of hypertension, patients with PA have a higher frequency of LVH than patients with EHT. If confirmed in larger studies, this finding suggests that early biochemical testing for PA, and specific treatment of PA, might contribute to the prevention of further progression of cardiovascular damage due to inadequately treated PA.

INTRODUCTION

Primary aldosteronism (PA) is characterized by unilateral or bilateral autonomous overproduction of aldosterone in the adrenal cortex. PA is the most common cause of secondary hypertension, with prevalences varying from 3% to 12% in primary care versus 1% to 30% in referral centres.[1,2]

According to the Endocrine Society guideline, the diagnosis of PA should be considered in specific patients with hypertension.[3] However, in daily clinical primary care practice, this recommendation on testing for PA is commonly omitted, as general practitioners are generally not aware of this guideline.[4] To complicate matters further, hypokalemia, previously considered to be a prerequisite for a diagnosis of PA, is present in only less than 40% of the PA patients. So, this biomarker has limited utility to incite appropriately testing for PA.[5] For these reasons the diagnosis of PA has been reported to be delayed for up to eight years.[6]

The delay in a timely diagnosis of PA is potentially harmful for patients for at least two reasons. First, treatment of PA differs from usual antihypertensive treatment in patients with essential hypertension (EHT). Patients with PA require specific treatment: those with a unilateral aldosterone-producing adenoma are advised to undergo adrenalectomy, whereas those with bilateral aldosterone overproduction are treated with a mineralocorticoid receptor antagonist (MRA), such as spironolactone.[3] Second, patients with PA have a higher risk of cardiovascular complications in comparison to patients with EHT with similar blood pressure levels.[7] This may be explained by a direct effect of aldosterone unopposed by appropriate treatment during the long pre-diagnostic phase, inducing cardiovascular organ damage well before diagnosis. The higher risk of cardiovascular complications in patients with PA suggests the need for timely biochemical testing for PA in (newly diagnosed) hypertensive patients to prevent further development of cardiovascular organ damage.

In this explorative study in the primary care setting, we prospectively assessed cardiovascular and renal damage in patients in whom PA was detected at the time when hypertension was diagnosed for the first time. A group of newly diagnosed patients with EHT, matched for gender, age and blood pressure, served as a control group.

METHODS

Study population

We included all patients over 18 years with newly diagnosed never treated hypertension from 55 primary care centres in the Netherlands from August 1st 2013 to December 31st 2015. In the context of a previous study on the prevalence of PA, the participating patients had plasma aldosterone and renin measured at the time of diagnosing hypertension, and before starting antihypertensive treatment. This study has been described in detail elsewhere.[8]

The diagnosis of hypertension was made according to the current guideline by the European Society of Hypertension.[9] In brief, hypertension was diagnosed: 1) if the average office blood pressure of at least two blood pressure measurements per day was ≥140/90 mmHg on two or more different visits within six months, or 2) if 24-hour ambulatory blood pressure measurement (ABPM) was ≥130/80 mmHg.

Patients with newly diagnosed hypertension were screened for PA by measurement of plasma aldosterone and renin. In case of an aldosterone-to-renin ratio (ARR) of >40 pmol/mU and a plasma aldosterone concentration of >400 pmol/L, an intravenous sodium loading test (SLT; two litres NaCl 0.9% in four hours) was performed. Diagnosis of PA was made when the aldosterone concentration exceeded 280 pmol/L after sodium loading. Patients were considered to have EHT if ARR values were ≤40 pmol/mU with a concomitant low baseline aldosterone level of ≤400 pmol/L (ARR_{neg}), or an aldosterone value of ≤280 pmol/L after SLT ($ARR_{pos}SLT_{neg}$). During biochemical testing, patients did not use medication that interfered with aldosterone and renin levels.[3]

We included all patients diagnosed with PA, and applied the following exclusion criteria: age <18 years, hypertensive crisis, heart failure classes II-IV (defined by the New York Heart Association[10]), diabetes mellitus, estimated glomerular filtration rate of <45 ml/min/1.73m^2, pregnancy or breast feeding, and severe comorbidity that would seriously interfere with study procedures. We applied similar exclusion criteria for the patients with EHT. We matched the patients with PA with the patients with EHT for gender, age, and baseline blood pressure. For every patient with PA, we included four control patients with EHT: one $ARR_{pos}SLT_{neg}$ patient, and three ARR_{neg} patients. This numeric relation corresponds to the ratio ARR_{pos}/ARR_{neg} that was found among patients with newly diagnosed EHT in primary care.[8]

This study was approved by the Ethics Committee of the Radboud university medical center. All patients provided written informed consent before enrollment. The study was conducted in accordance with Good Clinical Practices, and the Declaration of Helsinki, and was prospectively registered at ClinicalTrials.gov by number NCT01728493.

Clinical data

We collected the following clinical data: body mass index (BMI), medication use, smoking status (pack years (PY)), previous and family history of cardiovascular disease, alcohol intake per day (units of 10 g), physical exercise (standard defined as 30 minutes/day during five or more days/week), snoring (defined as light or heavy according to the patients' judgement) or diagnosis of obstructive sleep apnea syndrome (OSAS), and history of gestational hypertension. We expressed the antihypertensive medication in daily defined doses (DDD), as defined by the World Health Organisation.[11] From August 1st 2013 to December 14th 2014, plasma aldosterone was measured using the Coat-A-Count aldosterone radioimmunoassay (RIA) from Siemens Medical Solutions Diagnostics (United States of America). From

December 15th 2014 to December 31st 2015, plasma aldosterone was measured by the Active Aldosterone RIA kit from Beckman Coulter (Czech Republic). Plasma renin concentration was measured using the DSL-25100 active renin immuno-radiometric assay (IRMA) from Diagnostic Systems Laboratories (United States of America). Baseline plasma concentrations of creatinine, potassium, cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides were determined using standard assays in a central laboratory (SHO, Velp, the Netherlands).

Study protocol

In patients with PA and $ARR_{pos}SLT_{neg}$ patients with EHT, ankle brachial index (ABI) and urinary albumin-to-creatinine ratio (uACR) were performed during their admission for SLT. Echocardiography was carried out shortly after this. We performed measurements of the other cardiovascular risk markers during a separate visit. In ARR_{neg} patients with EHT, all measures were obtained during a single visit (Table 1).

Table 1 Duration from diagnosis to measurement of the primary outcomes (in months)

Assessment	PA (n=6)	EHT	
		$ARR_{pos}SLT_{neg}$ (n=6)	ARR_{neg} (n=18)
ABI, and uACR	1	1	18
Echocardiography	4	6	19
FMD, cIMT, central aortic blood pressure, and PWV	19*	12	18

*5 patients. ABI, ankle-brachial index. ARR, aldosterone-to-renin ratio. cIMT, carotid intima-media thickness. FMD, flow-mediated dilation. PA, primary aldosteronism. PWV, pulse wave velocity. SLT, intravenous sodium loading test. uACR, urinary albumin-to-creatinine ratio.

We measured the brachial blood pressure in the supine position using a manual sphygmomanometer (Welch Allyn, Leiden, the Netherlands), in a quiet room after a period of five minutes rest according to the guideline.[12] All patients abstained from caffeine, alcohol, and products rich on vitamin C and/or flavonoids 24 hours before the measurements. We performed the measurements at least six hours after fasting. We asked the patients not to smoke six hours before the experiments, and to refrain from exercise during 24 hours before the measurements. Patients took their medication after finishing all vascular measurements on the day of the experiments.[13,14] We assessed the following seven primary outcomes:

Ankle-brachial index (ABI)

For the measurement of the ABI we used the standardized technique as described by the American Heart Association.[15] In brief, we performed limb pressure measurements after at least five minutes of rest in the supine position. All limb pressure measurements were done by Doppler (Dopplex D900, Huntleigh Healthcare Ltd, Cardiff, UK) in the following sequence: right brachial artery, right tibial posterior artery, right dorsal pedal artery, left tibial posterior artery, left dorsal pedal artery, and left brachial artery. When the pressure between both brachial arteries exceeded 10 mmHg, we performed a second measurement of the right brachial artery and discarded the first measurement. We expressed the ABI as the highest lower-extremity blood pressure, divided by the highest blood pressure in both arms.

Echocardiography

Standard echocardiographic examinations were carried out with subjects in the partial left decubitus position using a commercially available instrument (GE Vivid E9, General Electric, Horten, Norway), equipped with the multifrequency 1.5 to 4.0 MHz M5S transducer. End-diastolic and end-systolic left ventricular internal diameters (LVIDd, LVIDs), interventricular septum and posterior wall thicknesses (PWT) were measured from two dimensional parasternal long axis view, from which left ventricular mass index (LVMI) was calculated according to the American Society of Echocardiography guidelines and normalized by body surface area.[16] Relative wall thickness (RWT) was calculated as $2 \times$ PWT/LVIDd. A normal LVMI was defined as $\leq 115\,g/m^2$ in men, and $\leq 95\,g/m^2$ in women. We defined eccentric hypertrophy as an increased LVMI ($>115\,g/m^2$ in men, and $>95\,g/m^2$ in women) with a RWT <0.42, and concentric hypertrophy as an increased LVMI with a RWT >0.42. Furthermore, concentric remodeling was defined as a normal LVMI, but increased RWT (>0.42).[16] Left ventricular (LV) filling, in casu diastolic LV function was assessed by the standard pulsed and tissue Doppler technique.[17] The following parameters were considered: the early diastolic mitral peak flow velocity (E), the late diastolic mitral peak flow velocity (A), their ratio (E/A ratio), and the average of both maximal early diastolic tissue velocity of the medial and lateral mitral annulus (E') and the average E/E'.

Flow-mediated dilation (FMD)

An experienced researcher of the Department of Physiology of the Radboud university medical center measured brachial FMD in a darkened, temperature-controlled room of 22.1 $\pm$ 0.4 °C using a 10-MHz multifrequency linear-array probe attached to a high-resolution ultrasound machine (Terason T3000, Burlington, USA) according to the guideline of Thijssen *et al*.[14] The researcher was blinded for the diagnosis. Briefly, a sphygmomanometer blood pressure cuff was positioned around the forearm and the brachial artery was imaged proximally of the antecubital fossa. After one minute of baseline recordings of diameter and blood flow velocity, the cuff was inflated for five minutes, at a pressure of 200 mmHg or at least 50 mmHg above systolic blood pressure

(SBP). We captured changes in brachial artery diameter and blood flow velocity 30 seconds before cuff deflation until three minutes post-deflation, and analyzed the recordings offline in a blinded fashion using computer-assisted software, utilizing edge-detection and wall-tracking. We expressed the FMD as the % change in diameter ((peak diameter after deflation minus baseline diameter)/baseline diameter x 100%).

Carotid intima-media thickness (cIMT)

The cIMT was measured by high resolution B-mode ultrasound with a 7.5-MHz linear-array transducer (Esaote Biomedica, Genoa, Italy). We measured the intima and media of the left and right common carotid artery far wall over a 1 cm segment caudally from the carotid bulb, in three different angles of 90, 120 and 180°. The integrated software of the Esaote platform uses radio-frequency technology to provide six measures, calculated as means from real-time values, obtained during six cardiac cycles. The standard deviation (SD) of these six mean measures was directly visible and the data were accepted if the SD did not exceed 20 μm. We calculated the mean diameter and cIMT from 18 measures (six means times three angles) of every patient for the left and right carotid artery. We thoroughly scanned the extracranial carotid arteries for the presence of plaques. A plaque was defined as a focal wall thickening of ≥50% compared to the surrounding vessel wall, or a local cIMT greater than 1.5 mm, according to the consensus statement from the American Society of Echocardiography.[18]

Central aortic blood pressure and pulse wave velocity (PWV)

With the patient in the supine position, we performed pulse wave analysis of the right radial artery using applanation tonometry (SphygmoCor, AtCor Medical, Australia). The SphygmoCor software generates the central aortic blood pressure and augmentation index (AIx) from a ten second recording after calibration for peripheral blood pressure. We discarded measurements that did not meet the quality control criteria of the software. We recorded the median of three valid central aortal blood pressure measurements. For the assessment of the aortic PWV, we measured the pressure waves at the sites of the right carotid artery and the right femoral artery. The SphygmoCor software automatically calculates the transit time as the delay between the R-spike in the electrocardiogram, and the arrival of the pressure waves at the recording sites. We estimated the travel distance by subtracting the distance from the carotid tonometer location to the sternal notch from the distance between the sternal notch to the femoral tonometer location.[19] In all patients we performed three measurements and recorded the median PWV. When the difference between the first and second PWV was ≤0.5 m/s, we did not perform a third measurement and recorded the mean PWV of these two measurements.[20] The quality of the pressure wave was directly analyzed by the Sphygmocor software. If the SD was ≥10% of the PWV measurement, we discarded the measurement and replaced it by a novel measurement, up to a maximum of six attempts.

Urinary albumin-to-creatinine ratio (uACR)

The uACR was measured in a single urine sample by the Department of Clinical Chemistry of the Radboud university medical center. Urinary albumin was measured using a nephelometric technology (BN II analyzer, Siemens, the Netherlands). Urinary creatinine was analyzed by Cobas 8000, Roche Diagnostics, the Netherlands).

Statistical analysis

For the analysis of the data, we used IBM SPSS Statistics 22. We expressed all values as mean ± SD. We considered a significance value of <0.05 (two-sided). Differences between patients with PA and patients with EHT were compared using an independent t-test. Because of the small sample size, we checked the robustness of the independent t-test with bootstrapping. Differences in proportions were compared using the Fisher's exact test (two-sided). For comparisons between more than two groups, we used a one-way ANOVA. We compared each of the single outcome measures between the patients with PA and EHT using a general linear model with correction for gender, age and blood pressure. Central aortic blood pressures were corrected only for gender and age, because the Sphygmocor software calculates the central blood pressure and AIx from the brachial blood pressure.

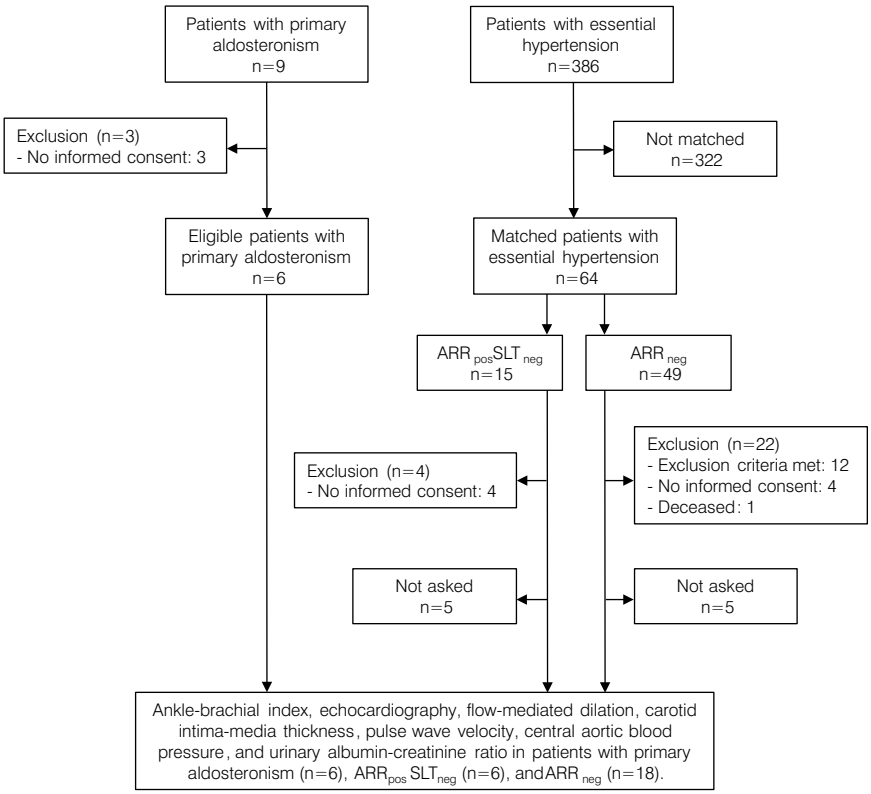
RESULTS

Patients and clinical characteristics

Of the patients with newly diagnosed hypertension, nine were diagnosed with PA.[8] Of these patients, three patients declined to participate in the present study. Therefore, we included six patients with PA and 24 matched control patients with EHT in our study. For an overview of the selection of our study population see Figure 1. In this total group of 30 patients, the diagnosis of hypertension was based on office blood pressure measurements in 26 patients, and on APBM in four patients.[9] Four patients with PA had bilateral aldosterone overproduction, and one had a unilateral aldosterone-producing adenoma. In one patient laterality could not be determined, as this patient refused adrenal venous sampling and computed tomography. One of the six patients with PA dropped out, because of recently diagnosed breast cancer. We did not perform FMD, PWV and central aortic blood pressure in this patient. Due to technical problems, we excluded the results of FMD in one patient with EHT. In two patients with EHT we did not obtain a valid PWV (SD >10% of the mean PWV) due to obesity.

There were no differences in baseline characteristics between the patients with PA and EHT regarding cardiovascular risk factors and laboratory screening, except for differences in plasma aldosterone, ARR, and potassium values (Table 2). None of the patients had hypokalemia.

Figure 1 Overview of the selection process of the patients



In the PA and the $ARR_{pos}SLT_{neg}$ patients, ABI and uACR were assessed after a mean period of one month after the diagnosis of hypertension (Table 1). The patients with PA did not use any antihypertensive medication at that time, and their SBP was significantly higher compared to ARR_{neg} patients (Table 3A). During echocardiography, two patients with PA used antihypertensive medication. In the other four patients with PA, antihypertensive treatment started after echocardiography. Echocardiography was performed 3.7 ± 2.9, 6.3 ± 9.2, and 19.1 ± 7.2 months after the diagnosis of hypertension in PA, $ARR_{pos}SLT_{neg}$ patients (with EHT), and ARR_{neg} patients (with EHT), respectively ($p<0.01$). During assessment of FMD, cIMT, PWV, and central aortic blood pressure, blood pressure levels and DDD of antihypertensive drugs were comparable between patients with PA and patients with EHT (Table 3B). The general practitioner had started antihypertensive drugs in 12 of the 24 patients with EHT without reaching target blood pressure levels of <140/90 mmHg (RR 151 ± 15/93 ± 10 mmHg).[9] The remaining 12 patients did not use antihypertensive agents, and their blood pressure was 164 ± 19/89 ± 6 mmHg.

Table 2 Baseline characteristics of all patients

Variable	PA (n=6)	EHT (n=24)	*P*-value
Demographics			
Male, n (%)	3 (50)	12 (50)	1.00
Age (years)	55.8 ± 9.1	56.6 ± 8.3	0.85
Cardiovascular risk factors			
BMI (kg/m^2)	26.6 ± 2.9	28.4 ± 3.4	0.23
Units alcohol/day	1.0 ± 1.2	1.2 ± 1.8	0.73
Smoking, n (%)			0.12
Current	1 (16.7)	0	
Former	1 (16.7)	12 (50)	
PY[a]	23 ± 30	21 ± 19	0.96
Daily exercise			
Less than standard (%)	2 (33.3)	6 (25.0)	0.21
1st degree family history			
CVD (%)	2 (33.3)	17 (70.8)	0.33
Unknown (%)	0	1 (4.2)	
2nd degree family history			0.35
CVD (%)	0	9 (37.5)	
Unknown (%)	0	2 (8.3)	
Gestational hypertension (%)	1 (33.3)	6 (50.0)	1.00
Snoring			0.08
Light (%)	1	15	
Heavy (%)	2	2	
OSAS (%)	1	0	0.20
Diagnosis			
Reason of visit			0.19
Complaints (%)	4 (66.7)	7 (29.2)	
High blood pressure at screening test (%)	2 (33.3)	8 ((33.3)	
At clinic for other reason (%)	0	9 (37.5)	
SBP (mmHg)	169 ± 9	164 ± 11	0.24
DBP (mmHg)	104 ± 7	97 ± 9	0.07
Laboratory screening			
Potassium (mmol/L)	4.1 ± 0.3	4.5 ± 0.3	0.03
Sodium (mmol/L)	141 ± 3	142 ± 2	0.36
MDRD (mL/min)	78 ± 8	74 ± 13	0.29
Aldosterone (pmol/L)	721 ± 90	338 ± 218	<0.01

Table 2 -Continued

Variable	PA (n=6)	EHT (n=24)	*P*-value
Laboratory screening			
Renin (pmol/L)	0.6 ± 0.3	2.1 ± 3.5	0.05
ARR (pmol/mU)	104.9 ± 57.6	26.8 ± 22.7	0.02
Fasting glucose (mmol/L)	5.4 ± 0.6	5.5 ± 0.7	0.88
Cholesterol (mmol/L)	6.8 ± 1.2	5.9 ± 1.0	0.15
HDL (mmol/L)	1.5 ± 0.5	1.5 ± 0.4	0.98
LDL (mmol/L)	4.6 ± 0.8	4.1 ± 0.9	0.23
Triglycerides (mmol/L)	2.8 ± 2.6	1.3 ± 0.6	0.22

Data are presented as mean ± standard deviation unless stated otherwise. [a]For current and former smokers. BMI, body mass index. CVD, cardiovascular disease. DBP, diastolic blood pressure. MDRD, 'modification of diet in renal disease', equation to estimate the glomerular filtration rate from serum creatinine. OSAS, obstructive sleep apnea syndrome. SBP, systolic blood pressure.

Table 3A Blood pressure and antihypertensive treatment during assessment of ABI, uACR and shortly before echocardiography

Variable	PA (n=6)	EHT		*P*-value
		$ARR_{pos}SLT_{neg}$ (n=6)	ARR_{neg} (n=18)	
Peripheral blood pressure				
Brachial SBP in mmHg	172 ± 15	172 ± 23	154 ± 10	0.01
Brachial DBP in mmHg	100 ± 10	91 ± 6	92 ± 7	0.05
Duration of hypertension (months)	1.0 ± 0.9	1.0 ± 1.1	18.0 ± 7.1	<0.01
Medication use				
Number of patients taking antihypertensive drugs (%)	0	1 (16.7)	11 (61.1)	0.01
Thiazides (%)	0	0	3 (16.7)	1.00
ACE inhibitors (%)	0	0	6 (33.3)	0.30
ARB (%)	0	0	1 (5.6)	1.00
CCB (%)	0	1 (16.7)	2 (11.1)	1.00
BB (%)	0	0	0	
MRA (%)	0	0	0	
Statin use (%)	0	0	2 (11.1)	1.00

Data are presented as mean ± standard deviation unless stated otherwise. ACE, angiotensin converting enzyme. ARB, angiotensin receptor blocker. ARR, aldosterone-to-renin ratio. BB, beta blocker. CCB, calcium channel blocker. DBP, diastolic blood pressure. EHT, essential hypertension. MRA, mineralocorticoid receptor antagonist. PA, primary aldosteronism. SBP, systolic blood pressure. SLT, intravenous sodium loading test.

Table 3B Blood pressure and antihypertensive treatment during assessment of FMD, cIMT, PWV, and central aortic blood pressure

Variable	PA (n=6)	EHT		*P*-value
		$ARR_{pos}SLT_{neg}$ (n=6)	ARR_{neg} (n=18)	
Peripheral blood pressure				
Brachial SBP in mmHg	139 ± 17.6	156 ± 26	154 ± 10	0.14
Brachial DBP in mmHg	86 ± 14	88 ± 10	92 ± 7	0.41
Duration of hypertension (months)	18.8 ± 7.4	12.3 ± 6.0	18.0 ± 7.1	0.20
Medication use				
Number of patients taking antihypertensive drugs (%)	5 (83.3)	2 (33.3)	11 (61.1)	0.09
Thiazides (%)	0	1 (16.7)	3 (16.7)	1.00
ACE inhibitors (%)	0	1 (16.7)	6 (33.3)	0.30
ARB (%)	0	1 (16.7)	1 (5.6)	1.00
CCB (%)	2 (40.0)	2 (33.3)	2 (11.1)	0.27
BB (%)	0	1 (16.7)	0	1.00
MRA (%)	4 (80.0)	0	0	0.00
Statin use (%)	2 (40.0)	0	2 (11.1)	0.17

Data are presented as mean ± standard deviation unless stated otherwise. ACE, angiotensin converting enzyme. ARB, angiotensin receptor blocker. ARR, aldosterone-to-renin ratio. BB, beta blocker. CCB, calcium channel blocker. DBP, diastolic blood pressure. EHT, essential hypertension. MRA, mineralocorticoid receptor antagonist. PA, primary aldosteronism. SBP, systolic blood pressure. SLT, intravenous sodium loading test.

Primary outcomes

The unadjusted primary outcome measures are presented in Table 4A. None of the patients had eccentric LVH, whereas two female patients with PA had concentric LVH on echocardiography (p=0.03). Concentric remodelling was present in two patients with PA (33.3%) and five patients with EHT (20.8%; p=0.60). LVMI was higher among patients with PA, but the difference with patients with EHT was not significant in the unadjusted analysis. After correction for blood pressure, gender, and age, LVMI was significantly higher in patients with PA compared to patients with EHT (90.50 ± 7.73 versus 70.70 ± 3.61 g/m^2, respectively; p=0.04). There was no increased frequency of diastolic dysfunction, atrial dilation, and carotid plaques in patients with PA compared to control patients with EHT.

The adjusted mean values of the single outcome measures, with correction for gender, age and baseline blood pressure are depicted in Table 4B. We did not observe differences in ABI, cIMT, FMD, central blood pressure, AIx, PWV, and uACR between patients with PA compared to patients with EHT (Table 4).

Table 4A Primary outcome measures, unadjusted

	PA	EHT	*P*-value
ABI			
Left ABI	1.1 ± 0.0	1.1 ± 0.1	0.76
Right ABI	1.1 ± 0.1	1.1 ± 0.1	0.53
Echocardiography			
Concentric hypertrophy (%)	2 (33.3)	0 (0)	0.03
Concentric remodeling (%)	2 (33.3)	5 (20.8)	0.60
LVMI (g/m^2)	83.48 ± 16.72	72.48 ± 16.92	0.19
Diastolic dysfunction (%)	3 (50.0)	11 (45.8)	1.00
Atrial dilation (%)	2 (33.3)	5 (20.8)	0.60
FMD*			
Baseline diameter (cm)	0.439 ± 0.114	0.407 ± 0.057	0.57
% change (diameter)	4.3 ± 3.3	4.6 ± 3.0	0.88
Time to peak (seconds)	54.4 ± 33.5	73.7 ± 47.9	0.32
cIMT**			
Carotid plaques (%)	1 (20.0)	8 (33.3)	1.00
Left cIMT (μm)	733.8 ± 188.8	747.0 ± 150.5	0.89
Right cIMT (μm)	732.7 ± 177.5	727.6 ± 135.2	0.96
Central aortic blood pressure**			
Central SBP (mmHg)	130 ± 18	145 ± 15	0.15
Central DBP (mmHg)	87 ± 14	92 ± 8	0.49
Central AIx (%)	28.4 ± 7.8	30.7 ± 8.0	0.55
PWV*** (m/s)	8.7 ± 1.3	9.6 ± 1.7	0.21
uACR (mg/mmol)	3.4 ± 3.6	3.9 ± 13.0	0.89

*in PA (n=5) and EHT (n=23). **in PA (n=5). ***in PA (n=5) and EHT (n=22). Data are presented as mean ± standard deviation unless stated otherwise. ABI, ankle-brachial index. AIx, augmentation index. cIMT, carotid intima-media thickness. DBP, diastolic blood pressure. EHT, essential hypertension. FMD, flow-mediated dilation. LVMI, left ventricle mass index. PA, primary aldosteronism. PWV, pulse wave velocity. SBP, systolic blood pressure. SLT, intravenous sodium loading test. uACR, urinary albumin-to-creatinine ratio.

Table 4B Primary outcome measures, adjusted for gender, age, and systolic blood pressure

	PA	EHT	*P*-value
ABI			
Left ABI	1.1 ± 0.0	1.1 ± 0.0	0.70
Right ABI	1.1 ± 0.0	1.1 ± 0.0	0.56
Echocardiography			
LVMI (g/m^2)	90.50 ± 7.73	70.70 ± 3.61	0.04
FMD*			
% change (diameter)	4.47 ± 1.48	4.63 ± 0.66	0.92
Time to peak (seconds)	67.0 ± 21.9	71.6 ± 9.7	0.85
cIMT**			
Left cIMT (μm)	748.8 ± 62.0	743.4 ± 26.8	0.94
Right cIMT (μm)	741.4 ± 62.9	725.3 ± 27.3	0.82
Central aortic blood pressure**			
Central SBP (mmHg)	131 ± 7	145 ± 3	0.07
Central DBP (mmHg)	87 ± 4	92 ± 2	0.25
Central AIx (%)	29.0 ± 3.5	30.7 ± 1.6	0.67
PWV*** (m/s)	9.0 ± 0.7	9.5 ± 0.3	0.55
uACR (mg/mmol)	5.1 ± 5.6	3.4 ± 2.6	0.80

*in PA (n=5) and EHT (n=23). **in PA (n=5). ***in PA (n=5) and EHT (n=22). Data are presented as mean ± standard deviation unless stated otherwise. ABI, ankle-brachial index. AIx, augmentation index. cIMT, carotid intima-media thickness. DBP, diastolic blood pressure. EHT, essential hypertension. FMD, flow-mediated dilation. LVMI, left ventricle mass index. PA, primary aldosteronism. PWV, pulse wave velocity. SBP, systolic blood pressure. SLT, intravenous sodium loading test. uACR, urinary albumin-to-creatinine ratio.

DISCUSSION

In a Dutch primary care population we screened patients with newly diagnosed hypertension for PA.[8] We demonstrated that the proportion of patients with LVH was higher in patients with PA as compared to patients with EHT. There were no differences in ABI, FMD, cIMT, central aortic blood pressure, PWV, and uACR between patients with PA and EHT.

The prevalence of LVH in patients with PA is in the range of 20% to 60% in referral centres.[7,21-23] This high proportion of LVH in these studies may be due to persistent exposure to high circulating aldosterone levels, since the diagnosis and treatment of PA are delayed.[6] In our study, echocardiography was performed in patients with newly diagnosed hypertension, with hardly any delay in the diagnosis of PA. Our findings therefore suggest that in patients with PA, LVH is already present at the time of diagnosing hypertension.

In addition to the increased prevalence of LVH, LVMI was higher in patients with PA compared to patients with EHT after correction for gender, age, and baseline blood pressure (Table 4). LVMI is a strong and independent predictor of future cardiovascular events.[24] It has been shown that every g/m^2 increase in LVMI results in a hazard ratio of 1.013 to 1.015 for the risk of cardiovascular events in the general population.[24]

To prevent progressive cardiovascular damage, it might be important to screen for PA as early as possible. However, it remains challenging when and who to screen. In our study there were no differences in baseline characteristics between patients with PA and patients with EHT. Moreover, none of the patients with PA presented with hypokalemia, which is one of criteria to screen for PA according to the Endocrine Society guideline. This guideline recommends screening also in case of sustained blood pressure >150/100 mmHg.[3] In our cohort, 83% of the patients with PA had a blood pressure above 150/100 mmHg on separate visits at the time of diagnosing hypertension, which highlights the relevance of this clinical clue in the primary care setting.

Our findings of an increased risk of LVH in patients with PA, but lack of differences in other surrogate endpoints of target organ damage between patients with PA and EHT, suggests that the toxic effects of aldosterone affect mainly the heart but not other organ systems. However, others have shown that patients with PA have more morphological and functional vascular damage, and an impaired endothelial function, when compared to matched patients with EHT.[25-29] Importantly, the patients in these studies were not included at the time hypertension was diagnosed for the first time. Therefore, we speculate that aldosterone-mediated effects on the vascular system may become manifest later on, and thus might be prevented by early diagnosis and adequate treatment.

Strengths and limitations

The strength of our study is that the diagnosis of PA was made without a substantial delay. Furthermore, the diagnoses of PA and EHT were based on stringent criteria, according to the international guideline of the Endocrine Society.[3] Another strength of our study is that we assessed cardiovascular damage using the combination of seven different cardiovascular risk markers. The results of our study might be helpful in the design and sample size calculation of future prospective trials.

A major limitation of the study is the limited power to detect differences in outcomes between the study groups, except for LVH. During the two year inclusion period, an ARR was measured in less than 10% of the patients with newly diagnosed hypertension.[8] Only nine patients were diagnosed with PA, which resulted in a lower power than anticipated for the current study. Our study was designed as a prospective cohort study, but given the lagging recruitment it has a explorative character.

Other limitations are the timing of the vascular investigations due to logistic difficulties, and the ensuing difference in medication use across the groups. We assessed ABI, uACR, and echocardiography in the patients with PA and $ARR_{pos}SLT_{neg}$

patients with EHT shortly after their diagnosis. In patients with EHT, these outcomes were assessed approximately 18 months after the diagnosis (Table 1). Fifty percent of these patients used antihypertensive drugs during a mean period of 16 ± 8 months at the time of the vascular investigations. One might argue that therapy-related improvements in the EHT group may have accounted for the differences in LVH and LVMI between patients with PA and patients with EHT. However, half of the patients with EHT were not treated despite being hypertensive with, in some cases, a stage 2 hypertension (systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥100 mmHg). Moreover, in the treated patients, blood pressure control appeared to be poor. Therefore, the longer duration of hypertension and the inadequate treatment of 50% of the patients with EHT, may have stimulated LVH in the control group. The fact that we still found an increased prevalence of LVH in patients with PA argues for aldosterone being the culprit of LVH.

During FMD, carotid ultrasonography, PWV and central aortic blood pressure, blood pressure levels did not differ between patients with PA and patients with EHT, as shown in table 3B. However, both patient groups used antihypertensive medications that may have altered the outcomes of these vascular risk markers (Table 3B).[30]

CONCLUSIONS

We found a higher prevalence of LVH in newly diagnosed hypertensive patients with PA compared to newly diagnosed patients with EHT in whom PA was excluded. This finding suggests that screening for PA with subsequent treatment at the time of diagnosing hypertension might be useful to prevent further progression of cardiovascular damage.

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Is the plasma aldosterone-to-renin ratio associated with blood pressure response to treatment in general practice?

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ABSTRACT

Background

Individualized antihypertensive treatment based on specific biomarkers such as renin may lead to more effective blood pressure control in patients with newly diagnosed essential hypertension. Recent studies suggested that the plasma aldosterone-to-renin ratio (ARR) may also be a candidate predictor for this purpose.

Objective

To assess whether the ARR is associated with the blood pressure response to antihypertensive treatment in patients with newly diagnosed hypertension.

Methods

In this prospective cohort study in primary care, we determined the ARR in patients with newly diagnosed hypertension prior to starting treatment. Treatment was categorized in five groups: no medication, use of angiotensin-converting-enzyme inhibitor or angiotensin receptor blocker, use of calcium channel blocker, use of diuretic, or use of beta blocker. We examined the relation between the ARR and blood pressure response within one year of treatment, taking into account the type of antihypertensive treatment and adjusting for gender, age, baseline blood pressure, and comorbidity.

Results

Out of 304 patients, we used 947 measurements (727 no medication, 220 medication) for analysis. There was no association between the ARR and the response in blood pressure, and this applied to each treatment group. Target blood pressure, defined as systolic blood pressure <140 mmHg, was reached in 31% of patients. There was no association between the ARR and reaching target blood pressure (OR 1.002, 95% CI 0.983 to 1.022).

Conclusion

The ARR is not associated with the response in blood pressure within one year of antihypertensive treatment in primary care.

INTRODUCTION

Despite a wide variety of treatment options, target blood pressure is not reached in many patients with hypertension.[1-3] This can be explained by various factors such as poor therapy adherence, secondary hypertension, comorbidity, and biological factors. Although numerous studies have demonstrated that all classes of antihypertensive drugs are similarly effective in reducing blood pressure,[4,5] it is also known that subgroups of patients respond better to specific classes of antihypertensive treatment. For example, in young hypertensive patients blood pressure is better controlled with an angiotensin-converting-enzyme inhibitor (ACE-I), angiotensin receptor blocker (ARB) or a beta blocker,[6,7] and in black patients with hypertension blood pressure response is superior to a calcium channel blocker (CCB) or a diuretic.[8] It is plausible to assume that personalized treatment guided by specific individual characteristics or biomarkers may lead to more effective blood pressure reduction.[9,10]

Of the studies that assessed biomarkers, several studies focussed on treatment guided by the activity of the renin-angiotensin-aldosterone system. In particular renin has been studied as a biomarker to guide antihypertensive treatment. Patients with low renin levels were considered as having a high (intravascular) volume hypertension and therefore proposed to be candidates for diuretic treatment, while patients with high renin levels were considered more sensitive for treatment with a renin-lowering beta blocker.[11,12]

The plasma aldosterone-to-renin ratio (ARR) reflects the level of aldosterone secretion in relation to renin secretion. The ARR is predominantly used as a screening test for primary aldosteronism, the most frequent cause of secondary hypertension.[13,14] The prevalence of primary aldosteronism is estimated to be 2-5% in primary care, but this varies between studies due to differences in diagnostic methods.[15,16] Apart from primary aldosteronism some patients with essential hypertension may also display high ARR levels, although still in the normal range.[17] Patients with a high ARR display an inappropriate high level of aldosterone in relation to renin. From the patho-physiological point of view, the high blood pressure in patients with a high ARR is considered to be due to an increased renal retention of sodium and water. Therefore these patients may be particularly sensitive for treatment with diuretics and mineralocorticoid receptor antagonists.[18] However, it is unclear whether or not the ARR can be used as a patient-specific biomarker in patients with newly diagnosed essential hypertension to predict the response to antihypertensive treatment.

For several reasons it would be very helpful if an easy to use and cheap test would be available to predict blood pressure response to antihypertensive treatment. First, such a test, if effective, could markedly contribute to a more rapid optimization of antihypertensive treatment. Second, it could lead to less side-effects as not all drugs would need to be tried. Finally, such an approach might result in lower health care costs as patients achieve the target blood pressure more rapidly, thus reducing the associated hypertensive complications.

In this primary care observational study, we measured the ARR in patients with newly diagnosed hypertension prior to starting antihypertensive treatment. Our primary objective was to assess whether the ARR is associated with the blood pressure response in patients after a maximum of one year of treatment, and whether this association varied over the main specific classes of antihypertensive medication used in primary care (ACE-inhibitors, ARBs, beta blockers, CCBs, thiazide diuretics). Our secondary objective was to study the association between baseline ARR level and the number of patients that achieved a target systolic blood pressure of <140 mmHg within one year of treatment.

METHODS

Study setting and study population

In this prospective cohort study we recruited patients from 55 primary care centres in the Nijmegen region in the Netherlands from August 1st 2013 to December 31st 2015. We included all patients over 18 years with newly diagnosed never treated hypertension. In the context of a previous study on the prevalence of primary aldosteronism, the participating patients had a measurement of plasma aldosterone and renin before starting antihypertensive treatment. This study is described in detail elsewhere.[15] All patients had an office blood pressure measurement at baseline, at least two weeks of antihypertensive treatment, and at least one visit with office blood pressure measurement within twelve months of follow-up. For generalizability of the study results to patients with essential hypertension, patients with primary aldosteronism were excluded from this patient cohort as they require specific therapy according to the Endocrine Society guideline.[19] Other exclusion criteria were: hypertensive crisis, heart failure class II-IV (according to the New York Heart Association), estimated glomerular filtration rate of <45 ml/min/1.73m^2, pregnancy, breast feeding, diabetes mellitus, and severe comorbidity (defined as seriously interfering with diagnostics or therapy). All patients were on a liberal salt diet.

This study was approved by the Ethics Committee of the Radboud university medical center and all patients gave informed consent. All general practitioners (GPs) approved extraction of their Electronic Health Records (EHRs) by written permission and informed their patients. Patients were given the opportunity to decline the use of their de-identified data. This method complies with the Code of Conduct for Health Research which has been approved by the Data Protection Authorities in conformity with the applicable Dutch privacy legislation. Reporting of this study is in concordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.[20]

Procedures

Hypertension was defined according to the guideline of the European Society of Hypertension.[1] In brief, hypertension was diagnosed if average office blood pressure of at least two blood pressure measurements per day was ≥140/90 mmHg on two or more different visits within six months.

In the period from August 1st 2013 to December 14th 2014, plasma aldosterone was measured using the Coat-A-Count aldosterone radioimmunoassay (RIA) from Siemens Medical Solutions Diagnostics (Unites States of America). From December 15th 2014 to December 31st 2015, plasma aldosterone was measured by the Active Aldosterone RIA kit from Beckman Coulter (Czech Republic). Plasma renin concentration was measured using the DSL-25100 active renin immunoradiometric assay (IRMA) from Diagnostic Systems Laboratories (United States of America).

Data collection and processing

We extracted data from the EHRs of the participating centres. The dataset included demographics, clinical characteristics, biochemical test results and prescribed medications. As in this study secondary analyses were performed, we refer to the parent study for the sample size calculation, and a full overview of the extraction process.[15] In brief, patients were included if they had: 1) an International Classification of Primary Care (ICPC)[21] code hypertension (K86 or K87) between August 1st 2013 and December 31st 2015, or 2) when an ICPC code was not available, if they had had two visits to their general practice documenting elevated blood pressure values as described in 'Procedures'. For each included patient we extracted all office blood pressure values at baseline (i.e. the date of diagnosis of hypertension), and those obtained during one year after the initial diagnosis. Antihypertensive treatment was initiated by the GP, and comprised the use of an antihypertensive agent in combination with lifestyle advice according to the guideline. Blood pressure measurements were categorized in five treatment groups, in which each blood pressure measurement and the corresponding antihypertensive treatment was set. Because treatment could change over time, patients could change from treatment categories throughout the observation period and thus contribute to the analysis of more than one antihypertensive treatment. For the follow-up we excluded blood pressure values that were measured within less than two weeks prior to the start or change of an antihypertensive agent, because we considered a time lapse of less than 14 days too short to achieve a maximal antihypertensive effect.[1]

Medication was encoded using the Anatomical Therapeutic Chemical (ATC) classification system: 1) angiotensin-converting-enzyme inhibitor or angiotensin receptor blocker, 2) calcium channel blocker, 3) diuretic, or 4) beta blocker (Supplementary File 1). Blood pressure measurements without an ATC code were set in the 'no medication' group.

Data analysis

We used the statistical package IBM SPSS Statistics version 22.0 to analyse the data. In case of normally distributed data, mean and SD were given. In case of skewed distribution, we calculated median and interquartile ranges. Next to patient level analyses, we analyzed a dataset using all blood pressure measurements for each patient during follow-up. Relevant differences in baseline characteristics have been described. For the outcome variable delta systolic blood pressure, the mean differences with its 95% confidence interval (95% CI) between the medication group(s) and the no medication group is presented. Because of the hierarchical structure of our study (repeated blood pressure measurements nested within patients), we performed multilevel analyses for the ARR and the blood pressure response. Statistical significance level was set at two-tailed *P*-value <0.05.

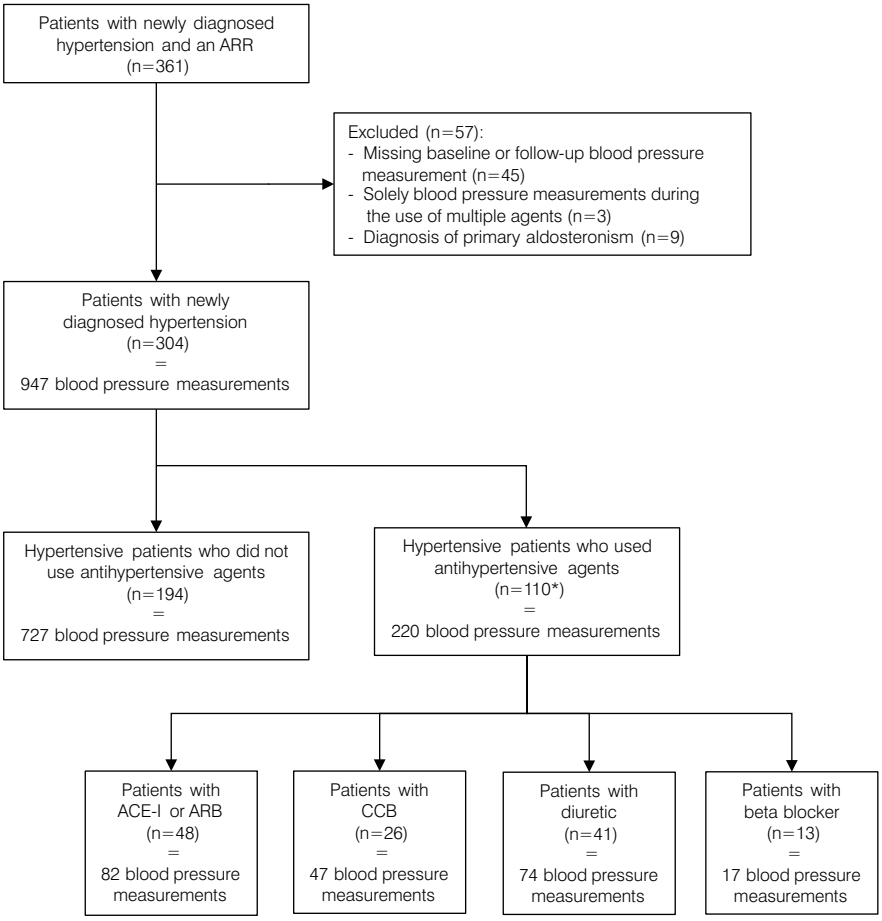
To assess if the ARR was associated with the response in blood pressure in relation to antihypertensive treatment, we used a multivariate multilevel linear regression analysis adjusted for gender, age, baseline systolic blood pressure and comorbidity entered into the random intercept model.[22] The interaction term (treatment group by ARR) tests the difference in the association between the ARR and the response in systolic blood pressure for the five groups. The response in systolic blood pressure is defined as the baseline minus the follow-up systolic blood pressure measurement. We performed a sensitivity analysis to be able to compare our results with other literature, using logarithmic scale of ARR in relation with blood pressure decline.[23]

To assess the association between the ARR and the number of patients on target blood pressure after one year of treatment, we used logistic regression analysis. Patients had reached target blood pressure if their last blood pressure measurement within twelve months from baseline was $<140/90$mmHg.

RESULTS

Of the initial 361 patients in whom an ARR was available, 57 patients had to be excluded from the analyses for the following reasons: 1) missing baseline or follow-up blood pressure measurements (n=45), 2) only blood pressure measurements during the use of multiple agents (n=3), or 3) a diagnosis of primary aldosteronism (n=9). Consequently, 304 patients with a total of 947 blood pressure measurements were used for the analysis. Baseline characteristics were comparable between included (n=304) and excluded patients (n=57; Supplementary Table 1). The majority of patients had an ICPC code for hypertension (n=210; 69.1%). Median number of blood pressure measurements per patient after baseline was two (interquartile range one to four measurements), with a range of one to ten measurements. During the follow-up period, 110 patients (36.2%) used antihypertensive medication and 194 did not. Figure 1 presents a flow diagram of the included patients in relation to blood pressure measurements. Table 1 shows patients characteristics for the medication and no medication group on patient level.

Figure 1 Flow diagram for included patients in relation to blood pressure measurements



*Some patients have been treated by >1 antihypertensive agent. ACE-I, angiotensin converting enzyme inhibitor. ARB, angiotensin receptor blocker. ARR, aldosteron-to-renin ratio. CCB, calcium channel blocker.

The number of blood pressure measurements for the medication and no medication group are shown in Table 2. Baseline characteristics between both groups were comparable. For delta systolic blood pressure, there was a clinically relevant difference between the groups of -13.0 mmHg (95% CI -15.5 to -10.5).

Table 3 shows the baseline characteristics of each of the five groups. Prescribed medication (n=220) consisted of 37.3% ACE inhibitors or ARBs, 21.4% CCBs, 33.6% diuretics, and 7.7% of beta blockers. In the 'ACE-I or ARB' group more blood pressure measurements were available in males than in females, patients with blood pressure

Table 1 Baseline characteristics of included patients with newly diagnosed hypertension

Variable	Patients with available data (total/no medication/ medication)	Total (n=304)	No medication (n=194)	Medication (n=110)
Demographics				
Male, n (%)	304/194/110	154 (50.7)	99 (51.0)	55 (50.0)
Age (years)	304/194/110	53.4 ± 11.4	52.8 ± 11.3	54.6 ± 11.7
BMI (kg/m^2), n (%)	205/134/71			
≤25		44 (21.5)	32 (23.9)	12 (16.9)
>25 - ≤30		93 (45.4)	61 (45.5)	32 (45.1)
>30		68 (33.2)	41 (30.6)	27 (38.0)
Smoking status, n (%)	149/102/47			
Current		34 (22.8)	23 (22.5)	11 (23.4)
Former		53 (35.6)	35 (34.3)	18 (38.3)
Never		62 (41.6)	44 (43.1)	18 (38.3)
Blood pressure				
Systolic BP (mmHg)	304/194/110	163.8 ± 13.1	162.4 ± 12.2	166.4 ± 14.3
Diastolic BP (mmHg)	301/192/109	96.3 ± 9.6	96.0 ± 9.6	96.8 ± 9.8
Heart rate (beats/min)	181/113/68	75.3 ± 12.7	73.5 ± 12.7	78.3 ± 12.0
Biochemical parameters				
Potassium (mmol/L)	302/193/109	4.43 ± 0.34	4.42 ± 0.34	4.44 ± 0.35
Sodium (mmol/L)	302/193/109	141.8 ± 1.99	141.8 ± 1.9	141.7 ± 2.1
Creatinine (μmol/L)	302/193/109	78.8 ± 14.4	79.5 ± 14.3	77.5 ± 14.6
Aldosterone (pmol/L)*	304/194/110	345.5 (214.8 - 460.0)	337.5 (200.5 - 485.5)	346.5 (233.5 - 440.3)
Renin (pmol/L)*	304/194/110	0.77 (0.58 - 1.31)	0.75 (0.58 - 1.26)	0.86 (0.59 - 1.43)
ARR (pmol/mU)*	304/194/110	25.2 (14.2 - 45.2)	25.2 (14.2 - 49.7)	25.5 (13.8 - 41.7)
Glucose (mmol/L)	279/180/99	5.4 ± 0.9	5.3 ± 0.8	5.5 ± 1.2
Cardiovascular morbidity				
Atrial fibrillation, n (%)	304/194/110	2 (0.7)	1 (0.5)	1 (0.9)
OSAS, n (%)	304/194/110	4 (1.3)	3 (1.5)	1 (0.9)
MI, n (%)	304/194/110	2 (0.7)	1 (0.5)	1 (0.9)
Stroke, n (%)	304/194/110	1 (0.3)	0	1 (0.9)

Data are presented as mean ± standard deviation, unless stated otherwise. *median (interquartile range). ARR, aldosterone-to-renin ratio. BP, office blood pressure. BMI, body mass index. MI, myocardial infarction. OSAS, obstructive sleep apnea syndrome.

Table 2 Baseline characteristics of all blood pressure measurements and response in systolic blood pressure for the medication and the no medication group

	All medication (n=220)	No medication (n=727)
Male, n (%)	117 (53.2)	359 (49.4)
Age	55.3 ± 11.6	53.3 ± 11.7
Aldosterone¶	362.0 (241.3 - 441.0)	359.0 (231.0 - 475.0)
Renin¶	0.86 (0.61 - 1.33)	0.77 (0.58 - 1.29)
ARR¶	27.2 (13.5 - 44.3)	27.0 (14.5 - 46.7)
Baseline SBP	166.3 ± 13.9	164.0 ± 13.3
Delta SBP#	-18.3 ± 18.4	-5.3 ± 16.0

Data are presented as mean ± standard deviation, unless stated otherwise. ¶median (interquartile range). #Delta SBP: the response in systolic blood pressure, defined as the baseline systolic blood pressure minus the follow-up systolic blood pressure. ARR, aldosterone-to-renin ratio. SBP, systolic blood pressure.

measurements in the diuretic group were slightly older, patients who receive CCBs had a higher aldosterone, patients who receive beta blockers had a higher renin, patients in the CCBs groups had a higher ARR, and baseline systolic blood pressure was comparable between all groups. Within each treatment group systolic blood pressure showed a significant decline. Mean differences of response for each medication group compared to the no medication group (reference) are -16.4 (95% CI -21.3 to -11.6) for the CCB group, -15.2 (95% CI -18.9 to -11.4) for the ACE-I or ARB group, -9.7 (95% CI -17.6 to -1.7) for the beta blocker group, and -9.3 mmHg (95% CI -13.3 to -5.3) for the diuretic group.

Multivariate multilevel linear regression analysis showed no association between the ARR and the blood pressure response within one year of treatment in the total group (regression coefficient 0.050, (95% CI -0.009 to 0.110), Table 4). There was no association between the ARR and the blood pressure response for the medication and no medication group (Table 4), but there was a significant difference in starting value of blood pressure response (i.e. a significant difference in intercept, $p<0.001$; Figure 2, Supplementary Tables 2 and 3). Subgroup analysis showed no significant association of the ARR and the response in blood pressure within each of the groups (Table 4). The interaction term between the ARR and the five treatment groups was non-significant ($p=0.77$) with a significant intercept ($p<0.001$, Figure 2, Supplementary Tables 4 and 5).

In our sensitivity analysis we found no correlation between log ARR and the response in blood pressure for all groups ($p=0.61$).

During the last blood pressure measurement 87 patients used antihypertensive medication (35 ACE-I or ARB, 14 CCB, 28 diuretic, and 10 beta blocker). Of these patients target systolic blood pressure was reached in 27 patients (31%). There was no association between ARR and the number of patients reaching target blood pressure (odds ratio 1.002, 95% CI 0.983 to 1.022).

Table 3 Baseline characteristics of all blood pressure measurements and response in blood pressure for the different classes of antihypertensive agents and the no medication group

Medication	ACE-I or ARB (n*=82)	CCB (n*=47)	Diuretic (n*=74)	Beta blocker (n*=17)	No medication (n*=727)
Male, n (%)	62 (75.6)	19 (40.4)	28 (37.8)	8 (47.1)	359 (49.4)
Age	54.2 ± 11.7	52.7 ± 10.6	59.3 ± 10.6	50.0 ± 13.7	53.3 ± 11.7
Aldosterone¶	345.0 (262.3 - 443.8)	415.0 (359.0 - 482.0)	314.0 (212.3 - 437.8)	363.0 (250.0 - 551.0)	359.0 (231.0 - 475.0)
Renin¶	1.1 (0.6 - 2.0)	0.7 (0.6 - 1.2)	0.8 (0.6 - 1.0)	1.8 (0.8 - 3.1)	0.8 (0.6 - 1.3)
ARR¶	18.2 (11.9 - 39.7)	41.7 (27.7 - 51.6)	30.9 (13.9 - 41.7)	17.0 (8.1 - 24.8)	27.0 (14.5 - 46.7)
Baseline systolic BP	166.9 ± 13.3	170.1 ± 17.7	164.3 ± 9.4	161.4 ± 19.2	164.0 ± 13.3
Delta systolic BP‡	-20.5 ± 17.0	-21.7 ± 23.3	-14.6 ± 15.6	-15.0 ± 19.0	-5.3 ± 16.0

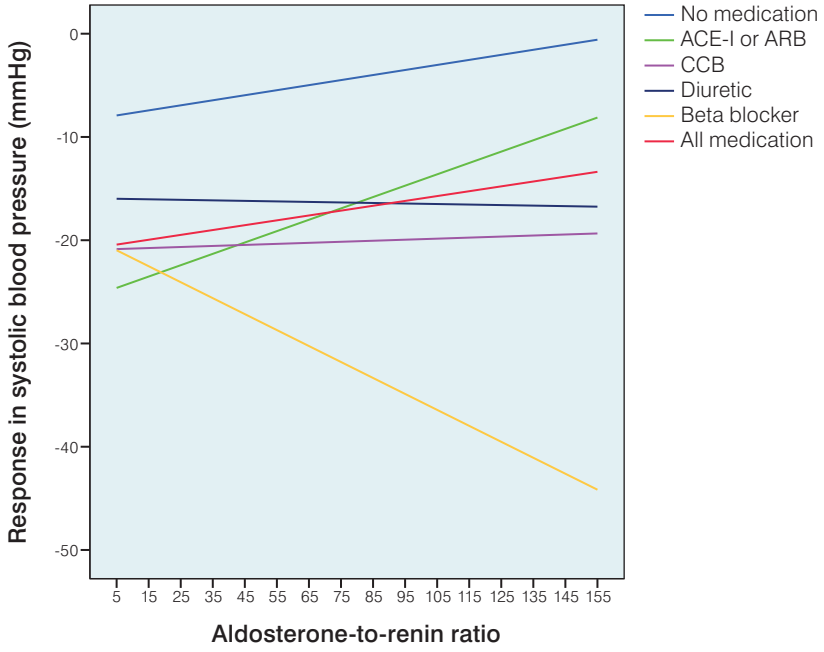
Data are presented as mean ± standard deviation, unless stated otherwise. *number of systolic blood pressure measurements. ¶median (interquartile range) ‡Delta systolic BP: the response in systolic blood pressure, defined as the baseline systolic blood pressure minus the follow-up systolic blood pressure. ACE-I, angiotensin-converting-enzyme inhibitor. ARB, angiotensin receptor blocker. ARR, aldosterone-to-renin ratio. CCB, calcium channel blocker. BP, blood pressure.

Table 4 Multivariate multilevel linear regression analysis of the aldosterone-to-renin ratio and response in blood pressure

	Blood pressure response* (n=947) ß# (95% confidence interval)
ARR	0.050 (-0.009 - 0.110)
ARR	
All medication	0.048 (-0.044 - 0.139)
No medication	0.047 (-0.014 - 0.108)
ARR	
ACE-I or ARB	0.110 (-0.019 - 0.239)
CCB	0.010 (-0.186 - 0.206)
Diuretic	-0.005 (-0.155 - 0.145)
Beta blocker	0.155 (-0.480 - 0.789)
No medication	0.049 (-0.012 - 0.110)

*Blood pressure response in systolic blood pressure, defined as the baseline minus the follow-up systolic blood pressure measurement. #Adjusted for age, gender and baseline blood pressure. ACE-I, angiotensin-converting-enzyme inhibitor. ARB, angiotensin receptor blocker. ARR, aldosterone-to-renin ratio. CCB, calcium channel blocker. ß, regression coefficient.

Figure 2 Association between the aldosterone-to-renin ratio and response in systolic blood pressure for the different treatment groups*



*adjusted for clustering, gender, age, and baseline blood pressure. ACE-I, angiotensin-converting-enzyme inhibitor. ARB, angiotensin receptor blocker. CCB, calcium channel blocker.

DISCUSSION

Summary

In this study we found no significant association between the ARR and the response in systolic blood pressure for the total medication group nor for the subgroups of antihypertensive agents after one year of treatment. Therefore, the ARR has no predictive value for the response in blood pressure to antihypertensive treatment in this primary care patient group. In patients that used antihypertensive agents target blood pressure was reached in 31% within one year after the initial diagnosis.

Strengths and limitations

A strong aspect of our study is the study setting of primary care as in many countries treatment of hypertension is started and monitored in primary care.[24,25] Moreover, all included patients had not previously been treated with antihypertensive drugs at the time of aldosterone and renin measurement, which eliminates any confounding effects of these drugs on the ARR.[19] Finally, we set relevant exclusion criteria by excluding groups which might confound the results and interpretation of the ARR, e.g. patients with diabetes.

As we included only patients with newly diagnosed hypertension and guidelines recommend to start lifestyle advice in newly diagnosed patients, the size of our study is limited to the medication groups. Since we did not include patients who needed referral to the hospital we did not have follow-up measurements of blood pressure in the primary care setting for these patients. Finally, 68 patients (22.4%) with less than one year follow-up were included, because they were included in the last phase of our inclusion period.

Comparison with existing literature

Previous studies have suggested that hypertensive patients with a high ARR respond favourably to spironolactone.[26] Parthasarathy *et al.* studied the antihypertensive response of spironolactone compared to bendroflumethiazide in two groups of antihypertensive patients, one with high and one with low ARR, and found no predictive effect of the ARR. However, patients with high ARR were not tested for primary aldosteronism, and participants were not newly diagnosed hypertensive patients.[27,28] Prisant *et al.* performed an ad hoc analysis in which baseline ARR levels did not predict the antihypertensive response to eplerenone in combination with an ACE-I or ARB.[29] Mahmud *et al.* examined the effect of spironolactone on 30 hypertensive never treated patients, and found a significant correlation between the log ARR and the decline in blood pressure.[23] All studies so far are uncontrolled and performed in a small number of patients. It is therefore not possible to draw conclusions on the value of the ARR in relation to the blood pressure response when mineralocorticoid receptor antagonists are used as monotherapy in essential hypertension. In our sensitivity analysis we transformed the ARR in log ARR, but

results remained non-significant. It should be noted that in our study not one patient was treated by mineralocorticoid antagonists, because in the Netherlands mineralocorticoid receptor antagonists are not used in primary practice for monotherapy in essential hypertension.[30] As we provided no instructions about the use of specific antihypertensive agents, our study reflects daily clinical practice.

Several large scale studies have promulgated the beneficial effects of thiazide diuretics in essential hypertension. Although a Cochrane review concluded that achieving target blood pressure is only partly responsible for the risk reduction of antihypertensive treatment, no inferences can be made about the association of the blood pressure lowering effect and the height of the ARR.[31] In our study, the antihypertensive effect of diuretics was similar over the entire range of ARRs (Figure 2).

Although lifestyle advice is recommended by the guideline for each patient with hypertension we were not able to monitor this from the EHR data, however the decline in blood pressure in the no medication group could have been the result of this.[32,33]

Implications for research and practice

This explorative study in primary care does not provide evidence that a simple and cheap test like the ARR is helpful in predicting whether and which antihypertensive treatment in primary care is effective. In our study no patients were treated with a mineralocorticoid receptor antagonist such as spironolactone, although previous studies have shown that a high ARR does predict a favourable blood pressure response to this drug.[23,26-28] Overall, these results cast doubt on the value of the ARR as a therapeutic marker.[34] However, the ARR remains useful as a diagnostic marker for primary aldosteronism in hypertensive patients.[13,19]

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Declaration

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Ethical approval. The study protocol was approved by the Medical Review Ethics Committee Region Arnhem-Nijmegen (ID: NL40133.091.12).

Competing interests. The authors have declared no competing interests.

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SUPPLEMENTARY FILES

Supplementary File 1. Treatments groups

- Treatment 1: no use of antihypertensive agents, treatment consists of lifestyle advise (salt reduction etc.).
- Treatment 2: use of angiotensin-converting-enzyme inhibitors and angiotensin receptor blockers. This category contains the agents with ATC-code C09A*, C09C*, and C09X*.
- Treatment 3: use of calcium channel blockers. This category includes ATC-codes C08C*, C08D*, and C08E*.
- Treatment 4: use of diuretics. This category contains ATC-code C03A*, C03B*, C03C*, and C03D*.
- Treatment 5: use of beta blockers. This category contains ATC-code C07A*.

ATC, anatomical therapeutic chemical.

Supplementary Table 1 Baseline characteristics of all patients

Variable	Number (%) of included patients		Number (%) of excluded patients		Number (%) of patients with available data of the prevalence study	
Demographics						
Male, n (%)	304	154 (50.7)	57	27 (47.4)	361	181 (50.1)
Age (years)	304	53.4 ± 11.4	57	53.9 ± 9.0	361	53.5 ± 11.1
BMI (kg/m^2), n (%)	205 (67.4)		30 (52.6)		235 (65.1)	
≤25, n (%)		44 (21.5)		6 (20.0)		50 (21.3)
>25 - ≤30, n (%)		93 (45.4)		15 (50.0)		108 (46.0)
>30, n (%)		68 (33.2)		9 (30.0)		77 (32.8)
Smoking status, n (%)	149 (49.0)		27 (47.4)		176 (48.8)	
Current, n (%)		34 (22.8)		4 (14.8)		38 (21.6)
Former, n (%)		53 (35.6)		6 (22.2)		59 (33.5)
Never, n (%)		62 (41.6)		17 (63.0)		79 (44.9)
Blood pressure						
Systolic BP (mmHg)	316	163.8 ± 13.1	55	161.9 ± 13.6	359	163.5 ± 13.2
Diastolic BP (mmHg)	313	96.3 ± 9.6	55	95.7 ± 10.7	356	96.2 ± 9.8
Heart rate (beats/min)	181	75.3 ± 12.7	28	73.1 ± 11.7	209	75.0 ± 12.5
Biochemical parameters						
Potassium (mmol/L)	302	4.43 ± 0.34	57	4.38 ± 0.31	359	4.42 ± 0.34
Sodium (mmol/L)	302	141.8 ± 1.99	57	141.4 ± 2.2	359	141.8 ± 2.0
Creatinine (μmol/L)	302	78.8 ± 14.4	57	77.1 ± 15.2	359	78.5 ± 14.5
Aldosterone (pmol/L)*	304	345.5 (214.8-60.0)	57	335.0 (205.5-613.0)	361	345.0 (213.0-482.0)
Renin (pmol/L)*	304	0.77 (0.58-1.31)	57	0.72 (0.51-1.09)	361	0.76 (0.57-1.31)
ARR (pmol/mU)*	304	25.2 (14.2-45.2)	57	25.3 (12.8-66.5)	361	25.2 (14.0-47.0)
Glucose (mmol/L)	279	5.39 ± 0.93	49	5.59 ± 1.31	328	5.42 ± 1.00
Cardiovascular morbidity						
Atrial fibrillation, n (%)	304	2 (0.7)	57	0 (0)	361	2 (0.6)
OSAS, n (%)	304	4 (1.3)	57	1 (1.8)	361	5 (1.4)
Myocardial infarction, n (%)	304	2 (0.7)	57	0 (0)	361	2 (0.6)
Stroke, n (%)	304	1 (0.3)	57	1 (1.8)	361	2 (0.6)

Data are presented as mean ± standard deviation unless stated otherwise. *median (interquartile range). ARR, aldosterone-to-renin ratio. BP, blood pressure. BMI, body mass index. Diastolic BP, office diastolic blood pressure. OSAS, obstructive sleep apnea syndrome. Systolic BP, office systolic blood pressure.

Supplementary Table 2 Tests of fixed effects of the aldosterone-to-renin ratio and response in blood pressure for no medication versus all medication

Source	Numerator df	F	*P*-value
Intercept	1	111,237	0,000
Medication	1	45,424	0,000
ARR	1	2,152	0,143
Medication * ARR	1	0,000	0,993
Gender	1	1,384	0,240
Age	1	0,000	0,996
Baseline systolic blood pressure	1	58,041	0,000

ARR, aldosterone-to-renin ratio.

Supplementary Table 3 Estimates of the fixed effects of the aldosterone-to-renin ratio and response in blood pressure for no medication versus all medication

Parameter	Estimate	Std. error	t	*P*-value	95% confidence interval	
					Lower bound	Upper bound
Intercept	-20,664078	2,120563	-9,745	0,000	-24,828872	-16,499283
No medication	12,737516	1,889910	6,740	0,000	9,028395	16,446638
All medication	0	0	.	.	.	.
ARR	0,047603	0,046421	1,025	0,306	-0,043541	0,138747
No medication * ARR	-0,000427	0,045545	-0,009	0,993	-0,089818	0,088963
All medication * ARR	0	0	.	.	.	.
Gender (M)	1,655311	1,406811	1,177	0,240	-1,114653	4,425275
Gender (F)	0	0	.	.	.	.
Age	-0,000284	0,059611	-0,005	0,996	-0,117676	0,117108
Baseline systolic BP	-0,395051	0,051854	-7,618	0,000	-0,497174	-0,292929

ARR, aldosterone-to-renin ratio. BP, blood pressure. F, female. M, male.

Supplementary Table 4 Tests of fixed effects of the aldosterone-to-renin ratio and response in blood pressure for the five treatment groups

Source	Numerator df	F	*P*-value
Treatment group	4	13,039	0,000
ARR	1	0,752	0,386
Treatment group * ARR	4	0,460	0,765
Gender	1	2,022	0,156
Age	1	0,030	0,863
Baseline systolic blood pressure	1	55,131	0,000

ARR, aldosterone-to-renin ratio.

Supplementary Table 5 Estimates of the fixed effects of the aldosterone-to-renin ratio and response in blood pressure for the five treatment groups

Parameter	Estimate	Std. error	t	*P*-value	95% confidence interval	
					Lower bound	Upper bound
Intercept	-8,160489	1,555027	-5,248	0,000	-11,220669	-5,100308
ACE-I or ARB	-17,014798	2,657359	-6,403	0,000	-22,229952	-11,799643
CCB	-12,750063	4,816451	-2,647	0,008	-22,202574	-3,297551
Diuretic	-7,803866	3,153428	-2,475	0,014	-13,992666	-1,615065
BB	-12,041658	7,078807	-1,701	0,089	-25,934105	1,850789
No medication	0	0	.	.	.	.
ARR	0,048929	0,031148	1,571	0,117	-0,012362	0,110219
(ACE-I or ARB) * ARR	0,061051	0,064766	0,943	0,346	-0,066062	0,188165
CCB * ARR	-0,038784	0,100725	-0,385	0,700	-0,236466	0,158898
Diuretic * ARR	-0,053978	0,075236	-0,717	0,473	-0,201638	0,093683
BB * ARR	0,105670	0,323515	0,327	0,744	-0,529253	0,740592
No medication * ARR	0	0	.	.	.	.
Gender (M)	2,014237	1,416594	1,422	0,156	-0,774687	4,803162
Gender (F)	0	0	.	.	.	.
Age	-0,010361	0,059849	-0,173	0,863	-0,128215	0,107493
Baseline systolic BP	-0,386034	0,051991	-7,425	0,000	-0,488423	-0,283645

ACE-I, angiotensin-converting-enzyme inhibitor. ARB, angiotensin receptor blocker. ARR, aldosterone-to-renin ratio. BB, beta blocker. BP, blood pressure. CCB, calcium channel blocker. F, female. M, male.



6

General discussion and summary

Some medical specialists support indiscriminate screening for primary aldosteronism (PA) in all patients with hypertension,[1-3] whereas others recommend selective screening in subgroups of patients with hypertension.[4-6] The aim of this thesis was to obtain data to assess whether screening for PA in all patients with newly diagnosed hypertension might be useful in Dutch primary care.

Because of reported wide variations in the prevalence of PA, we first needed to gain insight into the methodologies that were used to assess this prevalence. We performed a systematic review and meta-analysis to estimate the prevalence of PA in primary care as well as referral centres, and to determine factors that could explain the large variety in prevalences. Secondly, we needed to assess the prevalence of PA in Dutch primary care, as this information is pertinent for policy making. Thirdly, if an excess of cardiovascular damage in PA patients already exists at the time hypertension is diagnosed for the first time, this would be an additional argument to support early screening. We therefore also aimed to assess the extent of cardiovascular damage at the time of diagnosing hypertension. Finally we explored the potential of the aldosterone-to-renin ratio (ARR), which we used to screen for PA, to predict the presence or absence of an antihypertensive response to the main classes of antihypertensive agents in patients in whom PA was excluded.

In this closing chapter the main findings of our research are summarized, followed by methodological considerations, and a discussion on how our findings relate to the previous literature. The chapter ends with clinical implications, recommendations for future research, and final conclusions.

SUMMARY OF THE MAIN FINDINGS

In *Chapter 2* we present our systematic review and meta-analysis, in which we included 39 studies. These provided data on 42510 patients, of which 5896 patients (nine studies) were assessed in primary care. Prevalence estimates varied from 3.2% to 12.7% in primary care, and from 1% to 29.8% in referral centers. Heterogeneity was too high to provide point estimates ($I^2 = 57.6\%$ in primary care, and 97.1% in referral centers). Meta-regression analysis showed higher prevalences in studies: 1) published after 2000, 2) from Australia, 3) aimed at assessing prevalence of secondary hypertension, 4) that were retrospective, 5) that selected consecutive patients, and 6) not using a screening test. This study demonstrates that it is not possible to provide an accurate estimate of the prevalence of PA based on previous reports.

The prevalence of PA in a study in Dutch primary care is described in *Chapter 3*. In this cross-sectional study, we identified 3748 adult patients with a new diagnosis of hypertension. Of them, 343 patients were screened for PA according to the Endocrine Society guideline.[4] In nine out of 74 patients with an elevated ARR and increased plasma aldosterone concentration, the diagnosis of PA was confirmed by a saline infusion test. The resulting prevalence of 2.6% (95% confidence interval (CI) 1.4% to

4.9%) is lower than previously reported in primary care studies. Notably, all patients with PA were normokalemic, and eight out of nine patients had a sustained blood pressure of >150/100 mmHg. The low proportion of screened patients (9.2%) of the large cohort of eligible patients, reflects the difficulty of conducting prevalence studies in primary care clinical practice.

As it was unclear whether cardiovascular complications of excess aldosterone secretion are already present at the time of diagnosing hypertension and PA, we carried out an explorative study. In this study we assessed patients with PA and a matched group of patients with essential hypertension, in whom we applied a set of validated non-invasive measurements for cardiovascular function. We present this study in *Chapter 4.* Measurements were performed at the time when hypertension was diagnosed, or as soon as possible thereafter. Left ventricular hypertrophy was more frequent in patients with PA compared to patients with essential hypertension. We did not observe differences between patients with PA and patients with essential hypertension in all other parameters (ankle-brachial index, other echocardiographic features, flow-mediated vasodilation, carotid intima-media thickness, central aortic blood pressure, pulse wave velocity, and urinary albumin-to-creatinine ratio).

In *Chapter 5* we describe our findings of the level of the ARR in relation to the blood pressure response within one year of antihypertensive treatment in 304 patients. Treatment was categorized into five groups: no medication, use of angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB), use of a beta blocker (BB), use of a calcium channel blocker (CCB), or use of a diuretic. We did not find an association between the level of the ARR and the response in blood pressure within one year of antihypertensive treatment, irrespective of the type of antihypertensive treatment. There was also no association between the ARR level and the number of patients reaching target blood pressure (defined as systolic blood pressure <140 mmHg), and this target was reached in only 31% of the treated patients within one year of therapy. Consequently, the ARR cannot serve as biomarker to guide antihypertensive therapy.

METHODOLOGICAL CONSIDERATIONS

Systematic review and meta-analysis

A particular strength of the systematic review *(Chapter 2)*, is that we excluded studies that used a confirmation test in less than half of the patients with a positive screening test. Not performing a confirmation test indicates a potential of overdiagnosis of PA and is at variance with the stepwise approach to the diagnosis of PA, first screening and then confirmation, recommended by the Endocrine Society guideline.[4] Another strength is that we analyzed studies from primary care and referral centres separately, as we suspected that the variables that determine the prevalence in these two settings were different.

The systematic review has some limitations. It was not possible to build an explanatory model with the set of factors derived from the univariate analysis, as the number of studies performed in the primary care setting was too low. Another limitation is that we could not exclude articles of low quality, as the protocol that we followed for quality assessment (the Methodological evaluation of Observational Research, MORE) was not developed to 'weigh' or to exclude studies. However, it was possible to identify those studies which suffered from 'major flaws' according to the MORE protocol, and we found no difference in prevalences between studies with and without major flaws.

Studies performed in primary care

The major strength of both our prevalence study *(Chapter 3),* and the study in which we assessed the ARR as a therapeutic marker *(Chapter 5),* is the primary care study setting, as primary care is in most countries the setting in which hypertension is typically diagnosed, treated and monitored.[7-9] In contrast to prevalence studies in referral centers, screening in the primary care setting is not expected to be associated with referral bias. Another strength of our design is that specific subgroups in which PA may be more prevalent were excluded, such as patients with a hypertensive crisis.[10] Of additional relevance is the inclusion of patients with newly diagnosed hypertension, who were free from antihypertensive medication at the time of inclusion when biochemical testing was performed. This enabled us to exclude possible confounding effects of such agents on plasma aldosterone and renin.[4] Another strength was that we used a stringent prospective study protocol. However, despite this straightforward clinical protocol, an unbiased selection of patients for screening for PA appeared also very hard to achieve in a primary care setting.[11-13] Where this remains hidden in most studies, we were able to mine the Electronical Health Records (EHRs) of the total source population for incomplete screening and possible selection bias.[14] To our knowledge, this approach has never been reported before in primary care studies that addressed the prevalence of PA.

Our studies inevitably also have limitations. One of the largest bottlenecks we experienced was the limited compliance of general practitioners (GPs) to refer all patients with newly diagnosed hypertension for screening of PA, which resulted in a low proportion of patients who were really screened. This might be due to several causes, such as high administrative work load for GPs, and insufficient personnel support.[15] Since we had not expected this lack of 'inclusion compliance' at this scale, identifying the reasons for it was not part of our study. Another limitation concerns the reliability and validity of the screening test. The ARR is generally considered the best screening test for hypertensive patients who have a clinical suspicion of PA, and it is the screening test of choice as recommended by the Endocrine Society guideline.[4,16] Since its reliability as a screening test has been challenged,[17] we tried to overcome a potential insufficient sensitivity by decreasing the cut-off values for the ARR. In addition, a practical drawback was the change of the aldosterone assay

during the study, resulting in a slight change in reference values. However, we could not demonstrate any impact of this change on the results of our study.

Selection bias was assessed by the use of International Classification of Primary Care (ICPC) codes from the EHRs. These ICPC codes depend on the quality of recording. As GPs may not always assign an ICPC code for hypertension,[18,19] patients with elevated blood pressure at more than two occasions without an ICPC code were also included in our study. This case finding may have been too sensitive as we found 3748 patients with newly diagnosed hypertension, whereas based on the total study sample and the inclusion period we expected 2758 patients with newly diagnosed hypertension.[20] Moreover, we were only allowed to extract specific tables from the EHRs, as we had no access to free text due to privacy regulations. Hence, if GPs failed to note blood pressure measurements and laboratory results in the correct table of the EHR, this led to missing data in our baseline characteristics, thus to the loss of patients or data for the analyses. Finally, it was not possible to check in the EHRs whether lifestyle advice was indeed given, nor whether attention was paid to the adherence to the lifestyle advice as part of the follow-up.

Cardiovascular damage study

In our explorative study on cardiovascular damage we used multiple well-established and sensitive techniques to assess cardiovascular organ damage *(Chapter 4)*. In addition, the diagnoses of PA and essential hypertension were based on stringent criteria according to the Endocrine Society guideline.[4] Moreover, in the patients with PA and essential hypertension cardiovascular organ damage was assessed at the first time when the diagnosis of hypertension were made, or as soon as possible thereafter.

A major limitation is the low power for all outcome measures due to the small number of patients we could enroll. Although we expected to recruit 40 newly diagnosed patients with PA in the prevalence study of *Chapter 3*, only six patients could be included. This was due to: 1) the low recruitment of patients in our prevalence study, which resulted in a lower than expected absolute number of patients with PA, 2) the lower than expected prevalence we found,[21] and 3) a drop-out of three out of nine patients with PA for various reasons. Despite the small sample size, two patients with newly diagnosed hypertension and PA had left ventricular hypertrophy. This contrasts to the control group of hypertensive patients without PA, of whom none showed left ventricular hypertrophy. Another limitation was that some patients with hypertension had been treated for some time when the echocardiography was performed. Treatment related regression of eventual left ventricular hypertrophy might have resulted in underestimation of patients with early cardiac damage.[22,23]

HOW DO OUR FINDINGS RELATE TO PREVIOUS STUDIES?

Systematic review and meta-analysis

Previous reviews and meta-analysis studies reported mean prevalences of PA.[24-26] However, our study shows that calculating mean prevalences was not possible due to gross heterogeneity across studies, and this applies to both primary care as well as referral centres. Several factors were responsible for the reported variations in prevalence of PA. Studies carried out in referral centers published after the year 2000 showed approximately a nine times higher chance to find a higher prevalence than studies published before 2000, and this was independent from other factors. This might be explained by increasing awareness of the presence of PA over time, and by the use of different cut-offs for the screening and confirmation tests.[27] The very first studies that investigated the prevalence of PA were performed in centers in Australia in self-selected patients or on the basis of retrospective data.[28,29] This might partially explain why studies from Australia have a more than five times higher chance to find a higher prevalence than studies that were carried out in Europe. An alternative explanation is that the prevalence of PA is indeed higher in Australia.

Our finding that retrospective studies report higher prevalences than prospective ones suggests that the current 'epidemic' of PA is partly explained by reliance on retrospective studies.[30] It illustrates that prospective studies are more reliable to estimate prevalences.[31]

The Endocrine Society guideline advocates adjustment of antihypertensive agents before screening, as plasma aldosterone and renin may be affected by this medication.[4] In contrast, several studies have suggested that screening and confirmation testing is still reliable when patients continue their antihypertensive medication during testing.[32,33] Our meta-regression model confirms that adjustment of medication regimen has no effect on the prevalence of PA. Although this casts doubt on the recommendation of the Endocrine Society guideline, good comparative studies are lacking and therefore adapting the medication regimen as proposed by the Endocrine Society still seems the most appropriate way to screen for PA.

Prevalence study

The prevalence of PA of 2.6% in our study appeared to be lower than might be expected from previously published prevalence studies carried out in primary care. In the latter studies in which at least half of the patients had undergone a confirmation test for PA, the prevalence varied from 3.2% to 11.5%.[28,34-42] Several explanations for the lower prevalence in our study have to be considered. First, we restricted our study sample to patients with newly diagnosed hypertension. We found two studies that also restricted their study population to patients with newly diagnosed hypertension.[36,41] Omura *et al.* aimed to assess the prevalence of secondary hypertension among patients with newly diagnosed hypertension, and found a prevalence of PA of 6%.[36] According to our meta-analysis *(Chapter 2)*, studies

showed higher prevalences of PA if the study objective was to assess the prevalence of secondary hypertension. Although in this meta-analysis we were only able to determine responsible factors for higher prevalences in referral centres, it might be very well possible that (some of) these factors also apply to primary care. Moreover, the higher prevalence in this study may be explained by insufficiently defined confirmatory testing, which may have confounded their results. Another explanation is that the prevalence of PA is indeed higher in Japan. Westerdahl *et al.* confirmed a diagnosis of hypertension by a 24-hour ambulatory blood pressure monitoring (ABPM; inclusion if the blood pressure was >140/90 mmHg), and found a prevalence of PA of 5.5%.[41] The higher prevalence found in this study might be explained by the confirmation of a diagnosis of hypertension by an ABPM using a higher cut-off level for hypertension than usual.[43] As a result, their study population consisted of patients with more severe hypertension, and PA is known to be more prevalent in more severe hypertension.[4] In our study we did not confirm the diagnosis by ABPM, as in Dutch primary care an ABPM is not considered mandatory to confirm a diagnosis of hypertension.[9] Moreover, we aimed to interfere minimally in daily clinical practice in this study. Therefore, it might be possible that we performed screening of PA in patients with a false-positive diagnosis of hypertension (e.g. white-coat hypertension).

Second, our lower prevalence might be due to our quite strict cut-off for the confirmation test of 280 pmol/L (10 ng/dL) for aldosterone, which is in accordance with the recommendations of the Endocrine Society guideline.[4] Using a strict cut-off level may result in a lower prevalence, and in higher numbers of false-negative test outcomes. Lower cut-offs have also been proposed by several other studies.[44,45] However, as sensitivity is a prerequisite for screening, we deliberately chose a relatively low screening cut-off of 40 pmol/mU for the ARR, as the higher ARR cut-off as suggested by the Endocrine Society may lack sufficient sensitivity.[17,46-49] To improve specificity we added the extra requirement of a minimum plasma aldosterone level of 400 pmol/L to the screening test.[50]

Third, in our study 26 patients (7.6%) had an elevated ARR with a plasma aldosterone lower than 400 pmol/L. Therefore, these patients did not qualify for a confirmation test. Adding the 'elevated plasma aldosterone criterion' is subject to debate, as some patients with PA do not have increased aldosterone levels above the upper reference limit (400 pmol/L).[51] It remains to be established whether this group of 26 patients included some patients with PA. Therefore, adding this criterion might have contributed to our lower prevalence.

Fourth, in our study the screened patients were younger and had higher blood pressures as compared to non-screened patients. This indicates that GPs introduced some selection bias, as they tested mainly patients who were young and who had a more severely increased blood pressure. It seems that they intuitively followed the screening recommendations of the Endocrine Society guideline.[4] This might have resulted in an underestimation of the proportion of patients with PA. In addition, for unknown reasons GPs were less likely to perform biochemical screening in patients

with newly diagnosed hypertension who had suffered a stroke in the past. Possibly, GPs might have had the misconception that testing patients who already had a stroke was not useful anymore. Alternatively, it might be that GPs considered stroke as 'severe comorbidity', which was an exclusion criterion for participation in our study. It is conceivable that all this selective screening has contributed to an underestimation of the real prevalence of PA. Our experience in the current study raises the question whether similar potential selection bias might have confounded previously published primary care studies on the prevalence of PA.

Although previously thought to be a prerequisite for a diagnosis of PA, current literature shows that in general 9% to 37% of all patients with PA have hypokalemia.[52] In line with previous studies our results show that hypokalemia is not a reliable clinical clue for PA, as in our study none of the patients with PA had hypokalemia. This implies that normokalemia does not exclude PA and therefore, the current strategy in Dutch primary care to consider PA in hypertensive patients with hypokalemia, is no longer valid.

Cardiovascular damage study

Since the nineties it became increasingly clear that cardiovascular damage in patients with PA is more extensive than in patient with essential hypertension with similar blood pressure levels. Milliez *et al.* matched patients with essential hypertension and patients with PA for gender, age and systolic blood pressure, and found that cardiovascular damage was more prevalent in patients with PA.[53] It was hypothesized that this damage was causally related to the high level of aldosterone. Our study suggests that cardiac hypertrophy might be present at an early stage of PA, when hypertension is diagnosed for the first time, and that this is unrelated to the blood pressure level *(Chapter 4).* A recently published paper by Monticone *et al.* claims that there are alternative pathogenetic mechanisms to blood pressure or the mineralocorticoid receptor-mediated effects of aldosterone that enhance cardiovascular damage in patients with PA.[54] The variety of pathogenetic mechanisms for cardiovascular damage in patients with PA explains why regular antihypertensive therapy is ineffective, and mineralocorticoid receptor antagonist treatment is less effective than adrenalectomy.[55-58] Previous studies have also shown that young age and a short duration of hypertension are the most important predictors of successful outcome of treatment. [59-62] This all implies not only that there is a strong rationale for detecting PA as early as possible, but also that it is worthwhile to identify those patients with PA who benefit most from adrenalectomy.[63]

Study of aldosterone-to-renin ratio for guiding antihypertensive treatment

In our study with 304 patients we did not find an association between the level of the ARR and the response in blood pressure within one year of antihypertensive treatment, and this applied to all studied classes of antihypertensive agents *(Chapter 5).* It should be noted that in our study not a single patient was treated with a

mineralocorticoid antagonist, as in the Netherlands mineralocorticoid receptor antagonists are not used in primary care as monotherapy in essential hypertension.[64] As we provided no instructions to the participating practices about the use of specific antihypertensive agents, our study reflects daily clinical practice.

Although it has been suggested that hypertensive patients with a high ARR respond favourably to spironolactone,[65,66] several studies in hypertensive patients could not confirm this. Parthasarathy *et al.* studied the antihypertensive response of spironolactone compared to bendroflumethiazide in two groups of antihypertensive patients, one with high and one with low ARR, and the level of the ARR did not predict the anthypertensive effects of both drugs. However, patients with high ARR were not tested for PA, and participants were not newly diagnosed hypertensive patients.[67] Prisant *et al.* performed a placebo-controlled trial to assess whether baseline ARR levels could predict the antihypertensive response of add-on eplerenone to the use of ACE-I or ARB, but did not find one of the arms to be superior.[68] We did not find studies that assessed the association of the ARR and blood pressure response in newly diagnosed hypertensive patients, and in particular examining the effect of the blood pressure response to the classes of hypertensive agents that are used in primary care (ACE-I, ARB, BB, CCB, thiazide diuretic).

An additional and disturbing finding in our study was that the proportion of treated patients that had reached target blood pressure within one year after the diagnosis of hypertension was low (31%). This result is in agreement with other European studies, in which the proportions of patients reaching target blood pressure varied from 22% to 50%.[69,70] The low proportion of patients who had reached target blood pressure limits our conclusion regarding the predictive value of the ARR for the antihypertensive effect.

Although lifestyle advice is recommended for each patient with hypertension by the Dutch Guideline for Cardiovascular Risk Management (Dutch CVRM guideline), we were not able to extract these data from the EHRs. It remains possible that the decline in blood pressure in the group who received no medication at all, was the result of adherence to advised lifestyle changes.[71,72]

CLINICAL IMPLICATIONS

In our study described in *Chapter 3,* we found a prevalence of PA of 2.6% (95% CI 1.4% to 4.9%) in patients with newly diagnosed hypertension, in whom a few had already left ventricular hypertrophy *(Chapter 4).* Based on these data, systematic screening of new hypertensive patients would be expected to result in approximately 1100 to 3700 new patients with PA per year in the Netherlands.[73,74] The majority of these patients will not be discovered when the Dutch CVRM guideline is followed. This guideline recommendation for case finding of PA relies mainly on the presence of hypokalemia, and therapy resistant hypertension.[9] However, hypokalemia cannot

be used for this purpose, as it is only present in 9% to 37% of all patients with PA,[52] and in none of the patients we identified in our study. Consequently, most patients would be missed if one would rely on the presence of hypokalemia.

To assess whether implementing screening of all patients with newly diagnosed hypertension is useful, the criteria for screening as defined by Wilson and Jungner[75] should specifically be applied to PA. Although these criteria are fulfilled to some extent, the lack of reliable data precludes assessment of other criteria (Table 1). Indeed, PA confers an important health problem since it affects a large absolute number of new patients per year, who run an elevated risk of progressive cardiovascular damage, with an associated poor quality of life, when PA is not timely and properly treated. Initial diagnostic screening is feasible, as there is a simple blood test that can be easily incorporated in the current standard hypertension protocol in primary care. Further confirmation or exclusion of PA is already clinical practice in the referral centers, and there is clear evidence that the currently accepted treatment options are very effective, resulting in an improved long-term outcome for patients.[63,76-80]

Although several arguments support early screening of all patients with newly diagnosed hypertension, there are also several compelling and valid arguments to refrain from systematic screening. One is that health care facilities will face an increased work load for GPs as well as for specialists due to additional confirmatory testing and associated subsequent treatment, while benefit is still uncertain. In addition, the most important argument is the lack of convincing and reliable data that provide insight in the cost-effectiveness of such approach. Cost-effectiveness studies have already shown positive results of screening for PA in specific groups with an increased risk of PA, such as patients with resistant hypertension,[81,82] but are absent for the approach we explored in this study. The costs involve not only those of biochemical screening and confirmation testing, but also of subtype differentiation, including expensive procedures such as adrenal venous sampling. The costs of treatment (such as adrenalectomy) and proper follow-up should also be taken into account. The benefits for the patient of early screening for PA involves an improvement in quality of life, and a reduction in cardiovascular and renal complications on the long-term. Intuitively, this reduction in PA-related morbidity and mortality is expected to more than compensate for the healthcare and societal costs that are associated with early diagnosis and treatment of PA.

Taking all pros and cons into consideration, and applying the criteria of Wilson and Jungner,[75] we cannot advise screening for PA in primary care in all patients with newly diagnosed hypertension. As there are clearly defined subgroups of patients with an increased risk of PA, as described by the Endocrine Society guideline (Table 2), we do suggest to adjust the Dutch CVRM guideline by incorporating these subgroups that need to be screened for PA.[4] If such a strategy would be implemented in primary care, this would lead to screening for PA in approximately 50% of all hypertensive patients.[84]

Table 1 Arguments for early detection of primary aldosteronism in new hypertensive patients according to the criteria of Wilson and Jungner[75]

1.	*The condition sought should be an important health problem.* PA is an important health problem. As shown in our study, the prevalence of PA in newly diagnosed hypertensive patients in primary care is 1.4% to 4.9%, which translates in approximately 1100 to 3700 new patients with PA per year in the Netherlands. In addition, PA carries a high risk of cardiovascular complications, and untreated PA is associated with a poor quality of life.
2.	*There should be an accepted treatment for patients with recognized disease.* There are highly effective treatments available for patients with PA.
3.	*Facilities for diagnosis and treatment should be available.* In the Netherlands healthcare facilities for diagnosis and treatment of PA are widely available.
4.	*There should be a recognizable latent or early symptomatic stage.* Early symptoms consist of hypertension, and in some patients of hypokalemia. In addition, our study has shown that cardiovascular damage is already present in the initial phase of PA.
5.	*There should be a suitable test or examination.* A suitable test is available for screening: a venous blood sample for measurement of aldosterone and renin.
6.	*The test should be acceptable to the population.* The screening test can be performed in primary care by adding the test to the standard laboratory tests, which are performed when hypertension is diagnosed.
7.	*The natural history of the condition, including development from latent to declared disease, should be adequately understood.* The pathogenetic mechanism of PA, and development from latent to declared disease, are reasonably well understood.
8.	*There should be an agreed policy on whom to treat as patients.* After screening a confirmation test will confirm or refute the diagnosis of PA, thus guiding which patients will benefit of PA specific treatment.
9.	*The cost of case finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.* Previous studies in patients with resistant hypertension have shown that an early diagnosis of PA is cost-effective,[81,83] but it is unknown if this is valid for patients with newly diagnosed hypertension.
10.	*Case-finding should be a continuing process and not a "once and for all" project.* Screening for PA can be easily incorporated in standard hypertension care.

In *italic*, the original criterion as formulated by Wilson and Jungner.

Table 2 Recommendation for screening for primary aldosteronism according to the Endocrine Society guideline[4]

Patients with a high prevalence of PA include those with:
• sustained BP above 150/100 mmHg*
• resistant hypertension (BP >140/90 mmHg)#
• controlled BP (<140/90 mmHg) on four or more antihypertensive drugs
• hypertension and spontaneous or diuretic-induced hypokalemia
• hypertension and an adrenal incidentaloma
• hypertension and sleep apnea
• hypertension and a family history of early onset hypertension or cerebrovascular accident at a young age (<40 years)
• all first-degree relatives of patients with PA

*on different days. #resistant to three conventional antihypertensive drugs (including a diuretic). BP, blood pressure. PA, primary aldosteronism.

FUTURE RESEARCH

From all considerations described above we propose to perform further studies to determine the utility and benefits of screening for PA in all newly diagnosed hypertensive patients in primary care. To this end GPs and medical specialists should join efforts. Such collaboration of primary care and referral centres is necessary, as GPs have access to the target population of hypertensive patients, and medical specialists have the expertise for the diagnostic work-up and initiation of treatment. Such studies should adopt a similar design as the study described in *Chapter 3,* and should be carried out in patients with newly diagnosed untreated hypertension. A major and essential component is to incorporate the collection of data on costs and effectiveness. The latter should also include the assessment of quality of life. Therefore, active participation of patients or patients' advocacy groups in the design of such a study should be sought. Given the suboptimal recruitment rate and the biased inclusion of patients in the present study, exploration of reasons for that by focus groups with GPs is mandatory and the design and the execution of such studies should be adapted to prevent lagging and biased recruitment as much as possible.

CONCLUSION

Indiscriminate screening for PA of all patients who are diagnosed with new hypertension in primary care cannot be recommended based on the results of this thesis, as not all criteria of Wilson and Jungner for screening are fullfilled. The prevalence of PA is reasonably well established, and PA seems associated with left ventricular

hypertrophy at the time of the diagnosis of new hypertension. A cost-effectiveness analysis including all costs and long term benefits is required to determine whether screening for PA of all (new) hypertensive patients should be implemented in primary care. For the time being, focused screening of selected hypertensive patients with an increased risk of PA should be encouraged as recommended by the Endocrine Society guideline. To improve the detection rate, early treatment, and final outcome of patients with PA, the Dutch CVRM guideline should consider to adopt the recommendations of the Endocrine Society guideline.

Key messages

1) Primary aldosteronism is present in 1.4% to 4.9% of patients with newly diagnosed hypertension in primary care.
2) Focussing on only hypokalemia will miss most patients with primary aldosteronism, and therefore lack of hypokalemia cannot be used to exclude PA.
3) In newly diagnosed hypertensive patients with PA, left ventricular hypertrophy seems more prevalent than in a comparable group of patients with essential hypertension, suggesting more rapid development of cardiovascular damage than in essential hypertension.
4) At this moment, screening for PA in primary care should be preserved for specific subgroups as recommended by the Endocrine Society guideline. We suggest to adopt these recommendations for screening by the Dutch CVRM guideline.
5) The ARR is not a useful biomarker to guide antihypertensive treatment.

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7

Summary (Dutch)

SAMENVATTING

Dit proefschrift gaat over de vraag of het zinvol is om in de huisartspraktijk patiënten met een nieuw ontdekte verhoogde bloeddruk te screenen op primair hyperaldosteronisme (PHA). PHA is een aandoening die wordt veroorzaakt door een afwijking in één of beide bijnieren. Verhoogde bloeddruk, ook wel hypertensie genoemd, is vaak het enige symptoom van PHA. Deze Nederlandstalige samenvatting start met informatie over hypertensie. Daarna wordt beschreven wat PHA is en waarom het belangrijk is dat deze diagnose tijdig wordt gesteld. Aansluitend volgt de inhoud van dit proefschrift.

Hypertensie

Hypertensie is de grootste risicofactor voor ziektelast bij zowel mannen als vrouwen en is medeverantwoordelijk voor 9,4 miljoen doden per jaar wereldwijd. De prevalentie (=hoe vaak het voorkomt) van hypertensie in Nederland is ongeveer 30%. Dit betekent dat 30 van de 100 mensen een te hoge bloeddruk hebben. Met het stijgen van de leeftijd neemt de prevalentie toe tot ongeveer 45%. Van de mensen met hypertensie heeft 85% tot 90% een verhoogde bloeddruk waarbij geen specifieke oorzaak te vinden is. Dit heet essentiële hypertensie. Echter, in 10% tot 15% van de gevallen is er wel een oorzaak te vinden. Dit wordt secundaire hypertensie genoemd. PHA is één van die specifieke oorzaken van verhoogde bloeddruk.

De huisartsenrichtlijn in Nederland (=NHG-standaard 'Cardiovasculair risicomanagement') stelt dat hypertensie gediagnosticeerd wordt op basis van het gemiddelde van tenminste twee verschillende bloeddrukmetingen op twee verschillende momenten. Bij zo'n bloeddrukmeting wordt de meetwaarde weergegeven in twee getallen: de bovendruk (systolische bloeddruk) en de onderdruk (diastolische bloeddruk), waarbij de bovendruk altijd als eerste wordt genoemd. In de huisartspraktijk is de afkapwaarde voor een verhoogde bloeddruk een bovendruk >140 mmHg (150-160 mmHg bij patiënten vanaf 80 jaar).

Chronische hypertensie leidt tot atherosclerose (=(slag)aderverkalking)), verdikking van de hartspier en nierschade. Hierdoor stijgt de kans op het krijgen van hart- en vaatziekten (bijvoorbeeld een beroerte, of hartinfarct). De meeste mensen merken niet dat ze hypertensie hebben, omdat een verhoogde bloeddruk meestal geen klachten geeft. Het wordt opgemerkt tijdens een bloeddrukmeting op de huisartspraktijk, een keuring, of als langer bestaande hypertensie tot complicaties heeft geleid (bijvoorbeeld een hartinfarct).

De behandeling van hypertensie is gericht op preventie van hart- en vaatziekten. Omdat hart- en vaatziekten meestal het gevolg zijn van meer factoren dan alleen hypertensie, worden door de huisarts ook andere risicofactoren in kaart gebracht (bijvoorbeeld overgewicht, roken enz.). Op basis van dit 'cardiovasculaire risicoprofiel' wordt een behandeling ingezet. Hierbij behoren alle patiënten leefstijladviezen te krijgen die passen bij hun persoonlijke situatie. Het naleven en het effect van deze adviezen moeten geëvalueerd -en zo nodig bijgesteld- worden. Afhankelijk van het

resultaat van de leefstijladviezen en de hoogte van de bloeddruk, kan met bloeddrukverlagende medicatie worden gestart.

Om recht te doen aan het veelal internationale karakter van wetenschappelijk onderzoek, waarin gebruik wordt gemaakt van een internationaal geaccepteerde afkapwaarde voor hypertensie, wordt in dit proefschrift de definitie van hypertensie volgens de *European Society of Hypertension* gebruikt: bij een meting in de huisartsenpraktijk is sprake van hypertensie bij meerdere metingen met een systolische bloeddruk ≥140 en/of een diastolische bloeddruk ≥90 mmHg.

Primair hyperaldosteronisme

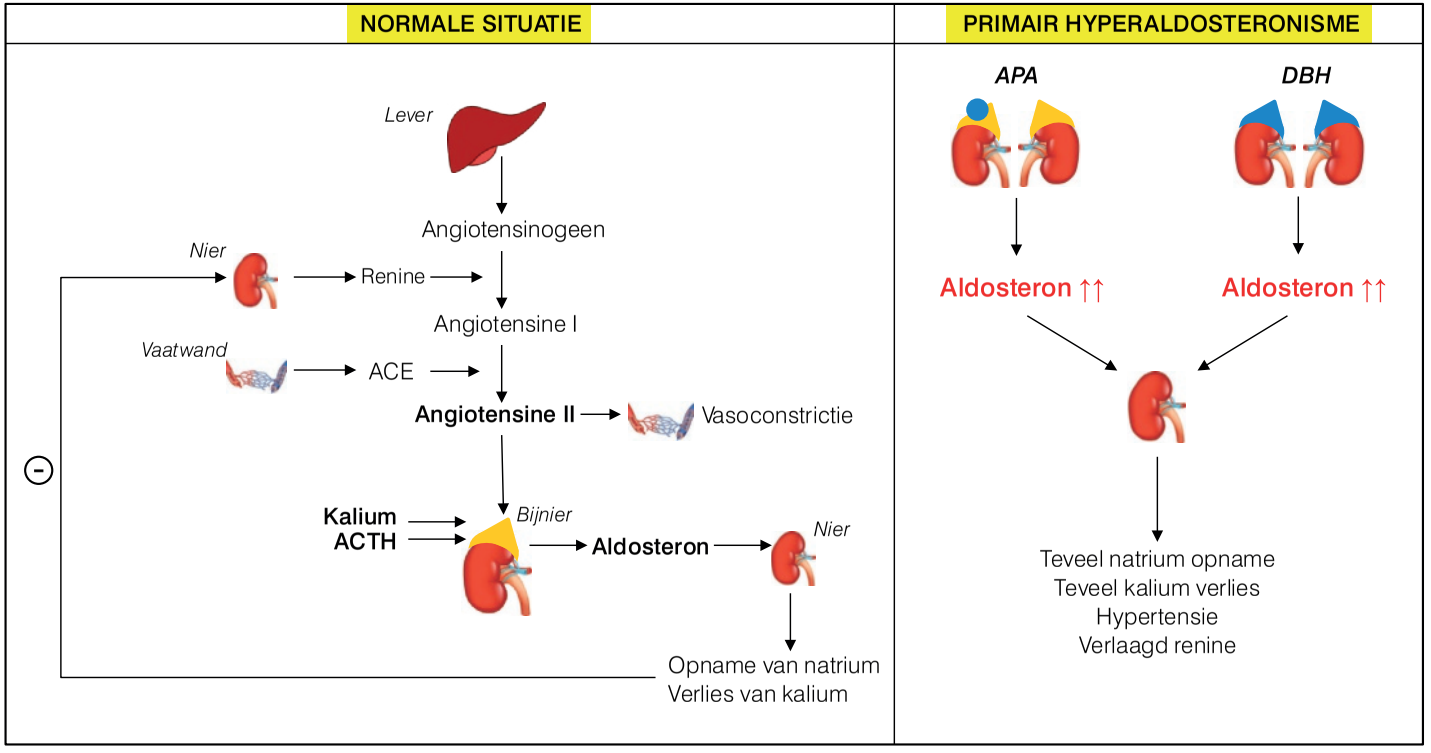
Bij PHA is sprake van autonome productie van aldosteron door één of beide bijnieren. De belangrijkste factoren die verantwoordelijk zijn voor de aldosteronproductie in de normale situatie zijn het renine-angiotensine-aldosteron systeem (RAAS), het kalium (=een mineraal dat we binnen krijgen met onze voeding) en ACTH (= een hormoon van de hypofyse (=hersenklier)).

De aanmaak van aldosteron begint met de omzetting van angiotensinogeen naar angiotensine I door het enzym renine (Figuur 1, linker kolom). De aanmaak van renine wordt met name gestimuleerd door een lage bloeddoorstroming van de nier. Angiotensine I wordt door het angiotensine-converterend enzym omgezet in angiotensine II. Dit angiotensine II zorgt voor het samentrekken van bloedvaten (=vasoconstrictie) en werkt daardoor bloeddrukverhogend. Daarnaast zorgt angiotensine II voor het vrijkomen van aldosteron in de bijnieren. Aldosteron werkt onder andere in de nieren waar het verantwoordelijk is voor het vasthouden van natrium (=bestanddeel van 'keukenzout') en daardoor ook van water, waardoor de bloeddruk stijgt. Bij patiënten met PHA reageert aldosteron niet langer op fysiologische signalen zoals renine en kalium (Figuur 1, rechter kolom). De ongereguleerde productie van aldosteron resulteert in een overmaat aan aldosteron met als gevolg een toename van het vasthouden van zout en water door de nieren. Dit leidt tot een blijvend verhoogde bloeddruk en een remming van de renineproductie.

Voorheen werd gedacht dat PHA zeldzaam was. Inmiddels is bekend dat PHA vaker voorkomt, namelijk bij ongeveer 5% tot 10% van alle mensen met hypertensie. De diagnose is niet makkelijk te stellen omdat, net als bij essentiële hypertensie, specifieke klachten ontbreken. Ook het klassieke concept dat patiënten met PHA altijd een verlaagd kalium hebben (=hypokaliëmie) blijkt achterhaald: een hypokaliëmie komt slechts voor bij ongeveer 30% van de patiënten met PHA.

PHA kent twee subgroepen: éénzijdig PHA, wat meestal wordt veroorzaakt door een adenoom (=zwelling) in één bijnier (ook wel 'ziekte van Conn' genoemd), en dubbelzijdige bijnierhyperplasie (=zwellingen in beide bijnieren) waarbij beide bijnieren teveel aldosteron produceren. Dit verschil is van groot belang voor de behandeling: bij éénzijdig PHA heeft operatief verwijderen van de bijnier de voorkeur, omdat hiermee in de meerderheid van de gevallen de overmatige aldosteronproductie genormaliseerd wordt. Wanneer beide bijnieren teveel aldosteron produceren is behandeling met

Figuur 1 Aanmaak van aldosteron bij gezonde personen en bij patiënten met primair hyperaldosteronisme



ACTH, adrenocorticotroop hormoon (=hormoon uit de hersenklier/hypofyse). APA, aldosteron producerend adenoom (=enkelzijdig primair hyperaldosteronisme). DBH, dubbelzijdige bijnierhyperplasie (=dubbelzijdig primair hyperaldosteronisme).

specifieke bloeddrukverlagende medicatie (mineralocorticoïd receptor antagonisten) nodig, waardoor de effecten van het teveel aan aldosteron geblokkeerd worden. Dit resulteert in een daling van de bloeddruk en een normalisering van een eventueel verlaagd kalium.

De tijd tussen het stellen van de diagnose hypertensie en de diagnose PHA is vaak lang (gemiddeld acht jaar). Een tijdige en juiste diagnose van PHA is belangrijk om vier redenen:

1) De kans op hart- en vaatziekten bij hypertensie door PHA is hoger vergeleken met de kans op hart- en vaatziekten bij essentiële hypertensie.
2) PHA behoort anders behandeld te worden, namelijk met een operatie of met specifieke bloeddrukverlagers (mineralocorticoïd receptor antagonisten). Hierdoor worden ook de schadelijke effecten van de overmaat aan aldosteron op bijvoorbeeld hart en bloedvaten tegengegaan.
3) Behandelresultaten zijn beter als patiënten jong zijn en de hypertensie nog niet lang bestaat, dit geldt met name voor patiënten die geopereerd worden.
4) De kwaliteit van leven van onbehandelde patiënten met PHA is slechter dan die van patiënten met onbehandelde essentiële hypertensie, maar deze verbetert of normaliseert na specifieke behandeling.

Diagnostiek van primair hyperaldosteronisme

De diagnostiek van PHA bevat drie stappen: 1) screening, 2) bevestigen (of uitsluiten) van de diagnose, en 3) onderzoek naar het aantal aangedane bijnieren (enkelzijdig, of dubbelzijdig). Screening op PHA gebeurt door meting van aldosteron en renine in een bloedmonster, waarna de ratio van beide berekend kan worden. Een verhoogde aldosteron-renine ratio is verdacht voor PHA en reden tot vervolgonderzoek. Zo'n vervolgonderzoek kan niet bij de huisarts plaatsvinden, hiervoor is een verwijzing naar een internist nodig. In het ziekenhuis krijgt de patiënt een zogenaamde 'confirmatietest', meestal een 'zoutbelastingstest', waarbij wordt gekeken of het aldosteron hoog blijft na toediening van zout. Hierna volgt vaak vervolgonderzoek naar het aantal aangedane bijnieren, tenzij bij voorbaat duidelijk is dat de patiënt niet geopereerd kan/wil worden: dan volgt direct behandeling met een mineralocorticoïd receptor antagonist.

Screening op primair hyperaldosteronisme

In Nederland behoort de diagnostiek en behandeling van hypertensie grotendeels tot het domein van de huisarts. De huidige richtlijn voor huisartsen formuleert twee redenen om aan PHA te denken: hypokaliëmie (=verlaagd kalium) en/of therapie-resistente hypertensie (=hypertensie waarbij de bloeddruk hoog blijft ondanks het gebruik van meer dan drie verschillende soorten bloeddrukverlagers) en adviseert in dat geval een verwijzing naar het ziekenhuis. Echter, aangezien zo'n 70% van de patiënten met PHA géén hypokaliëmie heeft, is het aannemelijk dat veel patiënten met PHA worden gemist. Dit leidt tot een vertraging van de diagnose, en een hogere

kans op cardiovasculaire complicaties. Het is derhalve de vraag of het wellicht zinvol zou zijn om alle patiënten met nieuwe hypertensie te screenen op PHA. De huisartsenpraktijk lijkt hiervoor de ideale setting, immers:

1) De diagnose hypertensie wordt meestal in de huisartsenpraktijk gesteld.
2) Screening op PHA moet bij voorkeur plaatsvinden zonder dat een patiënt al bloeddrukverlagende medicatie gebruikt, want deze medicatie beïnvloedt de meetresultaten van aldosteron en renine.
3) Ook bij een lage prevalentie is het absolute aantal patiënten met PHA hoog (2000 tot 5000 mensen per jaar).
4) Door PHA veroorzaakte schade aan hart en bloedvaten is deels omkeerbaar bij PHA-specifieke behandeling.

Alvorens te overwegen te screenen op een bepaalde aandoening, is het van belang naar een aantal criteria te kijken zoals geformuleerd door Wilson en Jungner (WHO, 1968). Om aan deze criteria te kunnen voldoen, is wetenschappelijke kennis nodig, waaraan dit proefschrift een bijdrage hoopt te leveren. In Tabel 1 zijn de criteria van Wilson en Jungner toegespitst voor screening op PHA.

Studies in dit proefschrift

Hoofdstuk 2 beschrijft de opzet en resultaten van een '*systematic review*' en '*meta-analysis*' naar de prevalentie van PHA. Toen uit verschillende studies bleek dat de prevalentie voor PHA zeer verschillend was, namelijk variërend van 1% tot 29%, zochten wij verklarende factoren voor deze discrepantie in prevalenties. Hiervoor verrichtten wij eerst een geprotocolleerd literatuuronderzoek. Vervolgens formuleerden wij mogelijke oorzaken voor de diversiteit in prevalenties. Op basis van de literatuur namen wij aan dat de prevalentie van PHA in de 1e lijn (=huisartspraktijk) verschilt van die in de 2e lijn (=ziekenhuis), daarom hebben wij deze apart geanalyseerd. Voor het literatuuronderzoek verzamelden wij alle prevalentiestudies over PHA die gepubliceerd zijn vanaf 1-1-1990 t/m 31-1-2015 uit verschillende medische databanken, namelijk PubMed, EMBASE, Web of Science, en Cochrane Library. Artikelen werden geïncludeerd (=insluiten voor onderzoek) als zij de prevalentie van PHA in volwassen patiënten (≥18 jaar) met hypertensie beschreven, mits de diagnose PHA was bevestigd door een confirmatietest in tenminste 50% van de patiënten met een positieve screening test. Negenendertig studies met in totaal 42510 patiënten werden geïncludeerd, waarvan negen studies met in totaal 5896 patiënten afkomstig waren uit de 1e lijn.

Prevalenties varieerden van 3,2% tot 12,7% in de 1e lijn, en van 1% tot 29% in de 2e lijn. Omdat de opzet van de studies te veel van elkaar verschilde, was het niet mogelijk om een gemiddelde prevalentie uit te rekenen. De meta-analyse toonde dat studies uit de 2e lijn met één of meer van de volgende factoren meer kans hebben op een hoge prevalentie: 1) gepubliceerd na 2000, 2) uit Australië, 3) doel van de studie was het onderzoeken van de prevalentie van secundaire hypertensie, 4) retrospectieve studies, 5) studies die hun patiënten opeenvolgend includeerden, en 6) studies die geen gebruik maakten van een screening test. Helaas bleek het niet mogelijk om de

Tabel 1 De criteria van Wilson en Jungner voor primair hyperaldosteronisme

1.	*De op te sporen ziekte moet een belangrijk gezondheidsprobleem zijn.* PHA is een belangrijk gezondheidsprobleem in een aanzienlijk aantal patiënten. Aangenomen dat de prevalentie 5% is, zijn er ongeveer 5000 nieuwe patiënten in Nederland met PHA. Daarnaast verhoogt PHA het risico op hart- en vaatziekten, en ervaren patiënten met onbehandelde PHA een lagere kwaliteit van leven.
2.	*Er moet een algemeen aanvaarde behandelingsmethode voor de ziekte zijn.* De behandeling voor PHA is afhankelijk van het subtype: bij een adenoom is het verwijderen van de aangedane bijnier de beste keuze, bij dubbelzijdige bijnierhyperplasie is behandeling met een mineralocorticoïd receptor antagonist de beste keuze. Beide behandelingen zijn effectief.
3.	*Er moeten voldoende voorzieningen voorhanden zijn voor diagnose en behandeling.* In Nederland zijn de gezondheidsvoorzieningen voor de diagnose en behandeling van PHA goed bereikbaar.
4.	*Er moet een herkenbaar latent of vroeg symptomatisch stadium van de ziekte zijn.* Vroege symptomen van PHA zijn hypertensie, en in sommige gevallen een verlaagd kalium.
5.	*Er moet een betrouwbare opsporingsmethode bestaan.* Screening op PHA bestaat uit een bloedtest waarbij de aldosteron-renine ratio wordt gemeten.
6.	*De opsporingsmethode moet aanvaardbaar zijn voor de bevolking.* De screeningstest kan worden toegevoegd aan het standaard bloedonderzoek dat de huisarts verricht als de diagnose hypertensie is gesteld.
7.	*Het natuurlijke verloop van de op te sporen ziekte moet bekend zijn.* Het verloop van PHA van latente tot manifeste ziekte is redelijk goed bekend.
8.	*Er moet overeenstemming bestaan over de vraag wie behandeld moet worden.* Bij een positieve screening vindt een confirmatietest plaats, waarna de diagnose wordt gesteld of verworpen.
9.	*De kosten van opsporing, diagnostiek en behandeling moeten in een acceptabele verhouding staan tot de kosten van de gezondheidszorg als geheel.* De kosten van screening blijken in meerdere onderzoeken op te wegen tegen de kosten van niet-screenen, maar deze studies zijn uitgevoerd bij specifieke groepen patiënten en daarom zijn de resultaten mogelijk niet geldig voor de patiënten met hypertensie in de huisartspraktijk.
10.	*Het proces van opsporing moet een continu proces zijn en niet een eenmalig project.* Screening op PHA kan vrij makkelijk worden geïmplementeerd in de standaard hypertensiezorg.

In *cursief:* het criterium zoals geformuleerd door Wilson en Jungner.
PHA, primair hyperaldosteronisme.

verklarende factoren voor studies met een hoge prevalentie ook voor de 1e lijn te berekenen, omdat het aantal geïncludeerde studies hiervoor te laag was.

De prevalentie van PHA in de Nederlandse huisartsenpraktijk wordt beschreven in *Hoofdstuk 3*. Het doel van deze studie was de prevalentie van PHA vast te stellen in mensen met nieuw ontdekte hypertensie. Hiervoor werd een aantal huisartsen uit de regio Nijmegen gevraagd om bij alle opeenvolgende patiënten met nieuwe hypertensie de aldosteron-renine ratio te bepalen. Bij een verhoogde ratio volgde een zoutbelastingstest, die de diagnose PHA bevestigde of juist verwierp. Van de totaal 3748 patiënten met nieuwe hypertensie werd bij 343 patiënten de aldosteron-renine ratio bepaald. In 9 van de 74 patiënten met een verhoogde aldosteron-renine ratio werd de diagnose PHA bevestigd door een positieve zoutbelastingstest. Dit resulteerde in een prevalentie van PHA in patiënten met nieuw ontdekte hypertensie van 2,6% (95% betrouwbaarheidsinterval 1,4 - 4,9). Alle patiënten met PHA hadden een normaal kalium. Bij 8 van de 9 patiënten was de bloeddruk bij herhaling >150/100mmHg. Deze bevindingen sluiten aan bij het advies van de richtlijn over PHA van de *Endocrine Society* (2016) welke patiënten te screenen op PHA. Hoewel de expliciete vraag aan de deelnemende huisartsen was om bij álle patiënten met nieuwe hypertensie de aldosteron-renine ratio te bepalen, viel het aantal gescreende patiënten met nieuwe hypertensie veel lager uit dan verwacht. Dit lage aantal gescreende patiënten (9,2%) weerspiegelt hoe lastig het kan zijn om prevalentie-onderzoek te doen in de 1e lijn. Daarnaast bleek op basis van de gegevens uit de huisartsendossiers, dat er sprake was van 'selectiebias'. Dit betekent dat de gevonden resultaten voornamelijk betrekking hebben op een deel (=selectie) van de patiënten. Uit onze studie bleek dat patiënten die jonger waren, of die een hogere bloeddruk hadden, een hogere kans hadden op een meting van de aldosteron-renine ratio. Het lijkt alsof huisartsen er intuïtief vanuit gingen dat de kans op het vinden van PHA bij deze patiënten het grootst is. Dit is in overeenstemming met het gegeven dat jongere mensen met hypertensie en mensen met een heel hoge bloeddruk daadwerkelijk een verhoogde kans hebben op een onderliggende oorzaak van die verhoogde bloeddruk. In eerder gepubliceerde studies werd de kans op selectiebias meestal genegeerd of niet onderzocht. Dit betekent dat de eerder gerapporteerde prevalenties kritischer bekeken moeten worden.

In *Hoofdstuk 4* onderzochten we in hoeverre het hart en de bloedvaten bij patiënten met nieuwe hypertensie en PHA zijn aangedaan in vergelijking met eenzelfde groep patiënten zonder PHA. Het was reeds bekend dat patiënten met hypertensie bij wie de diagnose PHA wordt gesteld meer schade aan het hart en de vaten hebben dan te verwachten op basis van de hoogte van de bloeddruk, maar we wisten nog niet of deze extra schade al zichtbaar is op het moment van het stellen van de diagnose hypertensie. Dit is zinvolle informatie, omdat de ernst van een aandoening meeweegt in een uiteindelijke beslissing om screening in te voeren. We hebben zes patiënten met nieuwe hypertensie en PHA vergeleken met 24 patiënten met nieuwe essentiële hypertensie. Deze twee groepen waren vergelijkbaar met

betrekking tot geslacht, leeftijd, en de hoogte van de bloeddruk. Zij kregen allen uitgebreide onderzoeken van het hart, de nieren en de vaten. Twee van de zes patiënten met PHA bleken reeds een verdikte hartspier te hebben, terwijl geen van de patiënten in de groep met essentiële hypertensie dit had. Er werden geen verschillen gezien bij de overige onderzoeken. Omdat het maar om kleine aantallen gaat, kan deze bevinding slechts als richtinggevend beschouwd worden. Er zijn grotere studies nodig om onze resultaten te bevestigen.

Ondanks vele studies naar zogenaamde '*biomarkers*' als hulpmiddel voor de keuze van een bepaald soort bloeddrukverlager bij de behandeling van hypertensie, worden tot op heden alleen grove maten als leeftijd en ras als onderscheidend beschouwd. *Hoofdstuk 5* beschrijft onze studie naar het gebruik van de aldosteron-renine ratio als mogelijke leidraad bij het starten van bloeddrukverlagende medicatie bij patiënten met essentiële hypertensie. Vóór de start van de behandeling werd de aldosteron-renine ratio bepaald, om na één jaar behandeling de bloeddrukverandering te evalueren. Er was geen protocol voor de keuze van de bloeddrukverlager anders dan de huisartsenrichtlijn. Van 304 patiënten hadden we 947 bloeddrukmetingen, waarvan 220 metingen behorend bij patiënten die bloeddrukverlagers gebruikten. Er werd geen verband gezien tussen de waarde van de aldosteron-renine ratio vóór de start van de behandeling en de bloeddrukverandering door de medicatie. Als belangrijke nevenbevinding vonden we dat slechts bij 31% van de patiënten met hypertensie na één jaar behandeling de streefwaarde van de bloeddruk was bereikt. Er was geen verband tussen de aldosteron-renine ratio en het (niet) bereiken van die streefwaarde.

Implicaties voor de dagelijkse praktijk

In dit proefschrift vonden wij een prevalentie van PHA van 2,6% (95% betrouwbaarheidsinterval 1,4-4,9) bij patiënten met nieuw ontdekte hypertensie. Gebaseerd op deze data, zouden we bij het invoeren van screening bij patiënten met nieuwe hypertensie in de Nederlandse huisartspraktijk per jaar tussen de 1100 en 3700 nieuwe patiënten met PHA vinden. Bij het grootste deel van hen wordt de diagnose op dit moment gemist, omdat de huidige huisartsenrichtlijn niet gericht is op het opsporen van PHA. Daarnaast is het waarschijnlijk dat reeds in de vroege fase van PHA hartschade bestaat bij een aantal patiënten.

Hoewel er verscheidene argumenten zijn om screening op PHA in te voeren, wordt op dit moment niet voldaan aan alle screeningscriteria van Wilson en Jungner. Het grootste probleem is het gebrek aan kosten-effectiviteitsstudies, waarin wordt aangetoond dat screenen op PHA effectief is als je kijkt naar wat het kost aan gezondheidzorg en wat het uitspaart aan kosten voor de gezondheidszorg wanneer PHA niet (eerder) was gediagnosticeerd. Op dit moment bestaan deze studies alleen voor patiënten met therapie-resistente hypertensie: screenen op PHA in deze groep is kosten-effectief. Naast kosten-effectiviteit op medisch gebied (invoeren screening, aanvullende diagnostiek en behandeling), zou ook gekeken moeten worden naar de maatschappelijke en financiële gevolgen van (onbehandelde) PHA, zoals werkverzuim

of arbeidsongeschiktheid. Tot meer duidelijkheid bestaat over dit kostenaspect, adviseren wij dat in de huidige huisartsenrichtlijn de aanbevelingen met betrekking tot screening op PHA worden overgenomen zoals geformuleerd in de richtlijn van de *Endocrine Society*. Als deze screeningsstrategie zou worden geïmplementeerd, zou ongeveer 50% van de patiënten met hypertensie in aanmerking komen voor screening op PHA.

Tabel 2 Aanbevelingen voor screening op primair hyperaldosteronisme volgens de richtlijn van de *Endocrine Society*

Patiënten met een verhoogd risico op onderliggend primair hyperaldosteronisme zijn zij met:
• aanhoudende bloeddruk >150/100 mmHg
• therapie-resistente hypertensie (bloeddruk >140/90 mmHg)
• gereguleerde bloeddruk (bloeddruk <140/90 mmHg) bij vier of meer soorten bloeddruk-verlagers
• hypertensie en spontane of diuretica-geïnduceerde hypokaliëmie
• hypertensie en een incidentaloom in de bijnier
• hypertensie en obstructief slaap apneu syndroom
• hypertensie en een familie-anamnese met hypertensie op jonge leeftijd of een beroerte op jonge leeftijd (<40 jaar)
• alle eerstegraads familieleden van patiënten met primair hyperaldosteronisme



8

Dankwoord
Curriculum Vitae
PhD portfolio

DANKWOORD

Graag wil ik allen die hebben bijgedragen aan het tot stand komen van dit proefschrift hartelijk danken. Een aantal mensen wil ik expliciet noemen.

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Lieve Mama, ik heb groot respect voor de wijze waarop jij Papa hebt verzorgd. Jij leerde mij de werkelijke betekenis van trouw en toewijding, waarbij het prima is om jezelf weg te cijferen als dit even nodig is. Dat heb je ook voor mij gedaan: ik heb dit proefschrift kunnen voltooien dankzij al die keren dat jij op Simon en Hugo paste, zodat ik kon werken als huisarts of aan mijn onderzoek. Farid en ik zijn je intens dankbaar voor alles wat je voor ons doet. Wat een zegen dat onze kinderen opgroeien met 'Nana' die hen zoveel warmte schenkt! Lieve Mama: een diepe buiging voor jou, ik dank je uit de grond van mijn hart.

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Dr. Bakx, beste Carel, wat was het een voorrecht om dit project te starten onder jouw behoedzame vleugels. Die eerste maanden ging mijn leercurve bijna loodrecht omhoog! Eén van mijn dierbare herinneringen is jouw gezichtsuitdrukking toen ik vol enthousiasme ons protocol in 12-voud kwam laten zien: je vroeg je af of de medisch-ethische commissie ooit eerder zo'n kleurrijk protocol had beoordeeld, maar was snel overtuigd van het nut van mijn efficiënte (inderdaad wat fleurige) indeling. En toen stierf je. Weet je dat mensen nog steeds een glimlach op hun gezicht krijgen als het over jou gaat? Ook ik voel mij een gezegend mens met jou in mijn herinnering. Heel veel dank voor jouw vertrouwen en inspiratie, waarmee ik uiteindelijk ook zonder directe begeleiding van een huisarts mijn weg heb weten te vinden in de wereld van huisartsgeneeskundig onderzoek.

Professor dr. Lenders, beste Jacques, na het overlijden van Carel en het afscheid van Mark was dit project een chaos. Jij kwam, je zag, en je nam het over: het is jouw verdienste dat de neuzen weer dezelfde kant op gingen staan. Jouw kritische blik en tomeloze inzet lijken onuitputtelijk, en ik kan de betrokkenheid die hieruit spreekt enorm waarderen. Je blijft continu in contact met het gezamenlijke doel, ongeacht de soms tegenstrijdige belangen binnen het team. Je stimuleert, doceert, inspireert en begrenst. Ik heb het als een zeer leerzame en prettige samenwerking ervaren. Het ziet er allemaal zo makkelijk uit als jij het doet! Mijn hartelijke dank voor de waardevolle adviezen op zowel medisch-inhoudelijk als persoonlijk vlak, je nauwgezette correcties en je standvastigheid.

Dr. Deinum, beste Jaap, jij bent het anker van dit project. Ik ontmoette je voor het eerst toen ik als student geneeskunde mocht deelnemen aan de masterclass interne geneeskunde en was zo onder de indruk dat ik bijna mijn nek verrekte met opkijken naar jou. En terecht: je bent een warm en integer persoon, een geduldig en kritisch clinicus, en een inspirerend en gedreven onderzoeker. Veel dank voor het vertrouwen dat jij mij vanaf het begin hebt gegeven. Bovenal veel dank voor jouw onvermoeibare en secure 'stofkam': daar werd ik altijd blij van!

Dr. Biermans, beste Marion, epidemiologie is duidelijk jouw expertise. Dankzij jouw opmerkzaamheid hebben wij de prevalentie van primair hyperaldosteronisme op de meest zuivere wijze berekend. Veel dank voor het overnemen van het copromotorschap, het leiden van het uitermate gecompliceerde proces van de data-extracties, jouw aanwezigheid bij de gezamenlijke overleggen en het kritisch commentaar bij de artikelen.

Dr. Schermer, beste Tjard, jouw jarenlange ervaring op het gebied van huisartsgeneeskundig onderzoek maakte je bijdrage aan dit project waardevol. Jouw uitgebreide Engelse woordenschat hebben menige zin verduidelijkt en je daagde me uit de tekst dusdanig helder te formuleren dat deze voor een ieder te volgen was. Veel dank voor jouw inzet en oplossingsgerichte adviezen.

Dr. van der Wel, beste Mark, het spijt me hoe het tussen ons is gelopen na het overlijden van Carel. We waren allebei erg verdrietig. Ik waardeer het ontzettend dat jij, ondanks onze meningsverschillen, bleef herhalen dat je dacht dat ik dit kon, dat dit 'mijn' project was. De herinnering aan jouw vertrouwen in mij heeft mij de afgelopen jaren gesteund, samen met de vastberadenheid deze 'tweede' kans zorgvuldig te benutten. En daarbij, het grootste deel van het werk (protocol, inclusie huisartspraktijken en samenwerking met SHO) hadden wij reeds samen gerealiseerd: hoe moeilijk kon het nog worden? Veel dank voor ons traject samen: ik kijk er met genegenheid op terug.

Dr. de Grauw, beste Wim, jouw bijdrage als huisarts-onderzoeker is zeer waardevol geweest. In 2014 werd het studieprotocol dusdanig aangepast, dat er ruimte kwam voor een nieuwe onderzoeksvraag vanuit de eerste lijn: *Hoofdstuk 5* is het resultaat van jouw explorerende geest. Veel dank voor dit zorgvuldig meedenken, als ook voor jouw inzet als huisarts én onderzoeker tijdens de gezamenlijke overleggen.

Veel dank aan het SHO, met name aan Dick, Eric, Carolien en Holger: ik heb onze samenwerking als zeer prettig ervaren. Dankzij jullie is de werkinstructie voor de afname van aldosteron en renine uitstekend geïmplementeerd. Gezien de resultaten van dit proefschrift, hoop ik van harte dat deze bepalingen in de toekomst regelmatig zullen worden aangevraagd. Evaluatie over een jaar?

Beste professor van der Wilt, veel dank voor uw begeleiding en kritische noten bij de uitvoering en het opschrijven van onze meta-analyse.

Beste Hans (Groenewoud), heel veel dank voor jouw hulp bij de meta-analyse. Ik heb altijd uitgekeken naar de overleggen met jou, waarin je me naast de biostatistiek ook onderwees in jouw passie. Sindsdien kijk ik toch anders naar nachtvlinders.

Beste Hans (Peters) en Waling, veel dank voor het extraheren van de HIS-data en het maken van de PAGODE-bestanden. Beste Waling, rust zacht.

Beste Hans (Bor) en Reinier, veel dank voor jullie hulp met de statistiek in de huisarts-praktijk. Jullie enthousiasme en expertise waren voor mij als twee boeien in de verraderlijke zee vol parametrische stormen en non-parametrische zeeslangen (ik word er poëtisch van). Gelukkig zijn er mensen zoals jullie die gewapend met logisch redeneren, en wellicht een specifiek gen dat ik mis, deze wateren wel kunnen bevaren.

Lieve Bianca, rots in de statistische branding. Ik blijf me verbazen over de eenvoudige wijze waarop jij de meest ingewikkelde berekeningen kan uitvoeren en uitleggen. Heel veel dank voor het maken van de koppelingen tussen vier (!) databases, het maken en toelichten van ingewikkelde analyses (dat gen zit dus niet alleen op de 'Y'), het nakijken en corrigeren van mijn eigen analyses, en het oplossen van (statistische) complicaties. Ik zal onze overleggen in de Hemelkamer missen!

Graag wil ik alle co-auteurs danken voor de vruchtbare samenwerking. Twee van hen wil ik in het bijzonder noemen.

Lieve Tanja, wat hebben we hard gewerkt in ons streven naar volledigheid en -vergeef mij mijn onbescheidenheid- wat is het een prachtig artikel geworden! Hartelijk dank voor een zeer prettige samenwerking. Veel succes met het afronden van jouw boekje!

Beste Daniëlle, ere wie ere toekomt: als internist met het aandachtsgebied vasculaire aandoeningen heb jij alle uitgebreide onderzoeken voor onze studie naar vaatschade verricht. Veel dank voor de fijne samenwerking. Jouw boekje is nu bijna af: succes met de laatste loodjes!

Beste collegae en medewerkers van de ELG, na een gastvrije start op de eerste etage (dankzij Annelies, Hilde, Wouter, Anne vd B, Riet, Joke, Lisette, Debby en Franca) ben ik via de tweede (Loes, Tilly, Twanny, Henk, Sietske, Marianne, Floris, Chris, Pim) geëindigd op de derde (Breg, Erik, Elza, Karin, Annette, Anouk, Kees). Graag wil ik jullie allen danken voor jullie steun, leuke anekdotes en praktische hulp (zoals een nieuw plafond toen het mijne tijdens een regenbui naar beneden kwam, dankjewel Marike!). Speciale dank aan Lea voor het maken van de Access database voor de PAGODE-praktijken.

Veel dank aan de studenten Jenneke, Tessa en Jasper voor het bijhouden van de PAGODE-data toen ik in de huispraktijk mijn opleiding afrondde.

Tot slot enkele woorden van dank gericht tot mensen uit mijn persoonlijke omgeving, die mij direct dan wel indirect hebben gesteund bij dit traject.

Lieve Max, wat een geluk dat ik het eerste jaar van mijn huisartsenopleiding bij jou kwam! Je liet mij zien hoe te relativeren en leerde mij wijze lessen over geneeskunst, waarin bekwaamheid en aardig zijn voor de patiënt hand in hand gaan. Veel dank voor jouw vertrouwen en het verbeteren van verschillende hoofdstukken in dit proefschrift. Ik ben trots één van jouw aios te zijn geweest.

Lieve Renske, ik vind je een fantastische opleider en bewonder je authenticiteit. Veel dank voor de manier waarop je meedacht toen ik tijdens mijn zwangerschap van Hugo iets rustiger aan moest doen. Je had voor mij de ideale oplossing: ik ging mijn patiënten met een rollende bureaustoel uit de wachtkamer halen.

Lieve Lidy, wat was je een inspirerende opleider en wat heb ik een goede tijd in jouw praktijk gehad.
Lieve Liesbeth, het door jou geïnitieerde gesprek tussen Lidy, jou en mij heeft groot verschil gemaakt.
Ik wil jullie beiden hartelijk danken voor jullie uitgesproken vertrouwen in mij en de tastbare betrokkenheid bij mijn onderzoek in mijn laatste jaar als aios huisartsgeneeskunde.

Mijn lieve vriendinnen Sabina, Carlijn, Rasha, Anne K, Vera, Neeria, Kerensa en Maaike, ik ben dankbaar voor jullie vriendschap, zorgzame adviezen, grappige anekdotes en bovenal jullie begrip voor het feit dat ik telefonisch contact vaak prefereer boven een bezoek met reistijd. Veel dank dat jullie deze ietwat heuvelige weg met mij bewandeld hebben. *To be continued.*

Lieve Raquel, hoe heet de grond onder mijn voeten ook werd, jij zag geen reden voor mij om te stoppen. Jij weet hoe het is om offers te brengen, en ik bewonder de wijze waarop jij dat doet: zorgvuldig, beheerst en vol bezieling. Wat fijn dat jij nu mijn paranimf bent! Veel dank voor jouw innige vriendschap en de bemoedigende kaarten (met chocola!).

Lieve Marloes, mijn supersociale vriendin en planwonder (zowel in tijd als in ideeën): ik ben heel blij dat jij mijn paranimf wilt zijn! Wat is het fijn om na een drukke dag samen uit te waaien tijdens een rondje fietsen. Onze mini-intervisie en recidiverende icc. Veel dank voor jouw warmte en wijsheid.

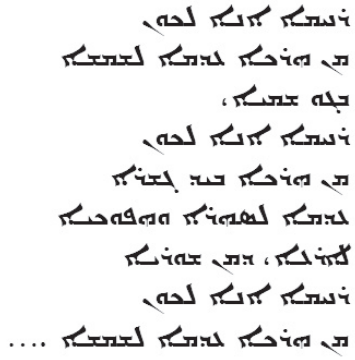
Lieve Lotje en Phuocie, van ons heb ik waardevolle herinneringen van samen een zolder delen in onze studententijd en gezondheidsadvies bij de McDonald's, tot speechen op mijn bruiloft en opvoedingstips. Wat koester ik onze vriendschap! Veel dank voor de zorgvuldige beoordeling van de Nederlandse samenvatting, zodat deze ook voor niet-medici te volgen is.

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Mijn lieve bijzondere zeer gedisciplineerde Papa, ik had je zo graag beter leren kennen. Tegelijkertijd voel ik mij enorm bevoorrecht dat ik, in tegenstelling tot velen, een fijne vader heb gekend. Wat hadden Mama en jij een lol samen, en wat hebben jullie hard gewerkt om mijn broertje en mij een zo goed mogelijke start te geven. Heel veel dank voor de wijze lessen en waardevolle herinneringen.

Lieve Simon en lieve Hugo, jullie zijn het grootste geluk van mij en jullie Papa. Wat ik jullie wil zeggen:





CURRICULUM VITAE

Sabine Christina Käyser werd in 1979 geboren te Nijmegen. Zij behaalde haar middelbare schooldiploma aan het Stedelijk Gymnasium, waarna zij vier keer werd uitgeloot voor de studie Geneeskunde. In 1996 volgde zij opleidingen tot aerobics- en stepsdocent (European Fitness and Aerobics Association), waarna zij werkzaam bleef als sportdocent. Na het intermitterend volgen van de studie Medische biologie (Universiteit van Amsterdam), werd zij in 2002 ingeloot voor de studie Geneeskunde (Radboud Universiteit). Naast de studie Geneeskunde volgde zij enkele modules in de Sociale psychologie (Radboud Universiteit). Na het behalen van het artsexamen werkte zij op de afdeling chirurgie van het ziekenhuis Gelderse Vallei (Ede). In 2011 startte zij met de opleiding tot huisarts. Gezien haar bijzondere belangstelling voor onderzoek op het gebied van hart- en vaatziekten, startte zij in 2012 haar promotietraject bij Carel Bakx† en Jaap Deinum met als resultaat dit proefschrift. Aanvankelijk combineerde zij opleiding en onderzoek met het geven van onderwijs, voornamelijk op het gebied van cardiovasculair risicomanagement. In 2013 ontving zij een posterprijs van de European Society of Hypertension. In 2015 rondde zij de huisartsenopleiding af en sindsdien werkt zij als waarnemend huisarts in regio Nijmegen. Van 2015 tot 2017 was zij als secretaris actief bij de WAGRO Nijmegen e.o. (WAarnemers GROep). Sabine is getrouwd met Farid Abdo en samen hebben zij twee zoontjes.

PhD portfolio

Institute for Health Sciences
Radboudumc

Name PhD candidate: SC Käyser-Abdo **Departments:** Primary and Community care & Internal medicine **Graduate School:** Radboud Institute for Health Sciences	**PhD period:** 01-03-2012 till 06-11-2018 (combined programme with GP residency) **Promotor:** Prof. dr. JWM Lenders **Co-promotors:** Dr. J Deinum, Dr. MCJ Biermans, Dr. TR Schermer

	Year(s)	ECTS
TRAINING ACTIVITIES		
a) Courses & workshops		
- PubMed introduction course	2012	0.1
- PubMed advanced course	2012	0.2
- Workshop Endnote	2012	0.1
- Workshop Medical legislation	2012	0.3
- NCEBP introduction course	2012	1.0
- Workshop Statistics and meta-analysis	2012	0.1
- SPSS introduction course	2013	0.2
- BROK course	2013	1.5
- Academic writing	2013	3.0
- Presentation skills	2013	1.5
- Coaching	2013	0.4
- Introduction to data-analysis	2013	1.5
- Management for PhD-students	2013	2.0
b) Seminars & lectures		
- AIOTHO *(arts in opleiding tot huisarts-onderzoeker)* seminar	2012	0.25
- NHG *(Nederlands Huisartsen Genootschap)* scientific seminar	2012	0.25
- HartVaatHAG *(HuisartsenGroep)* seminar (oral presentation)	2012	0.6
- Radboud Adrenal Centre (RAC) seminar (oral presentation)	2013	0.6
- Radboud Grand Rounds	2014-2017	0.3
c) Symposia & congresses		
- European Society of Hypertension (poster and oral presentation)	2013	2.0
- NHG congress	2014	0.3
- National hypertension congress	2014	0.25
- National hypertension congress	2015	0.25
- HartVaatHAG congress (poster and oral presentation)	2015	0.8
- National hypertension congress	2016	0.25
- National hypertension congress	2017	0.25
d) Other		
- Journal club (monthly), department of Primary and Community care (oral presentations)	2012-2014	2.0
- RAC meeting (every two weeks), department of Internal medicine	2012-2014	0.5
- Secretary of WAGRO Nijmegen e.o.	2015-2017	1.5
TEACHING ACTIVITIES		
e) Lecturing		
- Evidence Based Medicine (EBM) for medical students Radboudumc	2012-2013	0.5
- Cardiovascular risk management (CVRM) for medical students Radboudumc	2012-2013	0.5
- CVRM and primary aldosteronism for primary care practices	2013-2014	0.4
f) Supervision of internships / other		
- Supervision of medical students	2014-2015	0.5
TOTAL		23.9

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