A practical approach to diagnosing endocrine hypertension

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Abstract

Hypertension is a leading cardiovascular risk factor that remains difficult to manage in a large segment of the population. Secondary causes of hypertension, which are amenable to targeted treatment or even cure, may contribute to poor blood pressure control. The most common endocrine cause, primary aldosteronism, requires biochemical screening as there are often no symptoms or signs other than hypertension. Screening tests should be done after adequate preparation to optimise the results and minimise the confounding effects of diet and medications. Further testing beyond the initial screen generally requires input from specialist units to coordinate confirmatory tests and radiological procedures. Other less common or even rare endocrine causes of hypertension generally present with symptoms and signs of pituitary, adrenal, thyroid or parathyroid dysfunction rather than hypertension per se. Here, we review the endocrine causes of hypertension and provide a practical approach to their diagnosis.

SUMMARY AT A GLANCE

This review provides an up-to-date and practical clinical perspective to the diagnosis of endocrine causes of hypertension. Aldosteronism, Cushing’s Syndrome, pheochromocytoma acromegaly, thyroid dysfunction and hyperparathyroidism are considered.

Key words

Endocrine hypertension, primary aldosteronism, cushing’s syndrome, pheochromocytoma, subclinical cushing’s
Introduction

Hypertension has long been recognized as a major cardiovascular risk factor. Multiple clinical trials have demonstrated the benefits of treating hypertension with the SPRINT trial showing that intensive lowering of blood pressure significantly reduced rates of cardiovascular end points and the risk of death (1, 2). However, hypertension remains poorly managed. In Australia, of the 6 million adult hypertensive patients, 68% or 4.1 million are poorly controlled (ABS, National Health Survey: First Results 2014-2015). A contributing factor to inadequate blood pressure control may be undiagnosed secondary hypertension.

Secondary hypertension affects 5 – 10% of the general hypertensive population based on conservative estimates but could be more common than currently recognized (3, 4). Identifiable aetiologies can be broadly categorized into renal (renal artery stenosis, renal parenchymal disease), cardiovascular (coarctation of the aorta), endocrine (predominantly primary aldosteronism; less commonly Cushing’s syndrome and phaeochromocytoma; rarely thyroid dysfunction, acromegaly and primary hyperparathyroidism) and obstructive sleep apnoea. Routine biochemical tests and specialised radiological procedures used to investigate hypertension have been recently reviewed (5). The current review will focus on endocrine hypertension, with primary aldosteronism being the most common by far and the least likely to cause specific symptoms, and provide a practical approach to their diagnosis.

Primary aldosteronism

Primary aldosteronism (PA) was first described in 1955 by Jerome W. Conn, as a constellation of hypertension, hypokalemia, metabolic alkalosis and neuromuscular symptoms associated with increased levels of aldosterone (6). With improved diagnostic methods, it is now recognized that the symptoms described by Conn, aside from hypertension,
are only present in the minority of PA. Hypokalemia, for example, is only present in 20 – 25% of patients with PA when it was once thought to be a hallmark of the condition (7).

Biosynthesis of aldosterone from the adrenal zona glomerulosa is primarily regulated by the renin-angiotensin system, extracellular potassium concentration, and to a lesser extent, adrenocorticotropic hormone (ACTH) (8). The main effects of aldosterone are mediated by the mineralocorticoid receptor (MR) in the epithelial cells of the renal collecting duct (9). MR activation promotes sodium reabsorption, mainly via the epithelial sodium channel, and potassium excretion via Na+/K+-ATPase. Hydrogen ion loss also occurs which can cause a metabolic alkalosis. In PA, aldosterone production is autonomous, thereby leading to normal or elevated aldosterone levels that are not suppressible by sodium loading or volume expansion, together with low or suppressed renin.

PA is now recognized as the most common specifically treatable cause of hypertension with a prevalence ranging from 4.6% to 13.0% in patients with hypertension and up to 20% in those with refractory hypertension (10-13). Early detection is vital as undiagnosed and untreated PA is associated with increased cardiovascular, renal and metabolic morbidity and mortality related specifically to the effect of aldosterone excess on the MR in a range of target tissues (14, 15-17). Furthermore, treatment outcomes for PA are better in patients with a shorter duration of disease (18, 19). The early detection of PA crucially depends on screening by general practitioners who are the first port of call for hypertensive patients. However, a recent study performed in German and Italian general practitioners demonstrated a surprisingly low knowledge of PA and its diagnostic guidelines, resulting in substantial under-diagnosis of this disease (20).

PA is a heterogeneous collection of conditions, including aldosterone-producing adenoma (APA, 35% prevalence), bilateral adrenal hyperplasia (BAH, 60%), unilateral adrenal
hyperplasia (2%), aldosterone-producing adrenocortical carcinoma (< 1%), ectopic APA (< 1%) and familial hyperaldosteronism including Type I (< 1%), type II (1.2 - 6%), type III (rare) and type IV (rare) (21-23), and PASNA (primary aldosteronism, seizures, and neurological abnormalities), a rare syndrome featuring PA and neuromuscular abnormalities (24). APA and BAH constitute the majority of cases of PA although the exact proportion attributed to each condition depends on the availability of adrenal vein sampling (AVS), a diagnostic procedure used to lateralize aldosterone secretion. APA can be cured surgically and is therefore important to distinguish from BAH which is treated medically.

Screening for primary aldosteronism

Current international PA management guidelines recommend case detection for PA using the aldosterone to renin ratio (ARR) in the following groups (25):

a) Sustained BP > 150/100 on three measurements obtained on different days, or drug-resistant hypertension (BP >140/90 despite treatment with 3 anti-hypertensive medications), or controlled hypertension (BP < 140/90) on 4 or more anti-hypertensive medications;

b) hypertension and spontaneous or diuretic-induced hypokalemia;

c) hypertension with adrenal incidentaloma;

d) hypertension and obstructive sleep apnoea;

e) hypertension and a family history of early onset hypertension or cerebrovascular accident at a young age (< 40 y); and

f) all hypertensive first-degree relatives of patients with PA
The ARR was introduced in 1981 (26) and remains the recommended screening test for PA on the basis that isolated measurements of either plasma aldosterone concentration (PAC), direct renin concentration (DRC) or plasma renin activity (PRA) show broad overlap between normal patients and those with PA (26, 27). The ratio is more robust and less affected by diurnal variations and postural changes than individual measurements of PAC, DRC or PRA (28). Despite its routine use, there are laboratory-based analytical issues in the measurement of ARR. Plasma aldosterone is generally measured by radioimmunoassay which can be affected by antibody cross-reactivity with aldosterone metabolites, especially when using automated immunoassay platforms (29). DRC assays may also display cross-reactivity with non-target molecules such as pro-renin while PRA assays are subject to variability in the incubation period and non-standardized approach to angiotensinase inhibition (29).

Developments in high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) have allowed aldosterone and renin activity to be quantified in a more reproducible manner (30-33). An even more high-throughput method, immuno-MALDI (iMALDI)-based assay, has been proposed for PRA determination (34). However, the challenges of LC-MS assay development and calibration, as well as the substantial cost, means radioimmunoassays remain the predominant methodology.

At present, there are no firm recommendations for ARR cutoffs due to the variability of assays between laboratories and a range of units for measurement. PAC is reported as pg/mL, ng/dL, ng/L and pmol/L while PRA can be reported in ng/mL/h, pmol/L/min, nmol/L/h and DRC in ng/L or mU/L. As a result, published ARR thresholds appear widely discrepant. Furthermore, differences in patient populations and other confounding factors prevent generalization about the most appropriate ARR with thresholds between studies varying by more than 10-fold (35). As a guide, suggested ranges rather than definitive values are included in the international guidelines as thresholds for positive screening (25):
Recent studies performed with DRC (instead of PRA) measured by chemiluminescent immunoassays indicate that the ARR cut-off should be much lower than that indicated by the guidelines, that is, between 1.12 and 3.7 ng/dL:mU/L (36, 37). At the authors’ centre, ARR > 70 pmol/L:mU/L (PAC/DRC) is considered a positive screening test, leading to further confirmation testing.

Some centers also require an elevated PAC (usually > 410 pmol/L or > 15 ng/dL) as part of positive screening for PA, arguing that the ARR is very sensitive to the renin level and may be falsely elevated in patients with lowered renin, such as the elderly and the black African-American populations (38-42). However, while the addition of a threshold aldosterone level will increase the specificity of the ARR, it will reduce the sensitivity of the screening process (43). For example, 19% of patients with an APA and 43% of patients with BAH had PAC < 410 pmol/L amongst 63 patients with confirmed PA from the Princess Alexandra Hospital Hypertension Unit series (44). Therefore a threshold aldosterone level is not universally incorporated into the screening of PA. However, the aldosterone level should be at least higher than the cut-off for the subsequent confirmatory test (eg. >140 pmol/L or 5 ng/dL). Alternative diagnoses for patients with “positive ARR” in the context of a low aldosterone level and suppressed renin are discussed later.
Multiple factors including diet, posture and concomitant medications can significantly affect plasma aldosterone and renin, as summarized in Table 1 (7, 43, 45-47).

For an optimal result, patients should be carefully prepared before having their blood taken for an ARR. The same preparation is required before later confirmatory testing and subtype evaluation. Some salient points include (39, 54):

a) Correct hypokalemia with an aim of achieving K+ > 4.0 mM.

b) Encourage a liberal salt intake of around 100 - 150 mmol per day (this represents the average population intake on a normal diet; 6.4 g NaCl = 100 mmol Na+, 1 teaspoon table salt = 2.3 g NaCl).

c) Blood should be collected in the morning before 10:00 AM, after one hour or more of ambulation.

d) Pre-menopausal women should be advised to have the test during the follicular phase of their cycle (the first nine days from the start of menstruation).

e) Withdraw drugs which strongly interfere with ARR for at least 2 – 4 weeks:
   - spironolactone, eplerenone, amiloride (≥ 4 weeks)
   - potassium-wasting diuretics (≥ 2 weeks)

f) Stop the agents listed below for ≥ 2 weeks if hypertension (and other underlying cardiovascular disease) can be safely managed with alternative medications:
   - \( \beta \)-blockers
   - central alpha-2 agonists (clonidine & methyldopa)
   - non-steroidal anti-inflammatory drugs
   - angiotensin converting enzyme (ACE)-inhibitors
- angiotensin II receptor blockers
- dihydropyridine calcium channel antagonists (eg. amlodipine)

g) Use alternative agents which have less effect on the ARR to control hypertension:
- verapamil (90 – 240 mg daily)
- hydralazine (12.5 – 50 mg BD)
- prazosin (0.5 – 5mg BD or TDS)
- moxonidine (200 – 400 mcg daily): ARR not affected after 4 weeks of treatment in healthy normotensive adult men (55)

In most cases, it is enough to stop only diuretics (in point e) to have a diagnostic ARR and only when the results are still inconclusive that other interfering medications (in point f) should be withdrawn.

In patients with renal impairment, interpretation of the ARR is particularly difficult due to activation of the renin-angiotensin-aldosterone system with worsening renal failure (56). Reduction in renal mass, especially as creatinine clearance drops to < 70 m/min, is accompanied by a significant increase in plasma aldosterone level without a concurrent increase in renin which, if anything, falls due to reducing renin-producing juxtaglomerular cell mass and sodium/fluid retention, thus leading to falsely elevated ARR (57). A significant correlation has been noted between aldosterone level and rate of renal decay in a longitudinal study of diabetic patients (58) while earlier and more prominent renal damage has been noted in patients with PA compared to those with essential hypertension (59). These observation highlight the importance of early screening for PA, before end-organ damage occurs and before renal function deteriorates to the point of confounding test results.
Given the analytical complexities of measuring the ARR and the numerous factors that can influence its level, it is prudent to interpret the ARR in the full clinical context with attention to the medical and family history, severity of hypertension, resistance to antihypertensive medications, absolute aldosterone concentration and the presence of electrolyte disturbances (60). Furthermore, one should not rely on a single ARR but rather demonstrate a repeatedly elevated ARR over time before proceeding to confirmation testing, especially if the ARR is equivocal (61).

Differential diagnosis of suppressed renin in hypertension

Whilst PA is the most common cause of mineralocorticoid-driven hypertension, leading to suppressed renin with normal or elevated aldosterone, there are less common causes of hypertension with a suppressed renin. These are summarized in Table 2 (reviewed in 62).

Confirmatory diagnosis of primary aldosteronism

An elevated ARR is not diagnostic of PA, it is a screening test. The ARR is highly sensitive but not very specific (63, 64). Confirmation of PA requires the demonstration of at least partly autonomous aldosterone production in the presence of manoeuvres designed to suppress aldosterone in order to avoid false positive diagnoses and thus prevent inappropriate adrenal venous sampling or surgery. An argument could be made, however, for bypassing confirmatory testing in hypertensive patients with spontaneous hypokalemia, very suppressed renin levels together with PAC > 550 pmol/L, who may be diagnosed as having PA without further evaluation because of the very low likelihood of having the disorder (25).
For other ARR positive patients, a number of confirmatory tests are available. They include the fludrocortisone suppression test (FST), saline suppression test (SST) and oral salt loading, which all aim to expand plasma volume and hence suppress renin; or the captopril challenge test which aims to suppress aldosterone via inhibition of angiotensin-converting enzyme. Their characteristics, advantages and limitations are summarized in Table 3. Of note, the intravenous SST is traditionally performed in the recumbent position although a recent report suggested that a seated SST was superior for detecting those with bilateral disease (65). This study observed that all cases of bilateral adrenal hyperplasia (n = 11 in a cohort of 24 patients with PA) which tested positive with a FST and seated SST had post-saline aldosterone levels below the diagnostic threshold of 140 pmol/L for a recumbent SST. Further studies are required to substantiate this result. Currently there is no universal gold standard; it is recommended that a test, or even two confirmatory tests according to the Japanese Endocrine Society guidelines, should be selected based on patient status, compliance, cost and local laboratory expertise (64, 66).

**Subtyping of primary aldosteronism**

Once PA is diagnosed based on confirmed autonomous aldosterone production, further testing is required to identify its subtype. Unilateral disease in the form of APA is important to distinguish from bilateral disease such as BAH as it is surgically curable. For patients who are not suitable surgical candidates, treatment should proceed as for BAH without further testing. Young patients with PA or those with a family history of hypertension and early stroke should also be tested for familial hyperaldosteronism Type 1, also known as glucocorticoid remediable aldosteronism (GRA). The diagnosis can be reliably made by genetic testing for a hybrid gene mutation composed of 11β-hydroxylase gene regulatory
sequences and aldosterone synthase gene coding sequences, and which is regulated by ACTH (77, 78). If diagnosed, family members should be screened for GRA as it is inherited in an autosomal dominant fashion. Identification of this subtype of PA is important as the aldosteronism and hypertension can be readily controlled with low dose glucocorticoids (e.g. dexamethasone) which suppress expression of the hybrid gene. Early identification of GRA in pre-hypertensive stages may permit the early institution of dietary sodium restriction to delay the onset of hypertension and other sequelae (7).

All patients with PA who are potential surgical candidates should undergo a triple-phase adrenal CT scan to assist in subtype differentiation and to exclude large masses which may represent adrenal carcinoma. An MRI is slightly more sensitive but less specific and subject to motion artifact (79). However, there are limitations to the use of CT as the sole test to differentiate between unilateral and bilateral disease as small adrenal adenomas < 1 cm may go undetected or be interpreted incorrectly as adrenal hyperplasia, while areas of hyperplasia may be called adenomas. Furthermore non-functioning adrenal incidentalomas are not uncommon, especially in patients over 35 - 40 years of age and are indistinguishable radiologically from functioning adrenal adenomas.

Currently the most reliable method to differentiate unilateral from bilateral PA preoperatively is by adrenal vein sampling (AVS)(61). During AVS, adrenal and peripheral veins are sequentially or simultaneously catheterized through a percutaneous femoral vein approach under fluoroscopic guidance. Small amounts of contrast are injected to guide the catheter through difficult anatomy and to confirm catheter tip location. Blood is gently aspirated from the veins, accurately labelled and assayed for aldosterone and cortisol concentrations. The cortisol measurements are used to confirm successful cannulation of the adrenal vein and to correct the aldosterone measurements for dilutional effects. Calculations are done based on
left and right-sided adrenal and peripheral vein aldosterone/cortisol measurements to determine if there is unilateral dominance of aldosterone production.

AVS is technically challenging, especially catheterization of the right adrenal vein which is smaller than the left adrenal vein and empties directly into the inferior vena cava posteriorly at a right angle (80). Whilst an adrenal CT helps to define the anatomy of the adrenal veins, there is still considerable anatomic variability (63). The use of CT during angiography to aid catheter positioning has been reported to improve cannulation success (81, 82) while intra-procedural rapid cortisol analysis to confirm successful cannulation is also useful but has limited availability at most centers (83-85). Another factor that can significantly improve cannulation success rates is the focused expertise of one or two dedicated interventional radiologists who are responsible for all AVS at a large tertiary center (86-88). Success rate improves dramatically with the experience of the angiographer and can reach up to 96% in centers with a significant case load (89-91). At the authors’ center, one dedicated radiologist has improved the success rate of AVS cannulation from 40% in 2009 to 70% in 2014 (92), and in 2017 it has been 100%. Overall, AVS is a safe procedure with < 1% complication rate; even adrenal hemorrhage has a benign outcome causing either no or minor effects on adrenal function in a multicentric study (93).

The importance of AVS is highlighted by the poor concordance between the findings of adrenal CT and AVS (44, 89, 91, 94, 95) with one study showing that CT detected fewer than 25% of adrenal adenomas smaller than 1 cm and only correctly identified the adenoma in 53% (90). A review of 38 studies that compared localization by CT/MRI and AVS found that in 37.8% of 950 patients, the MRI/CT results disagreed with AVS results. Based on CT/MRI alone, surgery would have been done unnecessarily for bilateral disease in 14.6% of patients, medical treatment would have been given for unilateral disease in 19.1% and removal of the wrong adrenal would have occurred in 3.9% (96). A recent controversial study found that
despite the higher risk of incorrect subtyping by CT, there was little BP difference at 12 months’ follow-up in patients managed according to CT versus AVS (97). However, this study is limited by selection bias, lack of power, relatively short follow-up and multiple other analytical issues (98). A more stringent clinical trial is required before firm conclusions can be drawn about the adequacy of CT alone for PA subtyping and management.

Some centers do not advocate AVS for young patients with a solitary unilateral adrenal adenoma > 1 cm on CT (87, 90, 99), citing the incidence of adrenal incidentalomas of < 1% in those younger than 40 yr (100). This recommendation is supported by several studies where 100% of the young patients (< 35 or 40 yr) with unilateral adrenal nodules > 1 cm were found to have APAs, although the total number of young patients was limited to < 10% of each case series (101-103). This information should be provided to young patients who may choose to proceed to surgery without AVS.

**Summary of diagnosis of primary aldosteronism**

Screening for PA is a most worthwhile exercise in the investigation of hypertension as it is the commonest endocrine cause accounting for up to 13% of patients with hypertension in the community. Screening requires adequate patient preparation to achieve optimal results. It is ideal to screen patients before they are medicated to avoid the confounding effect of various antihypertensive drugs. If the patient screens positive, referral to a specialist unit (endocrinology or endocrine hypertension clinics) for confirmatory testing and subtype analysis is prudent. The diagnostic processes have continued to improve over time, especially in centers with focused expertise, which emphasizes the importance of seeking specialist input when making the diagnosis.
Cushing’s Syndrome – clinical and subclinical

Cushing’s syndrome refers to a state of glucocorticoid excess with multiple deleterious manifestations, such as hypertension, obesity, glucose intolerance; all conferring increased cardiovascular risk (104). The most common cause by far is iatrogenic from exogenous glucocorticoids, which initially needs to be excluded. Cushing’s syndrome is considered a rare endocrine disorder with an incidence of 0.2 – 0.5 per million per year with 80% attributed to ACTH-dependent causes and 20% due to ACTH-independent causes (105). Recent literature on gene mutations in Cushing’s disease such as USP8 and in adrenal Cushing’s syndrome such as ARMC5, PRKACA and PRKAR1A has been reviewed elsewhere (105-109). Initial diagnosis rests on clinical suspicions based on symptoms and signs, followed by multiple biochemical testing prior to any radiologic investigations (105).

Predictive features of Cushing’s syndrome include proximal muscle weakness, thin skin and easy bruising. Other features which suggest Cushing’s syndrome also include rapid weight gain, wide (> 1cm) violaceous striae, dorsocervical adiposity and facial plethora (110, 111). None of the presentations however are pathognomonic of Cushing’s syndrome hence, biochemical tests are required for diagnosis.

Evaluation

Hypertension may be present in up to 80% in adults with endogenous Cushing’s syndrome and the exact mechanisms are not fully understood (112). Significant hypertension with hypokalemia due to over-activation of the mineralocorticoid receptor may be seen with excessive cortisol burden from ectopic ACTH Cushing’s syndrome (113). Although it is a rare cause of endocrine hypertension, Cushing’s syndrome should be considered in the setting of resistant hypertension, hypertension at young age, or hypertension and any of the above-
mentioned predictive features, as well as the presence of adrenal incidentaloma (105, 111).

Biochemical screening includes 24-hour urine free cortisol (UFC) excretion, overnight 1 mg dexamethasone suppression test (DST) and midnight salivary cortisol. The diagnosis is made when overtly abnormal results are obtained with two different first-line tests. A low clinical probability accompanied by one negative screening test makes the diagnosis highly unlikely (105, 111).

**First line screening tests**

The 24-hour UFC is a reliable reflection of serum free cortisol concentration and offers an integrated cortisol secretion profile (111). It is collected in a plain 24-hour bottle and corrected for the urinary creatinine excretion. The UFC has the advantage of not being confounded by the level of cortisol binding globulin (CBG) which determines the serum (total) cortisol level. CBG level is raised by estradiol excess such as in pregnancy or taking oral contraceptive pill, which therefore raises the total serum cortisol (111, 114, 115). This test however, is not recommended for patients with moderate to severe chronic renal impairment due to falsely low levels that may occur (116). It may also be normal in mild cases of Cushing’s syndrome and may be elevated in patients with pseudo-Cushing’s syndrome (see below) (111, 117). The inconvenience and errors in carrying out the actual collection may also limit the utility or accuracy in some patients. Due to this potential variability, it is often recommended that it is done at least twice (111).

The 1 mg DST is a quick, accurate and convenient screening test whereby the patient is instructed to take 2 x 0.5 mg dexamethasone tablet at bedtime (11 pm) and to have an early morning (8 am) cortisol test the following day (105, 111, 118). It has a reported high sensitivity when using cut-off morning cortisol level of < 50 nmol/L, and is therefore a very good test for excluding Cushing’s syndrome (111, 119). Due to high false positive rates,
this test is not suitable for someone on estrogen-containing preparations (see above) or on medications such as anti-epileptics (e.g., carbamazepine) that increase the metabolism of dexamethasone leading to suboptimal serum dexamethasone level for desired adrenal suppression (120). It is the recommended test when evaluating patients with adrenal incidentaloma (see subclinical Cushing’s syndrome below).

The midnight salivary cortisol test evaluates the diurnal rhythm integrity of the hypothalamic-pituitary-adrenal (HPA) axis and it also reflects the free cortisol state in blood (111, 121). Cortisol production dips in the evening reaching a nadir overnight before peaking in early morning. Multiple readings above the normal level (assay-specific and age-specific reference range) suggest Cushing's syndrome and it is regarded to be as accurate as the UFC (111, 114, 118, 122). It has the advantage of being a convenient and easy test to perform; salivary samples can be collected on successive nights and stored in the fridge prior to submitting them to the laboratory due to the relative stability of the salivary cortisol (123, 124). It is also suitable for patients whom DST is not suitable or reliable (i.e., in pregnancy, on anti-epileptics) as it reflects the serum free cortisol level (114). However salivary cortisol measurements can be variable and may be influenced by recent food intake, cigarette smoking, and vigorous exercise (125-127). Therefore, avoidance of such factors prior to salivary collection and multiple sample collections are necessary for consistent and accurate results. It is also not suitable for evaluation in shift workers who are likely to have altered diurnal pattern.

**Definitive diagnosis**

Positive results in at least two of the three first-line tests should alert the clinician to the probability of Cushing's syndrome after excluding exogenous glucocorticoids which can occasionally be challenging to identify. We recommend a referral to an endocrinologist at this stage of evaluation for further diagnostics and management.
The endocrinologist may choose to repeat the first-line tests, arrange for further diagnostic tests such as the 2mg daily DST over 48 hours or the intravenous 4 mg DST, or pursue the differential diagnosis of Cushing’s syndrome (111, 128). Practices differ among centres; while some will be satisfied with two unequivocally positive different first-line screening tests, others including our centre will routinely pursue a more intensive and supervised diagnostic DST. Our centre performs the supervised, orally administered 2 mg daily (0.5 mg 4 times daily) DST over 48 hours, employing a cut-off cortisol of < 50 nmol/L at 48 hours (111). A diagnosis of Cushing’s syndrome has significant ramifications in terms of downstream invasive evaluation and treatment.

Once Cushing’s syndrome is diagnosed, the next step will be to investigate the cause of cortisol hypersecretion. A suppressed/undetectable plasma ACTH level (ACTH-independent) will suggest an adrenal form of Cushing’s and an adrenal CT will usually be performed to detect a unilateral adrenal mass or bilateral adrenal hyperplasia. Conversely, a normal or elevated ACTH suggests ACTH-dependent Cushing’s syndrome (Cushing’s disease or ectopic ACTH syndrome), which warrants a high dose DST (the 8 mg daily 48-h DST, using a > 50% suppression of cortisol from baseline level at the end of 48 hours to classify as Cushing’s disease), MRI of the pituitary or inferior petrosal sampling to localize the source of the ACTH hypersecretion (129).

**Physiological cortisol hypersecretion (pseudo-Cushing’s syndrome)**

This is a state of hypercortisolism in which patients may present with some features of Cushing’s syndrome (rarely cutaneous and muscular features) and mildly elevated (equivocal) biochemical results. This may be observed in a variety of conditions such as pregnancy,
chronic pain, obesity, and depression (111). Routine screening for Cushing’s syndrome is not recommended unless objective features (see above) are observed.

Subclinical Cushing’s syndrome (autonomous cortisol secretion)

This refers to biochemical hypercortisolism without the objective clinical manifestation of Cushing’s syndrome, and should be considered in the context of hypertension together with an adrenal mass lesion on CT or MRI (130, 131). The true nature of this condition remains controversial with a lack of consensus on the diagnostic criteria. Studies have demonstrated increased rate of hypertension, diabetes, atherosclerosis, osteoporosis as well as mortality associated with subclinical Cushing’s syndrome (130, 132-135). However, it is still uncertain whether treatment by unilateral adrenalectomy reduces mortality rate as compared to the benefits seen with hypertension and diabetes (132, 136). The first-line test of choice is the 1 mg DST as the UFC and the salivary cortisol test may not demonstrate sufficient sensitivity to detect subclinical Cushing’s syndrome (131, 137). There have been several proposed diagnostic criteria: positive 1 mg DST > 83 nmol/L with any one of low/suppressed ACTH, elevated 24h-UFC or midnight salivary cortisol, or a sole post 1 mg DST of > 138 nmol/L (138, 139). The combination of 1 mg DST > 83 nmol/L, ACTH below 10 pg/ml (2.2 pmol/liter) and elevated UFC showed the best balance between sensitivity and specificity in the evaluation of subclinical Cushing’s syndrome (138-140). A recent clinical practice guideline from the European Society for Endocrinology suggested that for a patient without overt Cushing’s syndrome, ‘autonomous cortisol secretion’ refers to cortisol level post 1mg DST of > 138 nmol/L, and between 51 – 138 nmol/L as ‘possible autonomous cortisol secretion’ (131). However, the guideline also suggested follow-up accessory tests such as ACTH level, UFC and midnight salivary cortisol. The use of calculated age- and gender-specific DHEAS ratios (derived by dividing the DHEAS by the lower limit of the respective reference range) has gathered interest as a non-inferior test to the DST (141, 142).
Pheochromocytomas and paragangliomas (PPGL)

Pheochromocytomas and paragangliomas (PPGL) are rare neuroendocrine tumours associated with hypertension due to the autonomous production of catecholamines such as adrenaline and noradrenaline (143). Phaeochromocytomas are derived from the chromaffin cells of the adrenal medulla whereas paragangliomas arise from the sympathetic ganglia, but the clinical presentations may be identical (143, 144). PPGL have an estimated annual incidence of 0.5 – 0.8 per 100,000 person-years and probably account for 0.2 – 0.6% of hypertensive individuals (143, 145, 146). While most are sporadic disease presenting in mid-life, underlying genetic mutations are common and approximately 30% are germline which often manifest as bilateral adrenal disease (143, 147). The germline mutations most commonly associated with PPGL are in the Von-Hippel Landau (VHL), RET proto-oncogene, Neurofibromatosis type 1 (NF-1) and genes encoding components of the succinate dehydrogenase (SDH) complex (143). In depth discussion on the increasing discovery of other mutations associated with PPGL is beyond the scope of this review.

Clinical features of PPGL may include hypertension which may be labile or paroxysmal, with non-specific headaches, ‘funny’ turns and dizziness, hyperhidrosis, anxiety and tachycardia (148, 149). Some may also be asymptomatic and present with an incidental adrenal mass on imaging for other reasons or with extreme hypertension at induction of anaesthesia (149, 150). It is therefore not uncommon for the disease to be diagnosed late with radiological finding of an adrenal tumour more than 4 cm (151). Indications for screening include onset of hypertension, especially resistant hypertension at a young age, symptoms of PPGL as illustrated above, adrenal incidentaloma, family history of PPGL and paroxysms in response to stimuli such as general anaesthesia, beta-blockers (if not associated with alpha-blockade due to catecholamine binding to α1-adrenargic receptors) and sympathomimetics (143).
Diagnosis: The test of choice to exclude PPGL is plasma free metanephrines taken in supine position, which measures the metabolites of catecholamines and is widely regarded as the most sensitive test of 96 – 100% (143, 152). A negative result effectively rules out PPGL. The “metanephrines” test collectively consists of normetanephrine (metabolite of noradrenaline), metanephrine (metabolite of adrenaline), and methoxytyramine (metabolite of dopamine). In our centre, we measure the plasma free metanephrines by LC-MS/MS as recommended by the Endocrine Society guideline 2014 (143). The specificity for plasma free metanephrines, although more variable from various centres, is regarded as non-inferior to other tests in the Endocrine Society guideline (143). However, it is notable that some had found much lower specificity of 85%, thus creating higher false positive result rates especially if plasma free metanephrines are less than four times the upper range of normal level (153-156). False positive results can be caused by interfering medications (i.e. sympathomimetic agents, clozapine and other psychotics, withdrawal from clonidine, tricyclic antidepressants), stress and illnesses, and inappropriate sampling in an upright position (143, 155, 157, 158). Interfering medications should be withheld (if and when safe and appropriate) for 2-3 weeks prior to biochemical testing. A mildly positive plasma metanephrine in a low-risk individual does not indicate PPGL; whereas in a high-risk individual it may, which includes one with a family history or previous history of PPGL, or one who have had highly suspicious features of PPGL on imaging (153, 154). We therefore recommend a referral to an endocrinologist when positive metanephrine test is encountered.

The 24-hour urine fractionated metanephrines, although more cumbersome, may offer a higher specificity and thus less false positive results in some centres (153, 154). At Mayo Clinic, it is the initial test of choice evaluating low-risk individual with suggestive symptoms. It may also serve as a useful test to verify a borderline positive plasma metanephrines test (156). The urine metanephrines undergo acid hydrolysis when collected in an acid container,
therefore yielding free metanephrines for measurement by LC-MS/MS (159). This test has superseded the 24-hour urine catecholamines and vanillylmandelic acid, which have been reported to have higher false negative rates (160).

False positive result with plasma free or urine fractionated metanephrines is a significant problem which can be difficult to resolve. The Clonidine suppression test had been proposed to differentiate between true and false positive in borderline elevated plasma metanephrines although proper validation of this test has not been performed (143, 155). Other centres utilize chromogranin A levels (also subject to multiple interfering factors, most commonly proton pump inhibitors and renal impairment), urine fractionated metanephrines or repeated plasma free metanephrines measurements at planned intervals to follow-up the less than convincing elevated plasma metanephrines (143, 156). Some patients with PPGL may present with normal or borderline elevated metanephrines in early disease, thus adding to the difficulty of interpreting any positive results (153).

Imaging is recommended after biochemical confirmation of PPGL. Contrast CT or MRI of abdomen and pelvis is a reasonable approach due to the high sensitivity (88 – 100%) of detecting PPGL (143, 161-163). CT has a higher spatial resolution compared to MRI and the use of contrast with CT is quite safe with PPGL (164). MRI may be superior for detecting metastatic PPGL and useful in patients when radiation exposure is contraindicated (143, 165).

Functional imaging is useful in patients with increased risk of extra-adrenal or metastatic disease, such as those with large primary tumours, bilateral adrenal tumours or paragangliomas (143). 123-I MIBG scintigraphy, a compound resembling noradrenaline, is commonly used for this purpose with a sensitivity of 75 – 95% and specificity of 70 – 100 % (143, 166, 167). Iodine administration prior to MIBG is recommended for thyroid protection. While it may be regarded as superfluous in the evaluation of sporadic single adrenal
phaeochromocytoma by conventional imaging, some centres including ours still include this test as part of the routine work-up for PPGL to exclude possible bilateral or extra-adrenal disease. There are however several limitations to this test. Normal adrenal glands do take up MIBG; therefore unless corroborated by structural imaging and biochemistry, the findings may be misleading (143). It has lower sensitivity for metastatic or recurrent PPGL, especially for SDHB-related PPGL (168-171). Studies have demonstrated that 18F-FDOPA PET to be superior to 123-I MIBG for localising PPGL (172). Recently, 18F-FDOPA PET/CT was shown to be excellent in detecting sporadic PPGL as well as head and neck PPGL, however there was a higher risk of false negative in SDHx related tumours (173, 174).

The Endocrine Society Guidelines recommend FDG-PET/CT as the functional imaging of choice for metastatic PPGL due to its superior sensitivity (143, 175, 176). More recently, Ga-68 DOTATATE PET/CT has been shown to be either superior or non-inferior to FDG-PET/CT for detection of metastatic PPGL (177, 178).

Genetic testing may be recommended when appropriate in the follow-up of confirmed PPGL and should follow a scheduled protocol based on probability of detection. Features such as young age, strong family history or syndromic presentation, paraganglioma and bilateral adrenal phaeochromocytoma warrant investigation of underlying genetic mutations; and several algorithms had been published for guidance (143, 179, 180). We recommend a referral to an endocrinologist and a clinical geneticist for a collaborative management.

**Rare endocrine causes of hypertension – acromegaly, thyroid dysfunction and hyperparathyroidism**

Apart from the adrenal causes of hypertension discussed thus far, other endocrine pathologies rarely present with hypertension. They are discussed here more for interest than practical use.
Acromegaly is a rare, insidious disease, commonly caused by a pituitary adenoma which causes the overproduction of growth hormone (GH) and subsequent increase in insulin-like growth factor 1 (IGF-1). It is associated with a range of comorbidities. Aside from somatic overgrowth evident in hand and feet enlargement, coarse facial features and soft tissue hypertrophy, other common comorbidities include diabetes, dyslipidemia, carpal tunnel syndrome, osteoarthritis and obstructive sleep apnea (181). The most prevalent cardiovascular complications of acromegaly include a cardiomyopathy, characterized by cardiac hypertrophy and diastolic and systolic dysfunction together with arterial hypertension, cardiac rhythm disorders and valve diseases (182). The diagnosis of acromegaly can be made based on an elevated serum IGF-1 level (range is age and laboratory-dependent) and a non-suppressed GH in response to oral glucose tolerance testing (181). GH itself is secreted episodically, hence an isolated GH level has little diagnostic value.

In hyperthyroidism, approximately one third of patients experience systolic hypertension due to a combination of increased heart rate, decreased systemic vascular resistance, and raised cardiac output (183, 184). In contrast, hypothyroid patients tend to have diastolic hypertension due to impaired endothelial function, increased systemic vascular resistance and extracellular volume expansion (185). Reported prevalence of hypertension in hypothyroid patients vary from 3 to 60%, depending on the diagnostic criteria for hypertension, degree of thyroid dysfunction and patient age (186). Hypertension resolves after achieving euthyroidism for both hyper- and hypo-thyroidism. Biochemical assessment includes measurement of the thyroid stimulating hormone (TSH) and free thyroxine (fT4).

Primary hyperparathyroidism (PHTP) is a relatively common endocrine disorder characterized by hypercalcemia resulting from the overproduction of parathyroid hormone (PTH) by parathyroid adenoma or hyperplasia. Mild PHTP is often detected incidentally on biochemical tests while PHTP with significant hypercalcemia may present with polyuria,
polydipsia, abdominal pain, renal colic, osteoporosis with fractures or renal impairment.

Hypertension is observed in 40-80% of patients with PHTP and is associated with lack of nocturnal dipping in at least half (187, 188). Significant reduction in blood pressure is observed after successful parathyroidectomy and normalisation of PTH, but only in some studies (189-191). The mechanisms of hypertension in PHTP are still unclear. There are some reports of an association between PHTP and primary aldosteronism (192, 193), although the increased PTH levels are attributed to calcium loss associated with PA (194). In fact PTH levels return to normal after PA treatment. Biochemical evaluation of PHTP includes measurement of PTH, calcium, phosphate, albumin and vitamin D, as well as 24 hour urinary calcium excretion. In the setting of hypertension, plasma aldosterone and renin should also be checked.

**Conclusion**

The most important endocrine condition to evaluate in the work up of secondary hypertension is primary aldosteronism which often presents without specific symptoms or signs.

Screening with the aldosterone:renin ratio is the crucial first step to diagnosis. Less common endocrine causes of hypertension include cortisol excess and catecholamine excess. The diagnosis can be very rewarding as these conditions are all curable or treatable with targeted therapy, which may significantly reduce the risk of adverse cardiovascular outcomes. Careful preparation for biochemical tests and meticulous interpretation of results are required to ensure an accurate diagnosis and optimal management.
References


172. Fiebrich HB, Brouwers AH, Kerstens MN, Pijl ME, Kema IP, de Jong JR, et al. 6-[F-18]Fluoro-L-dihydroxyphenylalanine positron emission tomography is superior to conventional imaging with (123)I-metaiodobenzylguanidine scintigraphy, computer


<table>
<thead>
<tr>
<th>ARR</th>
<th>Cause</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>False Positive</strong></td>
<td>β-blocker, α-methyldopa, clonidine, non-steroidal anti-inflammatory drugs</td>
<td>Decrease renin</td>
</tr>
<tr>
<td></td>
<td>Renal impairment (48)</td>
<td>Renin levels low due to reduced renin secretory mass and salt/water retention</td>
</tr>
<tr>
<td></td>
<td>Aging (49)</td>
<td>Decreases renin</td>
</tr>
<tr>
<td></td>
<td>Within 1 hour of assuming upright posture from lying</td>
<td>Increase in aldosterone &gt; increase in renin; more accurate ARR after 1-2 hours of upright ambulation</td>
</tr>
<tr>
<td></td>
<td>Luteal phase of menstrual cycle (50)</td>
<td>Increases ARR (only affect DRC, not PRA)</td>
</tr>
<tr>
<td></td>
<td>Combined oral contraceptives containing ethinyl-estradiol and drospirenone (51, 52)</td>
<td>Induce angiotensinogen production by the liver, increase angiotensin II which reduces DRC (PRA unaffected); Drospirenone has MR antagonist properties increases aldosterone and PRA</td>
</tr>
<tr>
<td><strong>False Negative</strong></td>
<td>Severe dietary salt restriction, malignant hypertension, pregnancy</td>
<td>Increase renin activity</td>
</tr>
<tr>
<td></td>
<td>Hypokalemia</td>
<td>Decreases aldosterone secretion</td>
</tr>
<tr>
<td></td>
<td>Potassium-wasting diuretics (thiazides) and potassium-sparing diuretics (spironolactone, eplerenone and amiloride)</td>
<td>Increase renin activity by causing volume contraction and sympathetic nervous system stimulation</td>
</tr>
<tr>
<td></td>
<td>Dihydropyridine calcium channel antagonists (e.g. amlodipine)</td>
<td>Decrease aldosterone level; Increase renin activity</td>
</tr>
<tr>
<td></td>
<td>Angiotensin II receptor blockers, ACE-inhibitors</td>
<td>Profound increase in renin due to interference with negative feedback of AngII on renin production; decrease aldosterone</td>
</tr>
<tr>
<td></td>
<td>Selective serotonin uptake inhibitors (sertraline, escitalopram) (53)</td>
<td>Increase renin activity</td>
</tr>
</tbody>
</table>
Table 2. Differential diagnosis of hypertension with a suppressed renin.

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Renin</th>
<th>Aldo</th>
<th>K⁺</th>
<th>Acid-base</th>
<th>Other features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Congenital adrenal hyperplasia</strong> with 11-β hydroxylase or 17-α hydroxylase deficiency</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>normal</td>
<td>Increased deoxycorticosterone (DOC, potent mineralocorticoid); virilization (only in 11β-hydroxylase deficient females); precocious puberty; cortisol deficiency</td>
</tr>
<tr>
<td><strong>Liddle syndrome</strong>: due to gain-of-function mutation in epithelial sodium channel</td>
<td>low</td>
<td>low</td>
<td>low</td>
<td>Metabolic alkalosis</td>
<td>Family history of early onset hypertension (autosomal dominant); responsive to amiloride but not MR antagonist</td>
</tr>
<tr>
<td><strong>Syndrome of apparent mineralocorticoid excess</strong>: due to mutation in 11β-hydroxysteroid dehydrogenase 2, which prevents cortisol inactivation at the MR</td>
<td>low</td>
<td>low</td>
<td>low</td>
<td>Metabolic alkalosis</td>
<td>Severe forms diagnosed in childhood; autosomal recessive; mild forms diagnosed in adulthood, based on elevated ratio of urinary metabolites of cortisol to cortisone</td>
</tr>
<tr>
<td><strong>Gordon syndrome</strong>: mutations affect thiazide sensitive Na-Cl cotransporter</td>
<td>low</td>
<td>Norm or high</td>
<td>high</td>
<td>Metabolic acidosis</td>
<td>Rare</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Test</th>
<th>Fludrocortisone Suppression Test (FST)</th>
<th>Intravenous Saline Suppression Test</th>
<th>Oral sodium loading</th>
<th>Captopril challenge test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Fludrocortisone acetate 100 mcg QID for 4 days; KCl supplement to keep K(^+) &gt; 4.0 mM; NaCl 1.75g TDS and high sodium diet to keep 24 hr urine Na &gt; 3 mmol/kg. Inpatient for 4 days with measurement of aldosterone and renin at 1000 hr of day 4 (patient seated).</td>
<td>2L normal saline infused over 4 hours, starting at 0800 – 0930 hr. Patient stays recumbent at least 1 hr before and during infusion. Measure aldosterone, cortisol and K(^+) at time 0 (baseline) and 4 hr (completion of saline infusion).</td>
<td>Slow Sodium, 1.8g tds with meals for 3 days, with 24 hr urinary Na(^+) &gt; 200 mmol/day; KCl supplement to keep K(^+) &gt; 4.0 mM; Measure 24 hr urinary aldosterone from morning of day 3 to day 4.</td>
<td>Maintain high sodium intake &gt; 7.6 g NaCl per day. Captopril 25 – 50 mg given orally after sitting/standing for 1 hr, between 0700 – 0900 hr. Measure renin, aldosterone, cortisol at time 0 and 1 – 2 hr after challenge with patient seated.</td>
</tr>
</tbody>
</table>

**Diagnostic PAC, unless specified otherwise for diagnosis (cut-offs differ at various centers around the world)**

- > 6 ng/dL (166 pmol/L) at 1000 hr on day 4
- > 8.13ng/dL (225 pmol/L) after 3 days of FST may also be appropriate (n=48) (67).
- > 5 ng/dL (138 pmol/L), sensitivity 90%, specificity 84% (68).
- > 7 ng/dL (194 pmol/L), sensitivity 88%, specificity 100% (69, 70).
- > 6.75 ng/dL (187 pmol/L) sensitivity 82.6%, specificity 75.1% (11, 71)
- > 10ng/dL (277 pmol/L) makes PA highly likely.

- > 24 hour urinary aldosterone > 12 ug (33.3 nmol)
- > 8.5 ng/dL (240 pmol/L) sensitivity 97% (72)
- > 12 ng/dL (330 pmol/L) or elevated ARR post captopril; but based on only 6 cases (73)
- > 13.9 ng/dL (382 pmol/L) sensitivity 69.6%, specificity of 74% (74)

ARR > 30 (ng/dL:ng/mL/h) (11)

Absence of suppression of aldosterone by 30% from baseline.
<table>
<thead>
<tr>
<th>Pros</th>
<th>Simple and specific. Requires only 4 hours in hospital. Inexpensive. May be more sensitive when done in seated position (65).</th>
<th>Inexpensive.</th>
<th>Suitable for patients with severe hypertension, cardiac failure, cardiac arrhythmia or severe renal insufficiency. Inexpensive.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pros</td>
<td>Very sensitive &amp; specific (63, 68).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cons</td>
<td>Inpatient stay x 4 days is expensive; difficult to maintain serum K⁺. Avoid if severe hypertension, cardiac or renal failure, or arrhythmia</td>
<td>Not as sensitive as FST (63). Avoid if severe hypertension, cardiac or renal failure, or arrhythmia</td>
<td>Urine collection may be incomplete; compliance with salt loading; Avoid if severe hypertension, cardiac or renal failure, or arrhythmia</td>
</tr>
</tbody>
</table>