PROGRESS IN ALDOSTERONISM

A review of the prevalence of primary aldosteronism in pre-hypertension and hypertension

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Abstract

Primary aldosteronism (PA) secondary to excessive and/or autonomous aldosterone secretion from the renin–angiotensin system accounts for ~10% of cases of hypertension and is primarily caused by bilateral adrenal hyperplasia (BAH) or aldosterone-producing adenomas (APAs). Although the diagnosis has traditionally been supported by low serum potassium levels, normokalaemic and even normotensive forms of PA have been identified expanding further the clinical phenotype. Moreover, recent evidence has shown that serum aldosterone correlates with increased blood pressure (BP) in the general population and even moderately raised aldosterone levels are linked to increased cardiovascular morbidity and mortality. In addition, aldosterone antagonists are effective in BP control even in patients without evidence of dysregulated aldosterone secretion. These findings indicate a higher prevalence of aldosterone excess among hypertensive patients than previously considered that could be attributed to disease heterogeneity, aldosterone level fluctuations related to an ACTH effect or inadequate sensitivity of current diagnostic means to identify apparent aldosterone excess. In addition, functioning aberrant receptors expressed in the adrenal tissue have been found in a subset of PA cases that could also be related to its pathogenesis. Recently a number of specific genetic alterations, mainly involving ion homeostasis across the membrane of zona glomerulosa, have been detected in ~50% of patients with APAs. Although specific genotype/phenotype correlations have not been clearly identified, differential expression of these genetic alterations could also account for the wide clinical phenotype, variations in disease prevalence and performance of diagnostic tests. In the present review, we critically analyse the current means used to diagnose PA along with the role that ACTH, aberrant receptor expression and genetic alterations may exert, and provide evidence for an increased prevalence of aldosterone dysregulation in patients with essential hypertension and pre-hypertension.

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Invited Author's profile

Dr Georgios Piaditis is director of the Department of Endocrinology, Metabolism and Diabetes Centre, at General Hospital of Athens. His research activities involve various areas of Endocrinology, such as diseases of the thyroid gland, the reproductive system and pituitary. However in the last 8 years his main research activity has been focused on endocrine causes of arterial hypertension.

Introduction

Primary aldosteronism (PA) is the most common form of endocrine hypertension secondary to excessive or inappropriately elevated aldosterone production from the adrenals that is partially or completely autonomous of the renin–angiotensin system (RAS) (1, 2). The prevalence of PA varies considerably between different studies among hypertensive patients, depending on patient selection, diagnostic methodology employed and severity of arterial hypertension. Thus, a 10% prevalence of PA was found when consecutive hypertensive patients were studied prospectively (3), whereas this figure raised to ~30% when the aldosterone to renin ratio (ARR) was used as a screening test in randomly selected hypertensive patients by general practitioners (4). Clinical trials, case series and meta-analyses published up to June 2014 were critically analysed to select most appropriate literature for this review.

Although aldosterone excess is directly linked to the development of hypertension through renal sodium and water retention, it also induces tissue inflammation and an increased central sympathetic drive, with subsequent fibrosis and remodelling in critical organs such as the kidney, heart and vasculature (3, 5, 6, 7, 8, 9, 10). As a consequence, PA leads to the development of renal impairment, atrial fibrillation, stroke and myocardial infarction (3, 11, 12). However, due to the absence of PA-related specific symptoms and signs, there is a substantial delay in the establishment of the diagnosis that may affect the response to treatment and overall prognosis. Thus, early diagnosis is of paramount importance in order to initiate specific treatment by either surgical removal of the hyperfunctioning adrenal lesion(s) or administration of targeted medical treatment with mineralocorticoid receptor antagonists (MRAs). This notion has clearly been proven in a recent prospective study of 180 patients with PA who were treated with either adrenalectomy or MRAs and after a median follow-up of 36 months achieved normalisation of blood pressure (BP) and reversal of the detrimental left ventricular changes as shown by echocardiography (13).

Considering the high prevalence of hypertension in the general population, reliable, easy to perform and cost-effective diagnostic tests are required for early diagnosis of PA. This is particularly relevant as the clinical signs and symptoms of PA are not specific, whereas hypokalaemia, which is regarded as the most distinctive biochemical feature of PA, is not always present and cases of normotensive PA have also been described (14, 15, 16, 17). However, currently available diagnostic

tests for PA are complex, time-consuming, relatively expensive and cannot be applied to all hypertensive patients. A further complexity in the diagnosis of PA is the notion that at least in a subset of patients, aldosterone secretion may be modulated by the presence of functioning aberrant receptors, the diagnosis of which requires an extensive and laborious investigating protocol (18). Thus, current standard practice for the diagnosis of PA is to initially apply a screening test using the calculation of basal ARR followed by a confirmatory test to document autonomous from the RAS aldosterone secretion, such as an oral sodium loading test, saline infusion test (SIT), fludrocortisone suppression test (FST) or captopril challenge test (16, 19) (Table 1). The use of ARR as a screening test has simplified the diagnosis of PA, as it permits the application of diagnostic tests only to a pre-selected hypertensive population with a high probability of PA (20, 21). However, ARR normal cut-offs vary significantly ranging from 20 to 100 ng/dl per ml per h using an immunometric method for estimation of plasma renin activity or 68–338 pmol/mU using an immunometric method for measuring direct plasma renin concentrations, with the most commonly employed being 20–40 ng/dl per ml per h or 68–135 pmol/mU, respectively, when performed in the morning on seated ambulatory patients (8, 20).

This wide variation in the normal ARR cut-offs affects the performance of diagnostic tests for PA and thus the estimated prevalence of PA in hypertension. As a result the Endocrine Society's clinical practice guidelines do not specify which of these four confirmatory tests should be regarded as the 'gold standard' test to definitely confirm or exclude PA (20), and thus different tests are used by different centres. For example, at the Mayo Clinic the 3-day oral salt loading test is used to diagnose PA when on the 3rd day 24-h urinary aldosterone measurement is higher than 12 µg/day, provided that 24-h sodium excretion exceeds 200 mEq/day (15). In contrast, in a large prospective study, the 50 mg captopril test was found to be equally effective to the SIT in hypertensive patients with aldosterone-producing adenomas (APAs). However, its diagnostic value is limited if the sodium intake is ≤130 mEq/day (3). On the basis of these findings, some recommend the use of the captopril test in patients with increased daily sodium intake (22), particularly when the ARR is high (23). On the other hand, others have shown that the captopril test may give misleading results (24, 25, 26). Although the FST is regarded by some as the

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Table 1 Tests employed for the confirmation of the diagnosis of PA.

Test	Methodology	Interpretation	Potential side effects, contraindications and limitations
Oral salt loading test (15, 16, 19, 21, 72)	Sodium intake >200 mmol (6 g) for 3 days substantiated by 24-h urine sodium collection (>200 mmol). Adequate potassium chloride supplementation to maintain potassium within normal range. 24-h urinary aldosterone is collected from the morning of day 3 till morning day 4	Normal: urinary aldosterone secretion <10 µg/24 h. PA: urinary aldosterone excretion >12 µg/24 h	Contraindicated in severe cardiac or renal failure. Reliability of the test is higher if urinary aldosterone is measured by mass spectrometry
Recumbent-saline infusion test (SIT) (16, 19, 21, 72)	Maintain recumbent position at least 1 h before and during the infusion of 2 l 0.9% normal saline infused intravenously over 4 h. Sampling for renin, aldosterone and potassium drawn before initiation of infusion and after 4 h with continuous monitoring of BP and heart rate	Post-infusion plasma aldosterone <5 ng/dl makes PA unlikely, whereas levels >10 ng/dl confirms the diagnosis. Values between 5 and 10 ng/dl are indeterminate	The test is contraindicated in patients with uncontrolled hypertension, cardiac and renal insufficiency, cardiac arrhythmias and severe hypokalaemia
Fludrocortisone suppression test (FST) (16,19, 21, 72)	Administration of sodium chloride (2 g three times daily with food) plus oral fludrocortisone (0.1 mg every 6 h for 4 days) along with potassium chloride (30 mmol three times daily) to maintain serum potassium >4 mmol/l	Upright plasma aldosterone >6 ng/dl on day 5 at 1000 h confirms PA (concomitant PRA <1 ng/ml per h) and plasma cortisol lower than the value measured at 0700 h (exclude a confounding ACTH effect)	The test can be conducted as in-patient for close monitoring or as out-patient in motivated/cooperative patients. Normal: plasma aldosterone level <6 ng/dl taken at 1000 h in an ambulatory patient on day 4 (single centre data)
Captopril test (16, 19, 21, 72)	Administration 25–50 mg captopril per os after remaining sitting or standing for at least 1 h. Measurement of PRA and plasma aldosterone and cortisol before and 1 and 2 h post captopril administration	Normal: post-captopril aldosterone <12–13.9 ng/dl or post-captopril A/R <26–30 ng/dl per ng per ml per h or <200 pg/ml per ng per ml/h	The test is recommended to be used in patients on an adequate sodium intake (>133 mEq or 6.3 g NaCl/day)
Fludrocortisone dexamethasone suppression test (FDST) (61)	Administration of sodium chloride (2 g three times daily with food) plus oral fludrocortisone (0.1 mg every 6 h for 4 days) along with potassium gluconate (4.68 g three times daily) to maintain serum potassium within the normal range (3.5–5.5 mEq/l). At midnight on the 4th day 2 mg of dexamethasone are added (2 h after dinner)	Upright plasma aldosterone >82 pmol/l and ARR >26 pmol/mU on day 5 at 0830 h confirms PA. Simultaneous cortisol measurements (<54 nmol/l) are required to confirm patients' compliance	The test can be conducted as in-patient for close monitoring or as out-patient in motivated/cooperative patients who are on anti-hypertensive treatment with calcium channel blockers with no or minimal effect on hormonal measurements.
Recumbent post-low dose dexamethasone suppression (LDDST)-saline infusion test (PD-SIT) (60)	LDDST: dexamethasone administration 2 mg/day (0.5 mg/6 h) for 2 consecutive days. Maintain recumbent position early in the morning of the 3rd day (0830 h) and during the i.v. infusion of 2 l 0.9% normal saline over 4 h. Sampling for renin, aldosterone, cortisol and potassium drawn before initiation of infusion and after 4 h with continuous monitoring of BP and heart rate	Post-infusion plasma aldosterone <68 pmol/l and ARR <10 pmol/mU confirms PA. Cortisol measurements (<30 nmol/l) are required to confirm patients' compliance	The test is contraindicated in patients with uncontrolled hypertension, cardiac and renal insufficiency, cardiac arrhythmias and severe hypokalaemia

PA, primary aldosteronism; BP, blood pressure; LDDST, low dose dexamethasone suppression test.

most reliable confirmatory test (16), it has recently been shown that the seated SIT is almost equally effective to FST, while the recumbent-SIT, which is used in the majority of studies, failed to diagnose PA in 67% of the

tested cases (27). However, similar to the wide normal cut-off variation in ARR, the cut-offs of post-SIT or post-FST aldosterone levels also vary widely, ranging from 139 to 277 pmol/l (15, 20, 24).

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Genetic determinants of PA

Besides the high prevalence of PA, specific genetic alterations involved in its pathogenesis had been identified only for the rare familial forms of the disease mainly familial hyperaldosteronism (FH) type 1. This form of PA results from an unequal crossing over between the highly homologous *CYP11B2* and *CYP11B1* genes that code for aldosterone synthase and steroid 11 β -hydroxylase, producing a chimeric gene that is under adrenocorticotrophin (ACTH) rather than RAS control (28, 29, 30). Another form of FH with no response to dexamethasone suppression, that is biochemically and clinically similar to sporadic PA, has also been described. This has been designated as FH type 2, being autosomal dominantly transmitted, is considered heterogenous and although no precise molecular basis has been found, there is a suggestion for a link with chromosomal region 7p22 (31). An important breakthrough in the molecular pathogenesis of PA was made by Choi *et al.* (32) by performing whole-genomic sequence identified mutations in the gene encoding the inwardly rectifying potassium channel Kir3.4 (*KCNJ5*) in patients with APAs (30). Subsequently, mutations in three further genes encoding for membrane proteins (the Na/K-ATPase (*ATP1A1*), the Ca ATPase (*ATP2B3*), and Ca1.3 (*CACNAID*)) have been described (33, 34, 35). The altered proteins are associated with Ca influx and/or activated Ca signalling pathways leading to hyperproduction of aldosterone by *CYP11B2* and in some cases zona glomerulosa cell hyperplasia or adenoma formation (28). In addition, *KCNJ5* mutations have been shown to represent the underlying genetic event in FH type 3 (32, 36). Further studies in a large number of patients with APAs revealed that somatic mutations in the *KCNJ5*, *ATP1A1*, *ATP2B3* or *CACNAID* genes are present in more than half of them (~40, 6, 1 and 8% respectively) (31). However, although in all these genetic alterations the end point is Ca influx and membrane depolarisation leading to aldosterone hypersecretion, there are substantial differences in the clinical and biochemical phenotypes related to specific genetic alterations. In particular, patients with PA harbouring *KCNJ5* mutations are more frequently female and generally younger compared with non-mutated patients, suggesting earlier onset and/or more severe disease (37, 38, 39, 40). In addition, in some studies higher aldosterone and lower potassium levels at diagnosis have been described and larger tumours were found compared with non-mutated cases (37, 38, 39, 40, 41). Conversely, in some but not all studies, it has been shown that patients harbouring ATPase mutations are more commonly men

and are associated with the most severe forms of PA and rather smaller size tumours (30, 39). These findings are particularly relevant in the familial form of PA (FH type 3) where specific mutations in the *KCNJ5* gene, as those altering the G151E amino acid, are associated with mild aldosteronism compared with those with *G151R* mutation that have a more severe clinical phenotype (28, 32). Likewise, histopathology is also quite heterogenous ranging from micro- or macronodular hyperplasia to adenoma formation, whereas the adjacent adrenal cortex may be atrophic, diffuse hyperplastic or nodular hyperplastic (37, 42). In particular, subjects with the *G151E* mutation have no hyperplasia in the surrounding tissue because there is increased cell death limiting the glomeruloma mass that is still able to produce aldosterone but does not evolve into progressive disease (28, 37). In contrast to APAs, no *KCNJ5* or *ATP1A1* and *ATP2B3* mutations have been found in 174 and 91 patients with PA due to bilateral adrenal hyperplasia (BAH) respectively (30, 37). More recently a common single-nucleotide polymorphism (c.-2G>C) of the *NR3C2* gene, coding for the MR, has been found to be associated with increased activation of the RAS and increased BP in the general population, connoting vulnerability for hypertension (43).

Evidence in favour of an ACTH role in aldosterone regulation

It is generally accepted that the main regulators of aldosterone secretion are the RAS and serum potassium levels, whereas ACTH is thought to exert a relatively minor effect. Previous studies have shown that chronic low-dose ACTH infusion in humans increases aldosterone levels within the first 12–36 h followed by a slow decline over the next several days (44) with the minimal effective dose of infused ACTH being extremely low (0.03 μ g) (45). Similar data have been obtained from *in vitro* studies that have demonstrated ACTH-responsive effects on the expression of genes responsible for aldosterone production (46, 47). Additional evidence of the ACTH role in aldosterone secretion is derived from patients with ACTH resistance syndrome who develop severe salt-losing adrenal hypoplasia due to loss-of-function mutations of the ACTH receptor (*ACTH-R*) (MC2R) (48). These data suggest that although ACTH may not be the principal regulator, it exerts a significant, at least short-lasting stimulatory effect on aldosterone secretion and a similar genomic effect on adrenal glomerulosa cells. In favour of such a potential ACTH effect is the finding that the highest *ACTH-R* mRNA expression in adrenocortical tumours is found in APAs

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(49, 50, 51). Indeed, aldosterone secretion, although relatively unresponsive to postural studies in such patients, exhibits an ACTH-dependent diurnal rhythm and responds to dexamethasone suppression (52, 53, 54). Moreover, the aldosterone response to ACTH-related physical and/or psychological stress has never been investigated in patients with hypertension. We have recently reported that the cortisol response to ACTH in patients with apparently non-secreting adrenal adenomas is correlated significantly with carotid intima-media thickness (IMT) and flow-mediated dilatation (FMD) in the brachial artery (55). Considering that ACTH is a natural stimulus of aldosterone secretion, similar ACTH-stress mediated alterations in aldosterone secretion could also be expected. This is supported by studies where the effect of graded intensities treadmill exercise (corresponding to 50, 70 and 90% of maximum oxygen uptake, $\text{VO}_{2\text{max}}$) on aldosterone secretion was examined (56, 57). A significant aldosterone response was observed even with mild exercise (50% $\text{VO}_{2\text{max}}$), while the maximum response was observed with moderate exercise (70% $\text{VO}_{2\text{max}}$) (57). During these studies, cortisol levels remained unchanged at mild exercise, suggesting that the adrenal cortex more readily secretes aldosterone than cortisol in response to physical stress. This ACTH-related effect could be further enhanced by the presence of particular polymorphisms of the *CYP11B2* gene associated with increased synthase activity leading to higher aldosterone levels and contributing to increased risk of developing aldosterone-related hypertension and cardiovascular disease in the general population (58, 59).

Considering these data, it can be reasonably assumed that even mild alterations in ACTH secretion, as encountered during physical or psychological daily stresses, can induce ACTH-mediated aldosterone hypersecretion. Indeed, some investigators have recognised this possible ACTH effect on aldosterone secretion from the adrenals as a potential confounding factor that could affect the performance characteristics of currently used confirmatory tests for PA (16). In order to overcome this potential confounding effect, the simultaneous aldosterone and cortisol measurement during the FST has been introduced to ensure that the test is not performed under stressful conditions secondary to ACTH fluctuations (16). Therefore, it is reasonable to suggest that if the diagnostic tests for PA are performed after dexamethasone administration in order to eliminate any ACTH stimulatory effect on aldosterone secretion, their diagnostic accuracy could be substantially improved. Based on this concept, we have modified the traditional FST by adding 2 mg of dexamethasone

(frudrocortisone dexamethasone suppression test (FDST)) at midnight of the 4th day of the test and the SIT by performing the test early in the morning following a low dose dexamethasone suppression test (LDDST) (post dexamethasone-saline infusion test (PD-SIT)) (60, 61). Furthermore, a number of other aberrant eutopic or ectopic membrane-bound G-protein-coupled receptors (GPCRs) have been found to be expressed in the adrenal cortex that could regulate aldosterone hypersecretion by an independent from the RAS albeit not autonomous manner (18). These aberrant GPCRs are expressed in all three steroidogenic zones of the adrenal gland and activate the same cascade of cellular events as ACTH following interaction with the ACTH-R (18). Using microarray analysis of APAs and cortisol-secreting adenomas in comparison with normal adrenals, the GPCRs that were found to have the highest expression were the luteinising hormone (LH) receptor, 5-hydroxy-tryptamine 4-receptor (5HT4R), gonadotrophin-releasing hormone (GNRH) receptor, glutamate receptor metabotropic 3, endothelin receptor type-B-like protein and ACTH-R, whereas their expression was higher in APAs compared with cortisol-secreting adenomas (62). Furthermore, *in vivo* and *in vitro* studies in patients with PA showed that aldosterone secretion was aberrantly regulated by several GPCRs albeit with substantial diversity of the receptors involved; of interest, aldosterone hypersecreting tissues exhibited ectopically expressed *GNRH* (4/15) and thyroid-releasing hormone (*TRH*) (1/15) mRNA that correlated with *in vivo* responses in a subset of patients studied (18, 51, 54). In favour of the role of these receptors are cases of PA that developed during pregnancy and were responsive to LH stimulation (63). The notion that the expression of aberrant receptors may be directly involved in the pathogenesis of PA is attractive as treatment targeting such receptors could have a therapeutic effect. However, the application of non-specific 5HT4R inhibitors (cyproheptadine and ketanserin) (64, 65) had only a transient and not sustainable effect on aldosterone secretion similarly to V1-receptor antagonist (66) and endothelin-1 ETA, B receptor inhibitors administration (67). Considering these findings and the recent advances in molecular pathogenesis of APAs, it is rather unlikely that the expression of aberrant GPCRs is directly linked with the development of at least APAs. However, their presence could potentially have an effect in some of the diagnostic tests of PA, such as the ARR with regard to posture and menstrual cycle in females, saline infusion in the presence of AVPR1A, whereas a link between the expression of such receptors and potassium and calcium channel mutations cannot be excluded.

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Potential limitations of currently employed diagnostic tests of PA

Currently existing cut-offs of both screening and diagnostic tests for PA have been obtained from apparently 'normal' individuals, in whom no imaging studies of the adrenal glands were performed. However, considering the high prevalence of adrenocortical adenomas in the general population (68) and the presence of dysregulated aldosterone secretion in a proportion of them (69), it is possible that individuals with adrenocortical adenomas and subclinical aldosterone excess could have been included among those regarded as 'normal controls'. This view is supported by the findings of our recent study which revealed that seven out of 79 (8.8%) normotensive individuals who served as controls had a single adrenal adenoma on abdominal computerised tomography (CT) (61). In addition, functional studies revealed that four of them had also PA, as their post-FDST aldosterone levels were significantly higher, ranging from 82 to 168 pmol/l, compared with the cut-off level of 74 pmol/l obtained from individuals with normal adrenal imaging (61). Even more, there is evidence suggesting that PA may also be present in normotensive individuals without adrenal adenomas, resulting in even higher aldosterone cut-offs used to exclude PA, and therefore an underestimation of the overall prevalence of PA in patients with hypertension (17). These findings necessitate the adoption of strict selection of a control group by recruiting individuals with normal BP and normal adrenal imaging (60, 61). Furthermore, the interpretation of currently available diagnostic tests could also affect the prevalence of PA. To date the procedure adopted by almost all studies is to estimate the ARR using pre-screened hypertensive populations, in whom the diagnosis of PA has been made by a confirmatory test and/or adrenal venous sampling (AVS). However, this approach may be hampered by the fact that the final diagnosis of PA is highly dependent on the specificity of preceding screening tests, mostly the ARR (70). A more appropriate approach would have been to diagnose PA retrospectively by performing confirmatory testing to both hypertensive and non-hypertensive (control group) populations regardless of the results of a screening test. There is only a single study that has adopted this approach using a community-based sample of 118 Caucasian hypertensive patients (71). The estimated specificity of ARR was found to vary from 71 to 84%, with a concomitant sensitivity ranging from 73 to 93%, whereas the prevalence of PA was found to be 13% (71). However, in that study the diagnosis of PA was only made by measuring 24-h urinary

aldosterone excretion, whereas the control group was not tested for the presence of adrenal adenomas, and the ACTH effect on aldosterone secretion was not considered.

All these factors contribute to the current difficulty in making the diagnosis and the wide variation of the recorded prevalence of PA. In addition, the available diagnostic tools are cumbersome and disease's epidemiology appears to be a continuous spectrum from low-renin hypertension, normotensive forms of normokalaemic PA to hypokalaemic PA, making the establishment of cut-offs used to make the diagnosis rather arbitrary (28, 72).

Prevalence of PA in hypertension and pre-hypertension

Recent epidemiological studies have shown that serum aldosterone and renin levels and the ARR correlate with increased BP and the incidence of hypertension in the general population (73, 74), suggesting that alterations in aldosterone production may be directly related to BP elevation in a significant proportion of hypertensive individuals. As already mentioned, several cross-sectional and prospective studies in unselected hypertensive populations have shown that the prevalence of PA is much higher than previously believed, although it varies significantly between studies ranging from 4.6 to 16.6% when confirmatory tests to diagnose PA were used (Table 2) (5, 6, 7, 8, 21, 71, 75, 76, 77, 78, 79, 80, 81, 82). However, in patients with resistant hypertension, the prevalence of PA is reported to be even higher ranging from 17 to 23% (16, 83). This view of a step-wise increase in the prevalence of PA according to the severity of systolic and diastolic BP elevation (SBP and DBP respectively) is substantiated further by the findings of Mosso *et al.* (77) who reported that the prevalence of PA increases with the severity (stage) of hypertension as defined by the Joint National Committee VI. According to their findings, the estimated prevalence of PA for stage 1 hypertension (SBP 140–159 mmHg, DBP 90–99 mmHg) is 1.99%, for stage 2 (SBP 160–179 mmHg, DBP 100–109 mmHg) 8.02%, and for stage 3 (SBP >180 mmHg, DBP >110 mmHg) 13.2% respectively. In addition, NAHANES from 1999 to 2006 estimated that 30% of adults above 20 years of age have pre-hypertension, which is defined as an untreated SBP of 120–139 mmHg, or an untreated DBP of 80–89 mmHg, and that ~15% of them have secondary hypertension the most common cause being PA (84). This is important as the prevalence of cardiovascular complications associated with PA is disproportionally higher than predicted by BP measurement alone, whereas PA is considered as a

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Table 2 Prevalence of primary aldosteronism in hypertension.

Reference	Number of patients	Threshold of confirmatory test	Prevalence (%)	APA (%)	Origin
(5)	199	FST ALD > 166 pmol/l	8.5	5/17 (29)	Hypertension Unit
(75)	495	FST, ALD > 140 pmol/l	9.2	5/43 (12)	Hypertension Unit
(76)	350	Seated-SIT, ALD > 277 pmol/l	5.0	6/16 (37.5)	Primary Care Clinic
(6)	305	FST, ALD > 140 pmol/l	9.5	1/29 (3.5)	Primary Care Clinic
(7)	300	FST ALD > 166 pmol/l	18	15/49 (31)	Hypertension Unit
(77)	609	FST, ALD > 140 pmol/l	6.1	2/37 (5.4)	Primary Care Clinic
(81)	345	SIT, ALD > 236 pmol/l	14.2		Hypertension Unit
(8) Torino, Italy	7343	SIT, ALD > 140 pmol/l	8.0	170/587 (29)	Hypertension Unit
(8) Rochester, Minnesota	1112	Oral sodium load/post-test 24 h-urinary ALD > 12 µg/day	10.8	34/120 (28)	Endocrine Unit
(8) Singapore	3850	SIT, ALD > 277 pmol/l	4.6	88/177 (50)	Endocrine Unit
(8) Santiago, Chile	914	FST, ALD > 140 pmol/l	7.2	6/66 (9.1)	Endocrine Unit
(71)	118	Oral sodium load/post-test 24 h-urinary ALD > 12 µg/day	13.0		Population-based Cohort
(79)	1125	Captopril test (50 mg)	11.2	54/126 (43)	14 Hypertension Centres
(82)	105	SIT, ALD > 140 pmol/l	0.9		Hypertension Unit
(80)	200	FST, ALD > 160 pmol/l	5.5		
(78)	327	FDST, ALD > 74 pmol/l plus ALD/REN > 32 pmol/mU	29	13/69 (19)	Endocrine Unit

FST, fludrocortisone suppression test; SIT, saline infusion test; FDST, fludrocortisone dexamethasone suppression test.

multisystem disease where aldosterone excess directly or indirectly affects several organs (85).

Many methodological factors may be responsible for this wide variation in the prevalence of PA in patients with hypertension, as they can interfere with renin and/or aldosterone measurements affecting the diagnostic accuracy of both screening and diagnostic tests. Factors such as posture at the time of sampling, serum potassium levels, renin/aldosterone assays employed, renal function, gender, age and use of anti-hypertensive drugs are all known to be implicated and have extensively been analysed in many recent relevant reviews (15, 16). In addition, heterogeneity in clinical expression of genes recently found to be implicated in PA secondary to APAs and/or presence of aberrant receptors could lead to alterations of aldosterone biosynthesis and differences in aldosterone levels (18, 28, 86). Indeed, in the Framingham Heart Study on the aldosterone heritability levels, the proportion of variation in the population that is related to genetic differences was 11% and that of renin's and ARR was 22 and 40% respectively (73).

Evidence in favour of an increased prevalence of PA in hypertension and pre-hypertension

The genetic variability of APAs, the unaccounted ACTH effect in currently existing tests and the absence of a well-defined control group could all operate to shift

the normal cut-offs of both screening and diagnostic tests of PA to higher values leading to a significant underestimation of the prevalence of PA in patients with hypertension. We have recently studied 180 unselected hypertensive patients and compared them with 72 matched non-hypertensive individuals with normal adrenal imaging (61). The diagnosis of PA was confirmed using the FDST, thus eliminating any ACTH effect on aldosterone secretion. This test was performed on both controls and hypertensive patients, regardless of ARR values, and the calculated normal cut-off of basal ARR was found to be 84 pmol/mU. The simultaneous estimation of both post-FDST aldosterone levels and ARR was employed to define the diagnosis of PA, using as normal cut-offs the 74 pmol/l and 32 pmol/mU respectively obtained from the control group (61). Using this approach, the mean post-FDST aldosterone levels and ARR were found to be much higher in hypertensive patients than controls (Fig. 1) and correlated significantly with both SBP and DBP (Fig. 2), whereas the estimated prevalence of PA was found to be 31% (56/180). In contrast, relying solely on basal ARR, only 7.2% (13/180) of the hypertensive individuals were considered to have PA which was confirmed by the FDST in ten of them (10/180, 5.5%). In the remaining three patients, the diagnosis of PA was not confirmed either due to an unaccounted ACTH surge or due to a false-positive ARR increasing the population requiring unnecessary confirmatory testing to ascertain

Figure 1

Basal and post-FDST aldosterone levels (ALD) and aldosterone:renin ratio (ARR) in controls (C) and hypertensives (H).

the diagnosis. The sensitivity and specificity of basal ARR were found to be 18 and 98% respectively, considerably different from those published so far (16, 70, 87). These findings suggest that the use of basal ARR alone permits the detection of more advanced cases of PA leaving milder forms undiagnosed. A major limitation of this study is that the FDST was not compared directly with the classical FST in order to document that the addition of dexamethasone indeed improves the performance characteristics of the test. However, when the PD-SIT was compared with the classical SIT in a previous study, a much higher prevalence of PA, 24% vs 11%, was found in 151 patients with adrenal adenomas (60). These results suggest that dexamethasone administration is expected to have a similar beneficial effect on the performance characteristics of FST and support the view that both the ACTH and renin stimulatory effects on aldosterone secretion need to be considered for making the diagnosis (60, 61).

These results have recently been confirmed by expanding our initial study including a much higher number of individuals (327 hypertensive and 100 controls) (78).

However, the normal basal ARR cut-off obtained from the latest study was significantly lower than previously defined (67.5 pmol/mU vs 84 pmol/mU), resulting in a higher sensitivity (35% vs 7.2%). Similarly, the cut-off value of post-FDST ARR was also lower (26 pmol/mU vs 32 pmol/mU), whereas the post-FDST aldosterone levels were higher (82 pmol/l vs 74 pmol/l) albeit without alteration in the prevalence of PA that was found to be 29% (78). The observed different test characteristics in the last study may be attributed to the higher number of controls included and the stricter selection criteria adopted. We subsequently followed up 122 non-hypertensive individuals, 100 of whom have completed a mean follow-up period of 5 years (17). Thirteen of these individuals had findings consistent with PA at initial assessment and 11 (85%) developed hypertension, whereas only 20 of the 87 individuals without PA (23%) developed hypertension (odds ratio: 18.42, 95% CI: 3.76–90.10, P : 0.0001) (Fig. 3). On the basis of these findings, only individuals who remained normotensive during the

Figure 2

Linear relationship between systolic/diastolic blood pressure (SBP, DBP) and the post-FDST aldosterone levels (ALD) and aldosterone:renin ratio (ARR).

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Figure 3

Box and whisker-Tukey plot. Post-FDST aldosterone levels and aldosterone:renin ratio in all subgroups of controls ($n=100$) who were followed up for a mean of 5 years are shown. G-4 is the group of controls without PA who remained normotensive ($n=67$); G-1 is the group of controls with PA ($n=11$) who developed hypertension; G-3 is the group of controls without PA ($n=20$) who developed hypertension. The controls with PA (G-2, $n=2$) who remained normotensive are indicated by the two black circles. The horizontal lines represent the mean values, whereas the vertical ones represent the 2.5–97.5 percentiles.

follow-up period were used to define the new tests, characteristics and estimate the prevalence of PA in patients with hypertension. Sixty-nine patients who were considered to have PA were treated with MRAs, obtaining a significant reduction in both SBP and DBP levels (mean reduction 28 ± 18 and 14 ± 10 mmHg respectively).

Indirect data for MR activation in patients with hypertension has previously been reported by Levy *et al.* (88). In that double-blind, randomised, multicentre and placebo-controlled study of 397 patients with mild-to-moderate hypertension, the anti-hypertensive effect of titrated doses of eplerenone (50–200 mg/day) was evaluated. Approximately, 80% of patients obtained adequate BP control, with the majority using the lowest dose, and only 20% failed to respond (88). Although the presence of PA was not evaluated, these results highly suggest that MR activation may be implicated in the development of hypertension as eplerenone is a highly selective MR antagonist. Other studies have also shown that eplerenone or spironolactone, either alone or in combination with other anti-hypertensive drugs, are both highly effective in improving arterial BP, renal function, arterial stiffness and left ventricular function in patients with hypertension (89, 90). Although effectiveness of MRA could be related to relative or absolute aldosterone excess, cortisol could also be implicated as an additional MR

activator, as in some tissues such as the heart, the activity of 11β -hydroxysteroid dehydrogenase type 2 is too low (91). However, this notion remains to be investigated by further studies.

Considering that resistant hypertension is found in up to 16% (92) of hypertensive patients, it is prudent to suggest that with the currently employed diagnostic procedures a significant proportion of patients with PA remain undiagnosed. However, the wide application of confirmatory tests to reveal states of altered aldosterone dynamics in patients with hypertension although clinically meaningful is cost-ineffective. This view has previously been put forward by Funder (93) who proposed an alternative approach by introducing low-dose MRAs as part of first-line therapy for hypertension, reserving the use of screening and diagnostic tests for selected hypertensive populations.

Although the natural history of PA is well known, there is limited information regarding its evolution. It is reasonable to consider that PA develops from an initially small-sized lesion with undetectable (by current means) dysregulated aldosterone secretion which then evolves over time into the full biochemical and clinical phenotype of PA. During this period, autonomous from the RAS or altered aldosterone secretion may be present as a state of ‘subclinical PA’. Such sub-clinical forms of PA can be expected to exist even in apparently normal non-hypertensive individuals, resembling those found in subclinical primary hyperparathyroidism, hyperthyroidism and hypercortisolism that are also associated with dysregulated albeit within normal limit calcium, thyroid hormone and cortisol levels respectively (94, 95). However, the prevalence of subclinical or established PA in pre-hypertension, and even more its clinical significance, remains largely unknown as aldosterone and renin measurements are not performed routinely in normotensive individuals.

The first case of normotensive PA was described in 1972 in a young non-hypertensive man with a rapidly growing adrenal carcinoma, who was presented with disabling myopathy and severe hypokalaemia (96). Since then, several normotensive cases of PA have been published with hypokalaemia and adrenal adenomas being the most consistent findings (97). We have also recently reported that PA was found in 12% of normotensive and normokalaemic patients with adrenal incidentalomas (98). However, there is increasing evidence that normotensive PA may be found in individuals without hypokalaemia or adrenal adenomas. Stowasser *et al.* (99) have previously described ten patients with familial PA type I, who

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remained normotensive after a 5-year follow-up period. An interesting observation made by the same group was that increased left ventricular wall thicknesses and reduced diastolic function found in eight normotensive patients with genetically proven glucocorticoid-suppressible PA (100). This is the first clinical study showing that cardiac dysfunction is associated with aldosterone excess in the absence of hypertension.

Although this data indicates that PA may be found in pre-hypertension, reliable evidence suggestive of a high prevalence is derived from the Framingham Offspring Study that evaluated the evolution of BP and basal aldosterone levels in 1688 normotensive individuals from 1995 to 1998 (74). Multivariable logistic-regression models based on quartiles of serum aldosterone levels were used to test for a linear trend in the risk of BP outcomes across quartiles. Aldosterone levels were directly associated with an increase in BP and the development of hypertension, with a 17% increase in the risk of hypertension per quartile increment in the serum aldosterone level (74). However, the prevalence of normotensive PA has prospectively been estimated only in our study of 100 normotensive, normokalaemic individuals who were followed up for 5 years; 11 of the 13 diagnosed with dysregulated aldosterone secretion (85%) developed hypertension, compared with only 20 of 87 (23%) without the diagnosis of PA (17). The results of this study in association with the results of the Framingham Offspring and other studies suggest that a sub-clinical form of PA may be present in a substantial proportion of normal individuals causing subtle cardiac dysfunction in the absence of hypertension.

Conclusions

It is now widely recognised that the prevalence of PA is substantially higher than previously believed. The identification and elimination of factors that could affect the performance characteristics of both screening and confirmatory tests of PA can greatly improve their diagnostic accuracy and reveal an even higher prevalence of PA in patients with hypertension. This view is reinforced further by the substantial reduction in arterial BP following the administration of MRAs in unselected hypertensive patients. In addition, the role that unrecognised forms of PA and stress-related aldosterone hypersecretion may exert in the development of hypertension needs to be further clarified. Although further studies are needed to verify these findings, it is possible that MR activation may be commonly related to the development of arterial hypertension.

Declaration of interest

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

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