Review Article

Laboratory Investigation of Primary Aldosteronism

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Abstract
Availability and wider application of the plasma aldosterone/renin ratio (ARR) as a screening test for primary aldosteronism (PA) has led to the recognition that PA is the most common potentially curable and specifically treatable form of hypertension, possibly accounting for as many as 5–13% of patients. Aldosterone excess also has adverse cardiovascular consequences that go above and beyond hypertension development. These findings support the concept that PA plays an important role in cardiovascular disease states and should be systematically sought and specifically treated, and have led to the development of a US Endocrine Society clinical guideline for the detection, diagnosis and management of this condition. Reliable detection requires that interfering factors (including medications known to alter the ratio) are controlled before ARR measurement (or their effects taken into account), and reliable methods such as fludrocortisone suppression testing are used to confirm PA. Because computed tomography frequently misses aldosterone-producing adenomas yet demonstrates non-functioning nodules, adrenal venous sampling is the only dependable way to differentiate unilateral (surgically correctable) from bilateral (usually treated with aldosterone antagonist medications) forms of PA. For the glucocorticoid-remediable form of PA (familial hyperaldosteronism type I), genetic testing for the causative 'hybrid' 11beta-hydroxylase/aldosterone synthase gene has greatly facilitated detection. Laboratory assessment (including suppression testing post-operatively, and renin measurement during treatment with aldosterone antagonist medications) can assist in assessing therapeutic responses and in guiding ongoing management. Development of new, highly reliable high-throughput mass spectrometric methods for measuring aldosterone and renin should further enhance detection and reliability of diagnostic workup for PA.

Introduction

In PA, production of the salt-retaining hormone aldosterone by the adrenal cortex is excessive for the body's prevailing sodium and volume status and autonomous of one of its major regulators, renin/angiotensin II (AII), circulating levels of which are usually suppressed.[1] Over time, the resulting excessive retention of sodium at the distal tubule leads to the development of hypertension. In exchange for the retained sodium, potassium and hydrogen ions are excreted, and, if this is prolonged and severe enough, hypokalaemia and metabolic alkalosis may occur.[1] Case detection is of considerable potential benefit to affected individuals. Unilateral adrenalectomy results in cure or improvement in hypertension and correction of hypokalaemia (when present) in patients with unilateral PA and an improvement in quality of life (which is often marked),[2-4] while agents that antagonise aldosterone action have beneficial effects on control of hypertension and hypokalaemia in medically treated patients with PA.[5-7]

Once considered a rare cause of hypertension (accounting for <1% of patients) and not worth looking for unless patients were hypokalaemic, PA has become a much more avidly sought condition in recent times owing principally to two changes in understanding that have occurred over the past few decades.

First, the introduction of the plasma ARR as a screening test by Hiramatsu *et al.* in 1983,[8] and its broader application to include both hypokalaemic and normokalaemic patients (initially advocated by Gordon *et al*. in 1992 after experiencing a marked increase in detection rate after adopting this approach[9]), has led investigators to appreciate that PA is much more common than previously realised, possibly accounting for as many as 5–13% of hypertensives with the great majority being normokalaemic.[10-16]

Second, a large body of experimental and clinical research has demonstrated that aldosterone excess has adverse

consequences (inflammation, remodelling and fibrosis) on cardiovascular and renal tissues which appear to be independent of blood pressure elevation.[17-21] As a result, PA is associated with an excess in cardiovascular morbidity due to stroke, myocardial infarction and arrhythmias[22,23] and increased urinary albumin excretion[20,24] compared to matched essential hypertensives. Importantly, this excess is reversed when patients with PA are given specific treatment directed against aldosterone excess (either unilateral adrenalectomy in the case of unilateral forms of PA, or medications which antagonise aldosterone action) as opposed to non-specific antihypertensive medications.[25] Hence, case detection not only provides an opportunity, through institution of specific surgical or medical treatment, to achieve cure or marked improvement in hypertension control, but also to reverse (or at least limit) additional damage and morbidity due to non-blood pressure-dependent adverse effects of aldosterone excess.

In the wake of these developments, The Endocrine Society has recently published a clinical guideline for the case detection, diagnosis and management of PA which has helped to cement the notion that PA plays an important role in cardiovascular disease states and should be systematically sought and specifically treated.[26]

The current review will focus on the laboratory assessment of PA, from screening and diagnostic confirmation through to subtype differentiation and assessment and monitoring of treatment effect. While it draws primarily on our own experience, it acknowledges some of the alternative views and protocols employed by other groups, and that the choice of approach may vary depending on factors (such as local resources and expertise) relevant to each particular centre.

Screening

Who Should be Screened for PA?

The Endocrine Society guideline recommends the case detection of PA in patient groups with 'relatively high prevalence of PA' including patients with (a) moderate, severe, or resistant hypertension (in whom PA has been reported to be more common than among those with mild hypertension); (b) hypertension and spontaneous or diuretic-induced hypokalaemia; (c) hypertension with adrenal incidentaloma; or (d) hypertension and a family history of early-onset hypertension or cerebrovascular accident at a young age (<40 years) which is encountered in families with the rare, inherited, glucocorticoid-remediable form of PA; and, given that PA exists in familial forms, (e) all hypertensive first-degree relatives of patients with PA.[26] We have argued, however, that a case can be made for screening all hypertensives (that is, even those with mild hypertension who lack hypokalaemia or a significant family history) on the basis that (a) the longer the diagnosis is missed, the more likely patients will suffer irreversible consequences of hypertension and aldosterone excess, and the less likely their hypertension will respond to specific surgical or medical treatment; and (b) screening before commencement of medications avoids the difficulties of ARR interpretation associated with the confounding effects of drugs on aldosterone and renin concentrations (see below).[9,27,28]

Plasma Potassium Measurement

While its presence is highly suggestive of underlying PA, the absence of hypokalaemia certainly does not exclude PA. In most recent series which used the ARR to screen for PA among both hypokalaemic and normokalaemic hypertensives, the prevalence of hypokalaemia among detected patients with PA has been <25%.[6,10,13,15,16,29] A higher proportion (approximately 40–50%) is generally found among those with unilateral, surgically correctable forms of PA including aldosterone-producing adenoma (APA) than in those with bilateral PA (commonly termed 'bilateral adrenal hyperplasia' (BAH); approximately 0–15%), but failure to screen for PA among normokalaemic hypertensives would nevertheless lead to at least half of otherwise detectable APAs being missed.[6,15,30]

Factitious rises in measured potassium concentrations can mask the presence of hypokalaemia when it exists. Steps that can be taken in an attempt to avoid this include (a) measuring potassium in plasma rather than serum (serum levels are usually higher due to release of potassium from the cells during clotting); (b) using fist clenching only to achieve venepuncture; (c) releasing the tourniquet after venepuncture has been achieved; (d) waiting for at least 10 s before gently withdrawing any blood; (e) using a syringe and needle rather than a Vacutainer®, so that blood can be withdrawn in a slow and careful manner, and then, after removing the needle, gently discharged down the side of the opened sample tube; and (f) separating the plasma from the cells within 30 min of collection.[31,32]

Aldosterone/Renin Ratio Measurement

Factors Affecting Reliability of the ARR in Screening for PA

While the ARR is generally considered the most reliable means of screening for PA, its interpretation is not straightforward. Although renin is the main regulator of aldosterone production and plasma levels, other important regulators (such as potassium and ACTH) and changes in hepatic blood flow are also influential. This helps to explain why renin and aldosterone do not always move strictly in parallel in response to physiological manoeuvres and certain medications. Thus false positive and false negative ratios may occur and need to be avoided where possible, as discussed below.

Posture

The assumption of upright posture is associated with a rise in plasma aldosterone.[33] This results partly from an increase in renin, released from juxtaglomerular (JG) cells in response to a fall in renal perfusion pressure associated with the translocation of blood into the lower limbs, causing an increase in sympathetic output and beta adrenergic receptor stimulation.[34] In addition, reduced metabolic clearance of aldosterone occurs due to reduced hepatic blood flow. Because the effect of reduced hepatic clearance is more rapid than that of increased renin, the rise in aldosterone concentrations measured in samples collected before and shortly (less than 1 h) after assuming upright posture may not demonstrate close correlation with the rise in renin. Better correlation between changes in aldosterone and renin levels would be expected in studies using a longer period (at least 2 h) of ambulation.[28,34]

In patients with AII-responsive (AII-R) forms of PA, which includes all with AII-R APA and most with BAH, aldosterone demonstrates normal responsiveness to upright posture, defined as a rise in plasma concentrations of at least 50% above basal.[35,36] Patients with AII-unresponsive (AII-U) forms, including those with AII-U APA or the glucocorticoid-remediable form (familial hyperaldosteronism type I (FH-I)), demonstrate a lack of responsiveness or even a fall, because ACTH (which falls during the early morning hours when these posture studies are carried out) assumes a dominant role over AII in regulating aldosterone in those subtypes of PA.[35,36] It could be anticipated that samples collected for ARR measurement during upright posture may be more sensitive for detecting the AII-R forms, whereas samples collected during recumbency might be more sensitive for detecting the AII-U forms.

In practice, most centres use a mid-morning upright sample, usually after sitting for 5–15 min. These are more convenient than having to provide recumbent conditions for a period such as 1 h, and are likely to be more sensitive overall since the majority (around 70% of patients diagnosed by the Greenslopes Hospital (GHHU) and Princess Alexandra Hospital (PAHHU) Hypertension Units) of patients with PA are AII-R, and, although aldosterone concentrations in the AII-U forms fail to rise in response to upright posture, upright levels are similar to those of patients with AII-R forms (whose recumbent levels are usually much lower).

Time of Day

In patients with PA, whose renin levels are chronically suppressed, plasma aldosterone concentrations are strongly influenced by ACTH levels, which follow a striking circadian pattern with highest levels around 0800 h and falling rapidly thereafter.[37] The ARR is therefore more likely to be elevated in blood collected from patients with PA during the morning rather than in the afternoon.[38]

Dietary Sodium Intake

The stimulatory effect of habitual dietary salt restriction on renin production may lead to a lowering of the ARR.[34,39] Sensitivity of the ratio is therefore improved if patients maintain a liberal dietary salt intake prior to testing.

Plasma Potassium Concentration

Because potassium stimulates aldosterone secretion, hypokalaemia may be associated with false negative ratios in patients with PA.[34,39] This can be avoided by correcting hypokalaemia with supplemental slow-release potassium chloride tablets before ratio measurement. As pointed out above, however, the presence of hypokalaemia can be obscured if care is not taken during sample collection to avoid false elevations of potassium concentrations.[31]

Medications Capable of Causing False Positive Ratios

Because blockade of beta-adrenoceptor-mediated stimulation of renin production by JG cells brings about a profound suppression of renin levels,[40,41] and aldosterone concentrations tend to fall to a lesser degree (possibly because of the continuing stimulatory action of potassium and ACTH), treatment with beta-adrenergic blocking medications has the potential to be associated with false positive ARR values.[28,39-41] Although Mulatero *et al.* found that beta-blockers lead to further elevation in the ARR in some patients with PA,[42] Young has argued that beta-blockers, despite inducing renin suppression, are not likely to lead to false positive ARR values because aldosterone levels fall in parallel.[43] Further evaluation of the effects of these drugs in hypertensive patients without PA is required to settle this issue. Methyldopa[44] and clonidine[45] may have an effect similar to beta-blockers by reducing central sympathetic outflow. Non-steroidal anti-inflammatory agents suppress renin levels by inducing renal sodium and water retention and by suppressing renal prostaglandins which normally stimulate renin release, while at the same time promoting retention of potassium leading to stimulation of aldosterone production and further elevation of the ARR.[46]

Medications Capable of Causing False Negative Ratios

False negatives may be encountered in patients taking medications that stimulate renin production. These include diuretics[34,47] (including potassium-sparing diuretics such as spironolactone, amiloride and triamterene) which all induce volume contraction and sympathetic nervous system stimulation. Dihydropyridine calcium channel antagonists briskly stimulate renin, probably through reflex sympathetic stimulation as blood pressure falls, natriuretic effects and direct stimulation of calcium-dependent renin regulatory

pathways.[42,48] Dihydropyridine calcium antagonists can also reduce aldosterone production by interfering with intracellular, calcium-dependent steps in biosynthesis.[49] Angiotensin converting enzyme (ACE) inhibitors[42] and AII receptor blockers (ARBs)[42] interfere with negative feedback of AII on renin production. Non-potassium-sparing diuretics such as thiazides have the added effect of increasing renal potassium losses and lowering plasma potassium concentrations, leading to reduced aldosterone secretion. ACE inhibitors and ARBs would also be expected to inhibit aldosterone production in patients with AII-R forms of PA.[50]

Other Medications with the Potential to Affect the ARR

The newly introduced renin inhibitors have complex effects on renin levels which depend critically on how renin is measured.[51] These agents are likely to raise the ARR (and cause false positives) if renin is measured as plasma renin activity (PRA), and lower it (causing false negatives) if measured as direct active renin (DAR) concentration.

Contraceptive agents and other oestrogen-containing preparations have generally been thought to have little effect on the ratio when renin has been measured as PRA,[52,53] which measures the rate of generation of angiotensin I from endogenous renin substrate (angiotensinogen). However, patients receiving these agents may theoretically demonstrate falsely elevated ratios when immunometric measurements of DAR are used rather than PRA. Increased hepatic production of angiotensinogen, induced by oestrogen, would result in higher angiotensin I concentrations if renin remained constant, but leads to increased negative feedback by angiotensin-suppressing active renin production.[52-54] This usually prevents PRA from rising significantly in individuals taking these medications but would be expected to lead to suppressed renin concentration and increased ARR if measured using DAR.

Progesterone and some progestogenic agents used in contraceptive preparations have a stimulatory effect on renin and aldosterone levels, probably mediated primarily through their antagonist action at the mineralocorticoid receptor, causing natriuresis. Pizzolo and co-workers recently reported a normotensive 34-year-old female patient who demonstrated a falsely elevated ARR value (with renin measured as DAR) on day 28 of her menstrual cycle while taking a combined preparation of drosperinone (a relatively new progestogenic agent with potent antagonist action at the mineralocorticoid receptor) and ethynyl-oestradiol.[55] In a subsequent study, they reported a mean rise in the ARR in a cohort of 27 women commenced on a combined preparation of gestodene and oestradiol, with the number of positive tests increasing from four to nine.[56] Further work is required to determine whether other contraceptives have clinically significant effects on the ARR and whether choice of renin assay method (PRA versus DAR) will impact on these.

The effects of other commonly used agents (such as antidepressants) which have the potential to affect aldosterone and renin levels (for example, through effects on the sympathetic nervous system and hypothalamic-pituitary-adrenal axis) and thus the ARR, remain largely unexplored and are an area worthy of urgent attention.

Gender and the Menstrual Cycle

In a recently reported study, Pizzolo and co-workers reported a higher proportion of healthy, normotensive women than men (8 of 51 (14%) versus 1 of 43 (2%), $p<0.05$) to have elevated ARR.[56] Furthermore, among 81 hypertensive subjects found to have raised ARR, only 39% of the 54 women (compared with 85% of the 27 men) demonstrated non-suppressible aldosterone after intravenous saline suppression testing (used as a confirmatory test for PA). These findings led the authors to propose that female gender, probably via effects of sex steroids, predisposed to false positive ARR testing, and that females with elevated ARR should therefore be subjected to repeat testing before considering further diagnostic workup for PA.

Fommei *et al*. studied the effects of the menstrual cycle on the ARR (with renin measured as PRA) in 26 mildly hypertensive women with low renin levels.[57] The proportion of subjects with elevated ARR combined with an aldosterone concentration >15 ng/dL (>415 pmol/L) (a criterion required by some investigators for positive screening testing) rose significantly from 27% on day 7 to 68% on day 21. Because samples were collected in the supine position, it is difficult to extrapolate these findings to subjects studied seated. Furthermore, because confirmatory testing was not performed, it was not possible to identify which of these subjects actually had PA, and therefore which ARR test results represented false positives (when elevated) or false negatives (when not). Pizzolo *et al*. reported higher aldosterone and DAR concentrations in the luteal (compared to follicular) phase in 33 healthy, normotensive females, but no difference in mean ARR.[56]

In a study of 19 healthy, normotensive females, we reported luteal concentrations to be higher than follicular for plasma aldosterone, DAR, PRA and ARR calculated using DAR but not when calculated using PRA.[58] In two subjects luteal ARR was elevated when calculated using DAR but normal using PRA. Hence, it may be preferable to screen women for PA during the follicular rather than the luteal phase, and possibly best during the menses when oestrogen and progesterone concentrations are at their lowest. Clearly, further study is required in this area so that firm conclusions can be drawn as

to whether phase of the menstrual cycle needs to be taken into account when considering ARR testing, as well as the method of measuring renin.

Renal Dysfunction and Ageing

False positive ratios may occur in patients with renal impairment,[59] in which renin levels tend to fall as a result of reduced renin secretory mass and also salt and water retention, while any associated hyperkalaemia tends to elevate aldosterone. In the elderly, falling renin levels accompany gradually reducing renal function, while the fall in aldosterone levels is less marked, and false positive ratios are therefore frequently encountered.[60]

Effects of Co-existing or Other Conditions

Conditions which may co-exist with PA and lead to previously suppressed renin being released from suppression (thereby resulting in a false negative ARR) include pregnancy,[61] renal artery stenosis[62] and malignant hypertension.[63] False positive ratios occur in Gordon's syndrome (otherwise known as familial hypertension and hyperkalaemia or pseudohypoaldosteronism type 2), in which a primary defect in renal tubular function results not only in excessive resorption of sodium (leading to hypertension and renin suppression) but also potassium (causing chronic hyperkalaemia, which prevents suppression of aldosterone).[64-67] This contrasts with Liddle's syndrome,[68] the syndrome of apparent mineralocorticoid excess,[69] and hypertensive forms of congenital adrenal hyperplasia due to 11β-hydroxylase deficiency[70] or 17α-hydroxylase deficiency[71] in which both renin and aldosterone are suppressed and the ratio normal.

Measurement of the ARR - a Suggested Approach

A major area of difficulty in comparing or pooling laboratory results from different centres has been the fact that approaches used to control for factors potentially confounding the ARR have varied considerably from one centre to the next. This is perhaps not surprising given the complex array of factors and conditions that affect aldosterone and renin levels. The following is the approach currently used by GHHU and PAHHU.[28] While acknowledging that considerable room for improvement exists, we have nevertheless found this approach to result in large numbers of patients with PA being detected including those with surgically correctable forms (which make up around a third of patients in our experience) who have been either cured or had hypertension markedly improved following laparoscopic adrenalectomy. Bilateral forms have usually responded well to medical treatment with aldosterone antagonists,[30] and only rarely has unilateral adrenalectomy been indicated.[4]

A. Preparation for ARR Measurement

(i) Hypokalaemia is corrected, after measuring plasma potassium in blood collected slowly using a syringe and needle, avoiding fist clenching during collection, waiting at least 10 s after tourniquet released (if it was used to achieve insertion of needle), and ensuring separation of plasma from cells within 30 min of collection. Vacutainer® tubes are avoided.

(ii) Patients are encouraged to liberalise (rather than restrict) sodium intake.

(iii) Medications which significantly affect the ARR are withdrawn (at least two weeks before testing for beta-blockers, clonidine, methyldopa, non-steroidal anti-inflammatory drugs, ACE inhibitors, ARBs and dihydropyridine calcium blockers; at least four weeks for diuretics (including spironolactone)).

(iv) Where necessary to maintain hypertension control, other antihypertensive medications which have lesser effects on the ARR such as verapamil slow-release (with or without hydralazine) and/or prazosin are commenced.

(v) Contraceptive agents are not withdrawn unless confident of alternative effective contraception.

B. Conditions for Collection of Blood

(i) Blood is collected mid-morning, after the patient has been ambulant for at least 2 h and seated for 5–15 min.

(ii) Blood is collected carefully to avoid stasis and hemolysis (see A(i) above).

C. Factors Affecting Interpretation of Results

(i) Advanced age (over 65 years)

(ii) Time of day, recent diet, posture and length of time in that posture

(iii) All medications

(iv) Method of blood collection including any difficulty

(v) Concentration of potassium

(vi) Renal function

(vii) Phase of menstrual cycle in women.

It is important to emphasise the potential danger in ceasing medications in non-hospitalised patients to achieve wash-out. Although this can be achieved safely in mildly hypertensive patients who are seen frequently, it is more often necessary to commence a relatively renin-neutral drug such as those listed in A(iv) above. In cases where a potentially interfering medication cannot be withdrawn, useful information can still be obtained by taking into account its known effects when interpreting the ARR result. For example, a raised ratio in patients receiving a diuretic, ACE inhibitor, angiotensin receptor blocker or dihydropyridine calcium blocker would make PA very likely, whereas a normal ARR in the presence of beta-blocker treatment would make the diagnosis very unlikely.

What Should be the Cut-Off Point for the Ratio?

Lack of uniformity in diagnostic protocols and assay methods used for measurement of the ARR has resulted in substantial variability in cut-off values used by different groups ranging from 20 (when plasma aldosterone is expressed as ng/dL and PRA as ng/mL/h) to 100.[26,28]

Differences between laboratories in the units used for reporting aldosterone and renin levels have added to the complexity faced by the laboratory and practising physician. It is likely, however, that this problem will diminish with time as more laboratories adopt the Systeme Internationale (SI) method of reporting aldosterone concentrations (in which 1 ng/dL, the 'traditional' units, converts to 27.7 pmol/L) and change over from a PRA assay to an immunometric method for measuring DAR concentration (in which a PRA level of 1 ng/mL/h converts to a 'direct renin' concentration of 8.4 mU/L). Within GHHU and PAHHU, and using the protocol described above, we currently consider ratios of 100 or more (with plasma aldosterone measured as pmol/L and DAR as mU/L) to be highly suggestive of PA, and accept that a 'grey zone' exists between 70 and 110.[28]

A limitation of the ARR is that, in the presence of extremely low renin levels (for example, at PRA values of ≤0.1 ng/mL/h or DAR <2 mU/L), the ARR may be elevated and thereby raise the possibility of PA even when plasma aldosterone is also very low (for example, 110 pmol/L) and clearly not consistent with PA. In order to avoid this problem, some investigators have suggested the inclusion of a minimum plasma aldosterone concentration within the screening criteria. For example, William Young Jr, with extensive experience, has proposed a ratio >20 (plasma aldosterone as ng/dL and PRA as ng/mL/h) in combination with a plasma aldosterone concentration >15 ng/dL as a positive screen for PA.[47] This approach, however, would have resulted in many of our patients with PA (including some with APA) being missed because their plasma aldosterone concentrations fell below this cut-off level.[30] Within GHHU and PAHHU, we continue to follow (repeating the ARR and considering, from time to time, further diagnostic workup) all patients with elevated ARR other than those whose plasma aldosterone concentration is below the level used to define normal suppression during fludrocortisone suppression testing (FST) (165 pmol/L).

The Problem of Assay Reliability

Highly reproducible assays are essential for the diagnosis and management of PA. The ARR appears to be more dependent on renin than aldosterone,[72] especially when renin levels are low (as in patients with PA) in which case small absolute changes can result in large changes in the ARR. Therefore, it could be argued that it is more important to measure renin accurately than aldosterone. However, false positive and negative ARR values may also result from inaccurate aldosterone measurement, and reliable quantification is critical during subsequent suppression testing (in which the definitive confirmation or exclusion of PA is dependent on the aldosterone concentration) and adrenal venous sampling (AVS) (the results of which largely determine whether a patient is a candidate for unilateral adrenalectomy, or alternatively, treatment with aldosterone antagonist medication).[34]

Schirpenbach *et al.* compared four different aldosterone assay approaches and reported them to often give markedly different results.[73] Faster, more convenient methods of directly measuring active renin using immunometric techniques and automated machinery[38,74] have been adopted in many large, busy laboratories in favour of the more laborious but well-established PRA radioimmunoassay. This change has been accompanied by concerns regarding the validity of these methods, and whether they may provide misleading results in certain situations (for example, those in which concentrations of active renin are not reflective of the state of activation of the renin/AII system, such as during treatment with oestrogen-containing compounds or with renin inhibitors as explained above).

Where PRA assays are still in use, there is also variability among laboratories regarding whether they choose to adopt the well-substantiated advice of Sealey and Laragh to routinely extend the incubation time from 90 min (recommended by the manufacturer) to 3 h, and to 18 h for samples with levels <1 ng/mL/h, to permit enough generation of angiotensin I to ensure assay reproducibility at the lower end of the scale.[75]

These issues represent important impediments to diagnostic accuracy and standardisation of diagnostic criteria, and emphasise the need for individual laboratories to develop their own references intervals and employ careful quality control measures. For both renin and aldosterone assays, this would ideally include using aliquots from human plasma pools, carefully selected to cover the critical range of measurements, rather than the lyophilised controls provided by the manufacturer to monitor intra- and inter-assay reproducibility. A major step forward has been the development of new, high-throughput mass spectrometric methods of aldosterone measurement which have proven highly reliable within the clinically relevant range.[76] Hopefully, development of renin assays with the same capability for precision and specificity will soon follow.

Because of the critical role of validated assay techniques and the innate variability of both aldosterone and renin, a single

measurement of ARR should never be relied upon to guide management decisions.[28,34] Before deciding that PA is highly likely or highly unlikely, clinicians should repeat the ratio until confident that it is raised, meanwhile adjusting medications and conditions of collection if indicated. The next step, a definitive test involving salt loading, is not entirely risk-free in patients with severe hypertension or compromised cardiac or renal function.

Confirmation of the Diagnosis

Confirmation of PA usually involves demonstrating evidence of ongoing aldosterone production in the face of manoeuvres designed to bring about complete suppression of circulating renin (and therefore of aldosterone production that is significantly autonomous of its normal chronic regulator, AII). Of the various approaches described, the most common in use are the FST, oral sodium loading and saline infusion testing.

Fludrocortisone Suppression Testing

Although relatively labour-intensive, FST is the approach used within GHHU and PAHHU as it is generally regarded to be the most reliable (Figure 1).[34,39,77] Patients undergoing FST within our units are admitted to the hospital to ensure

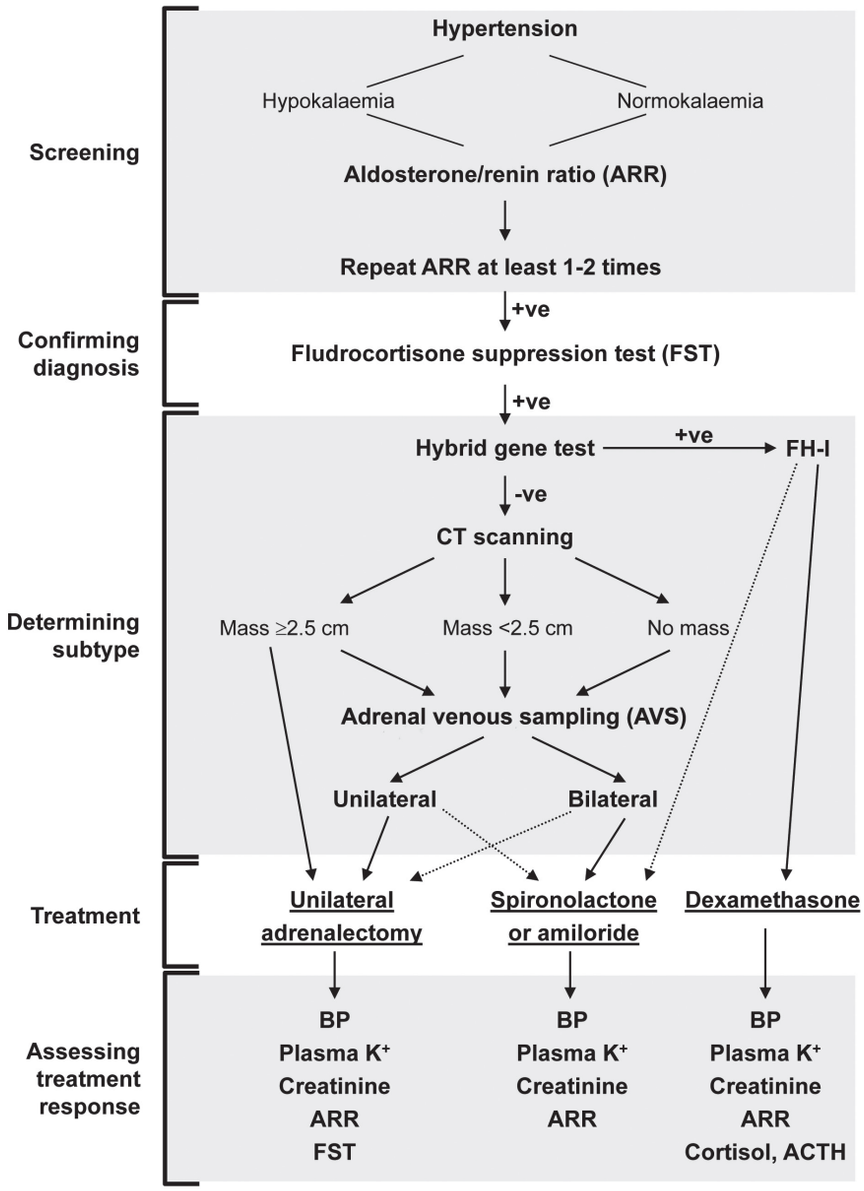

Figure 1. Algorithm describing the case detection, assessment and treatment of primary aldosteronism as performed in the Greenslopes and Princess Alexandra Hospital Hypertension Units. BP = blood pressure. CT = computed tomography; FH-I = familial hyperaldosteronism type I (glucocorticoid-remediable aldosteronism).

adherence to the dietary and posture requirements of the test protocol, and to facilitate monitoring of blood pressure, plasma potassium levels and other parameters.

Failure of upright (1000 h) plasma aldosterone to suppress to <165 pmol/L at the conclusion of four days administration of a high sodium diet and slow-release sodium chloride (Slow Sodium, 30 mmol thrice daily with meals) and of fludrocortisone acetate (0.1 mg every 6 h) is regarded as diagnostic, provided that:

(i) PRA is suppressed to <1 ng/mL/h (or DAR to <8.4 mU/L) on day 4 of the test. Failure to achieve adequate suppression of renin and AII complicates interpretation of a non-suppressed aldosterone level, since even normally regulated aldosterone production would be expected to persist under these circumstances. The maintenance of a dietary salt intake of at least 3 mmol of sodium/kg/day (confirmed by measuring urinary sodium excretion during the last 24 h of the FST) helps ensure that adequate renin/AII suppression is achieved, and is facilitated by having the patient see a dietician within 24 h of admission

(ii) Plasma potassium on day 4 is in the reference interval. This can be achieved by giving slow-release potassium chloride 6 hourly in sufficient doses to keep plasma potassium (ideally measured at 0700 h, 1000 h, 1600 h and 1900 h) as close as possible to 4.0 mmol/L. This avoids worsening or development of hypokalaemia (due to fludrocortisone maximally stimulating sodium retention and potassium excretion) and the possibility of a missed diagnosis due to hypokalaemia-induced suppression of aldosterone release, or conversely a false positive FST due to stimulation of aldosterone by hyperkalaemia from over-replacement

(iii) Plasma cortisol concentrations on day 4 are lower at 1000 h than at 0700 h, thereby excluding the occurrence of an acute rise in ACTH which may have prevented suppression of aldosterone (Figure 2).

Oral Sodium Loading

Several highly reputable groups, including Young and co-workers,[47] measure urinary aldosterone concentrations following oral salt loading to diagnose PA. For this protocol, patients are encouraged to consume enough dietary salt to achieve a urine sodium excretion >200 mmol/day and are given sufficient potassium supplementation to maintain normokalaemia. A 24 h urinary aldosterone concentration >12 μg/day on the third day is regarded as diagnostic. Our application of Young's criterion to urinary aldosterone concentrations measured in collections obtained on the fourth day of the FST permitted detection of 50 of 80 patients with a positive FST and 8 of 10 subsequently cured by surgery.[78]

Saline Infusion Testing

A common approach has been to administer intravenous 0.9% saline (usually 2 L over 4 h) with measurement of plasma aldosterone at the end of the infusion. Concentrations regarded as diagnostic for PA have varied from >5 to >10 ng/dL (>140 to >280 pmol/L).[79-81] This approach has the advantage of requiring only a brief outpatient visit. In the authors' experience, saline infusion (using a diagnostic post-saline plasma aldosterone of >220 pmol/L) detected only 17 of 97 patients confirmed as having PA by FST, and only 3 of 10 patients who were subsequently cured of PA following unilateral adrenalectomy, but these results may have been at least partly influenced by the use of a more rapid infusion protocol (2 L over 2 h).[78] Further work to refine definitive testing for PA would be worthwhile.

Because each of the methods described above is dependent on achieving 'complete' suppression of renin/AII (ideally confirmed by measuring renin during the testing protocol) to ascertain whether there is at least partial autonomy of aldosterone production, it is necessary to avoid drugs (such as diuretics, ACE inhibitors, angiotensin receptor blockers and dihydropyridine calcium blockers) which stimulate renin production during suppression testing. Practically speaking, this usually involves maintaining patients off such interfering medications after ARR testing has been completed and throughout the remainder of the diagnostic workup for PA, using alternative agents (such as verapamil slow-release plus or minus hydralazine, and/or prazosin) where necessary to maintain hypertension control. Fortunately, the bed rest obtained in hospital during the FST lowers blood pressure in most patients, and reduces associated risk by permitting close observation of blood pressure levels.

Subtype Differentiation

The histopathological expression of PA is highly variable, and includes adrenal cortical hyperplasia (which is rarely diffuse and more commonly micro- or macronodular, and occasionally of the 'giant macronodular' variety), a solitary nodule or 'adenoma' (which may or may not be associated with non-tumorous hyperplasia of zona glomerulosa), and rarely adrenocortical cancer. The considerable overlap that exists between these categories, and the fact that morphological appearance does not necessarily predict the precise site of the aldosterone overproduction, limit the usefulness of these pathological categories from a management point of view. Results from AVS studies, for example, have shown that autonomous aldosterone production may be bilateral or even contralateral to the side of an apparently solitary adrenal nodule or may be unilateral in patients with bilateral hyperplasia.[34,82,83]

(a)

DAY	TIME	ALDO (pmol/L)	RENIN (mU/L)	K^+ (mmol/L)	CORTISOL (nmol/L)
Day 0	07:00 h REC	640	2	2.6	340
	10:00 h UP	310	<2	3.0	170
Day 3	07:00 h REC	960	3	3.5	400
	10:00 h UP	330	<2	4.0	130
Day 4	07:00 h REC	940	<2	3.8	390
	10:00 h UP	410	<2	3.9	190

(b)

SAMPLE	TIME	ALDO (pmol/L)	CORTISOL (nmol/L)	CORTISOL AV/PV	ALDO/ CORTISOL
LAV1	09:01	2302	6346	14.4	0.4
Peripheral	09:01	665	441		1.5
LAV2	09:03	1886	6622	16.0	0.3
Peripheral	09:03	721	414		1.7
RAV1	09:12	100696	9105	19.4	11.1
Peripheral	09:12	777	469		1.6
RAV2	09:17	79891	9381	22.7	8.5
Peripheral	09:17	777	414		1.9

(c)

DAY	TIME	ALDO (pmol/L)	RENIN (mU/L)	K^+ (mmol/L)	CORTISOL (nmol/L)
Day 0	07:00 h REC	40	9	3.9	440
	10:00 h UP	140	19	4.1	380
Day 3	07:00 h REC	<40	4	3.8	320
	10:00 h UP	<40	2	3.6	230
Day 4	07:00 h REC	<40	2	3.8	340
	10:00 h UP	<40	<2	3.6	220

Figure 2. Results of (a) fludrocortisone suppression testing (FST); (b) adrenal venous sampling (AVS); and (c) post-operative FST (performed following right unilateral adrenalectomy) in a 39-year-old female patient with right aldosterone-producing adenoma (APA). The patient was referred with hypertension and unprovoked hypokalaemia and was found to have repeatedly elevated upright midmorning aldosterone/renin ratios (ARR) and suppressed renin levels off interfering medications (see day 0 of FST). FST confirmed the diagnosis of primary aldosteronism (PA) (failure of upright plasma aldosterone (aldo) to suppress to <165 pmol/L despite suppression of renin to <8.4 mU/L). The absence of a rise in cortisol between 0700 and 1000 h excluded a stress-induced rise in ACTH which may have prevented aldo suppression. Initial hypokalaemia was successfully corrected and normokalaemia maintained up to day 4 by administration of potassium supplements (Slow K, given 6 hourly). The failure of aldo to rise in response to upright posture (UP) following overnight recumbency (REC) on day 0 (prior to commencement of fludrocortisone) was suggestive of angiotensin II-unresponsive APA or familial hyperaldosteronism type I (FH-I), but not sufficient to exclude bilateral PA. Not shown here are results of genetic testing for the hybrid gene (negative, thereby excluding FH-I), or adrenal CT (13 mm lesion in the right adrenal gland). During AVS, adequate cannulation of both adrenal veins was confirmed by assessment of adrenal/peripheral venous (AV/PV) cortisol gradients which exceeded our criteria (>3.0) for cannulation success. Although both left (LAV) and right (RAV) adrenal venous aldo levels were greater than those in simultaneously collected peripheral blood, only the right aldo/cortisol gradients exceeded peripheral, while left aldo/cortisol gradients were less than peripheral (contralateral suppression), consistent with lateralisation of aldo overproduction to the right adrenal. Following right laparoscopic adrenalectomy, hypertension and hypokalaemia normalised without the need for medications. FST performed two months post-operatively showed normalisation of the day 0 ARR (with renin now 'unsuppressed') and normal suppression of aldo by days 3 and 4, consistent with biochemical cure of PA.

At GHHU and PAHHU, subtype differentiation involves addressing the following issues relevant to management decision-making (Figure 1).

(i) Does the patient have the inherited, glucocorticoid-remediable form of PA?[84] If so, an excellent clinical response to glucocorticoids, given in low doses, would be expected and family screening should be undertaken to identify similarly affected relatives.
(ii) If not, is the autonomous aldosterone production confined to one adrenal (in which case unilateral adrenalectomy would be expected to either cure hypertension or at least result in a substantial improvement in control) or bilateral (currently managed medically in most patients with aldosterone antagonists)?
(iii) Does the patient have a large adrenal mass lesion (>2.5 cm)? If so, it may warrant removal based on its malignant potential.
(iv) Any adrenal mass which is not removed should have repeat imaging (usually computed tomography (CT)) at six months, then 12 months (if no change at six months) and then at intervals no longer than every 3–5 years thereafter.

Testing for Glucocorticoid-Remediable Aldosteronism (FH-I)

Glucocorticoid-remediable aldosteronism or FH-I, a familial form of PA inherited in an autosomal dominant fashion, is caused by a 'hybrid' gene mutation composed of regulatory sequences derived from the 11β-hydroxylase gene (*CYP11B1*) and coding sequences mainly derived from the aldosterone synthase gene (*CYP11B2*).[85] Unlike FH-I, a second familial form of PA (FH-II) which we first described in 1990 is not glucocorticoid-remediable and not associated with the hybrid gene mutation.[86-88]

The hybrid gene in FH-I encodes an enzyme with aldosterone synthase activity, but unlike the wild-type aldosterone synthase gene (which is regulated primarily by AII), the hybrid gene is regulated by ACTH by virtue of its 11β-hydroxylase regulatory sequences.[89] The result is excessive aldosterone production which is regulated by ACTH rather than by AII.[84,90-93] The hyperaldosteronism and hypertension are therefore remediable by the administration of glucocorticoids given in small doses (for example 0.125–0.25 mg of dexamethasone daily) which are sufficient to partially suppress ACTH without causing Cushingoid side effects.[94] Alternatively, these patients can be treated with aldosterone antagonists such as amiloride or spironolactone.

Genetic testing for the presence of the hybrid gene in peripheral blood DNA using either a Southern blot approach described by Lifton *et al*.[85] or a faster, PCR-based method developed at the GHHU[95] has streamlined the diagnosis of FH-I. The demonstration of marked, sustained suppression of plasma aldosterone during four days administration of dexamethasone (0.5 mg 6 hourly) is the most reliable biochemical alternative, being highly sensitive and specific for FH-I,[96-98] but requires hospital admission or repeated visits and blood collections over several days. It is therefore difficult to perform in young children, and is not without occasional false positives and negatives.[97-99]

Severity of hypertension is highly variable in FH-I, with some patients severely hypertensive from childhood, while others remain normotensive well into adulthood.[100-102] Even normotensive individuals with FH-I demonstrate evidence of left ventricular remodelling compared with matched normotensive controls, and may therefore be at greater risk of cardiovascular disease.[103] Severely affected individuals, if left undiagnosed and not specifically treated, are at risk of early death from hypertensive complications (commonly cerebral haemorrhage).[100,104] Because of this, and because the blood pressure response to glucocorticoid therapy is usually excellent, genetic testing of relatives of patients found to have FH-I is mandatory.

Differentiating Unilateral From Bilateral Adrenal Forms of PA

Because FH-I is a rare form of PA, the great majority of patients with PA test negative for the hybrid gene. These patients then require further evaluation to differentiate forms associated with unilateral adrenal overproduction of aldosterone, which may be curable by unilateral laparoscopic adrenalectomy, from those with bilateral overproduction.

Adrenal Venous Sampling

At GHHU and PAHHU, all hybrid gene-negative patients with PA undergo AVS (Figures 1 and 2), which is the only reliable way to differentiate unilateral from bilateral forms. In a study describing increased rates of diagnosis of PA associated with wide application of the ARR in five centres, APAs constituted a much higher proportion (28–50%) of newly-diagnosed patients with PA in the four centres that employed AVS during diagnostic workup than in the centre that did not (9%).[15] As with ARR and suppression testing, medications which stimulate renin production are avoided leading up to AVS. This is because lateralisation of aldosterone production in patients with unilateral forms of PA may be lost (and AVS results instead give a mistaken impression of bilateral PA) in situations where renin/AII levels are not suppressed, as the circulating AII is likely to result in stimulation of aldosterone production by the otherwise suppressed contralateral adrenal.

Prior to AVS, all our patients undergo CT scanning because it can assist in localising the adrenal veins,[39,105] and will detect

any large masses which may warrant removal based on risk of malignancy. Patients are provided with detailed information sheets regarding the procedure and are instructed in relaxation techniques in an attempt to minimise effects of stress on steroid hormone production. Performing AVS in the morning following overnight recumbency avoids the confounding effects of changes in posture on aldosterone concentrations in patients with AII-R forms of PA and takes advantage of the effect of high early morning endogenous ACTH levels on aldosterone production.[106] Samples are collected from each of the presumed right and left adrenal veins by a radiologist highly skilled in this technique, and a peripheral venous sample (usually from the antecubital fossa, alternatively from the low inferior vena cava below the adrenal veins or iliac vein) is collected simultaneously with each adrenal vein sample. Because of the variable anatomical nature of the adrenal veins, and the inability of the radiologist to be certain that cannulation has been successful, at least two samples are collected from each side. The risk of adrenal haemorrhage associated with AVS is low (<2% of all AVS procedures at GHHU and PAHHU) provided adrenal venography is avoided.[105]

Examination of the ratio or 'gradient' between adrenal and peripheral venous cortisol concentrations permits an assessment of the adequacy of AVS. At GHHU and PAHHU, gradients of at least three are taken to indicate adequate sampling. Samples demonstrating gradients of between two and three may provide useful information, but those with gradients of less than two are always excluded from further consideration. In support of these recommendations, two recent studies have reported poor reproducibility in terms of AVS subtype diagnosis when samples with lower cortisol gradients (e.g. less than 2–3) have been included for analysis.[107,108]

The right adrenal vein is often harder to locate and successfully cannulate than the left as it is usually smaller and shorter and usually empties into the inferior vena cava rather than the renal vein.[109] Despite this degree of difficulty, in highly experienced units success rates reach 90% or higher.[105,110] Use of CT scanning to localise the right adrenal vein prior to AVS has contributed to the high rate of successful cannulation at the GHHU.[39,105] An additional refinement utilises 'on-the-spot' plasma cortisol measurement, permitting determination of adrenal venous cortisol levels within minutes of collection.[111,112] This offers a means of definitively establishing, at the time of AVS, whether adrenal venous cannulation has been successful, and thereby potentially reducing the requirement for multiple samples and the need for repeat procedures.

Debate exists as to the value of ACTH stimulation, which has been used by several groups to maximise adrenal/peripheral venous cortisol gradients, reduce fluctuations in steroid secretion resulting from changes in endogenous ACTH levels during non-sequential AVS, and stimulate aldosterone production by APAs and thus avoid sampling during a relatively quiescent period of secretion.[26] Seccia *et al*., however, in a recently-reported study examining the effects of three different currently-used ACTH stimulation protocols, found that (a) the lowest dose had no measurable effects on cannulation success or subtype diagnosis; and (b) while the higher two doses resulted in a higher proportion of samples that would be regarded as 'successful', they unfortunately had the potential to result in incorrect lateralisation of aldosterone overproduction.[113]

Adrenal venous samples differ greatly in the degree to which they are 'diluted' with non-adrenal venous blood. Because of this, direct comparison of aldosterone concentrations in these samples will frequently give misleading results. Because failure to cannulate the right adrenal vein is not rare, the most common misinterpretation of results occurs when the left adrenal vein aldosterone concentration is high (due to successful cannulation), while the right adrenal vein aldosterone concentration is low (due to failed cannulation). Without correction for cortisol concentration, this result can lead to removal of the left adrenal gland without leading to cure or improvement in hypertension. In a patient with APA, aldosterone concentrations on the side of the contralateral suppressed 'normal' gland are significantly lower than on the side of the APA, but quite often higher than peripheral, and can thereby give the mistaken impression of bilateral adrenal autonomous aldosterone production (Figure 2). This is presumably because the normal gland can still produce small quantities of aldosterone in response to secretagogues such as potassium and ACTH (despite renin/AII suppression), and, because adrenal venous blood is characteristically very slow flowing, only very small amounts of secreted aldosterone are required to render concentrations substantially higher than peripheral.

Calculating the aldosterone/cortisol ratio for each adrenal and peripheral venous sample corrects for differences in dilution and permits a more meaningful comparison of sample levels. At GHHU and PAHHU, if the average aldosterone/cortisol ratio on one side is significantly (at least two times) higher than the simultaneous peripheral venous ratio, with a ratio no higher than peripheral on the other side, the study is considered to show lateralisation, indicating that unilateral adrenalectomy should cure or improve the hypertension (Figure 2).[34,39]

Measurement of adrenal venous aldosterone and cortisol concentrations requires great care as concentrations may

be very high, and small errors in sample dilution and assay technique may have a major effect on results. The use of new generation, highly reliable mass spectrometric techniques should prove helpful in this regard.[76]

Adrenal Imaging

Because the application of AVS has been limited by its technical difficulty in inexperienced hands, many centres rely on adrenal imaging with CT or scintigraphy. However, imaging methods frequently fail to detect small APAs and can be frankly misleading, since not all mass lesions detected in patients with PA may be secreting aldosterone.[34,82,83,114] As described above, however, CT is nevertheless an important step in the diagnostic workup in that it may identify large mass lesions (e.g. 2.5 cm or more in diameter) that may warrant removal based on malignant potential and can assist in localisation of the right adrenal vein prior to AVS.

Measuring Aldosterone Response to Upright Posture, and 'Hybrid Steroid' Concentrations

The demonstration of responsiveness (defined as a rise of at least 50% above basal) of plasma aldosterone to upright posture and (where available) to AII infusion was once thought to obviate the need for lateralising procedures such as AVS because plasma aldosterone concentrations demonstrate normal responsiveness in most patients with BAH but not in those with classic, AII-U APAs, or those with FH-I.[35,115] However, these manoeuvres cannot distinguish BAH from AII-R APA.[35] AII-R APA was first described by the GHHU in 1987[35,36] and accounts for over half of all APAs currently removed by the unit. Examination of the response to posture in patients with PA is nevertheless worthwhile, because its absence almost always narrows the diagnosis to AII-U APA or FH-I.[35,92,93,96] Urinary concentrations of the 'hybrid steroids' 18-hydroxy-cortisol and 18-oxo-cortisol are usually elevated in patients with AII-U APAs and FH-I, but not in patients with BAH, and are usually normal in patients with AII-R APA.[35,116-120] Assays for 'hybrid steroids', however, are not widely available. Because patients with AII-R APA masquerade biochemically as BAH, yet, like those with classic AII-U APA, can be surgically cured of hypertension,[35] a careful search should be made for APA regardless of either the aldosterone response to posture or the hybrid steroid concentration. The most reliable way to do this is by AVS.

Assessment of Treatment Response

Available treatment modalities for PA include the following:

(i) unilateral laparoscopic adrenalectomy is usually reserved for patients with unilateral PA (as defined by AVS) in whom surgery results in cure of hypertension in at least 50% and improvement in virtually all the remainder.[2,3,6] It is occasionally also performed in carefully-selected patients with bilateral forms (for example, those in whom aldosterone antagonist medications have been poorly tolerated or failed to result in adequate hypertension control) in which case the blood pressure response is less predictable[121]

(ii) medications which antagonise aldosterone action, including spironolactone and eplerenone (mineralocorticoid receptor antagonists) and amiloride (a sodium epithelial channel inhibitor) are the treatment of first choice for patients with bilateral forms of PA, but are also utilised in those with unilateral forms who are unsuitable for (or decline) surgery or to facilitate control of hypertension and hypokalaemia while awaiting surgery[5,34,39]

(iii) glucocorticoids (in low dose) are the favoured treatment for patients with FH-I (although they can also be treated with aldosterone antagonists).[84,88]

Because blood pressure can be affected by multiple factors, residual hypertension following institution of one or more of the above specific treatment approaches does not necessarily mean that (a) aldosterone excess has not been corrected by surgery for unilateral PA or glucocorticoid treatment for FH-I; or (b) its effects have not been adequately blocked (by mineralocorticoid antagonist agents). Laboratory investigations help to assess responses to both surgical and medical treatment in patients with PA.

As is the case during the pre-treatment diagnostic workup of PA, it should be remembered that interfering medications (including several antihypertensives as listed above) can affect aldosterone and renin levels following adrenalectomy or during medical treatment of PA, and this should be taken into account when attempting to interpret these levels as an indication of treatment response. Ideally, non-interfering medications should be used, thus avoiding this important problem.

Laboratory Assessment Following Surgical Treatment

Following unilateral adrenalectomy for unilateral PA, resolution of hypokalaemia (when present pre-operatively) is almost invariable, and normalisation of the ARR is a reassuring sign that PA has been biochemically 'cured', even if blood pressure has not returned to normal. A rise in serum creatinine is commonly seen, but this reflects correction of the volume expanded state (caused by excessive sodium retention) that existed prior to surgery rather than a deterioration in renal function *per se*.[24] At GHHU and PAHHU, patients undergo FST one to three months post-operatively to detect any autonomous aldosterone production by the remaining adrenal. All patients lateralising pre-operatively on AVS have shown either biochemical cure (70%) of PA or significant improvement (30%) on post-operative FST.[3]

Laboratory Assessment During Medical Treatment

For patients demonstrating bilateral aldosterone production on AVS, treatment with aldosterone antagonists usually brings about marked improvement in hypertension control.[5,34,39] Only small doses (for example, 12.5–50 mg daily of spironolactone and/or 2.5–20 mg daily of amiloride) are usually required for optimal antihypertensive effect. In countries where eplerenone, a modified form of spironolactone with negligible blockade of the androgen receptor, is available, it can be used as a preferred alternative. Its widespread usage will depend on pricing. It is a little more than half as effective as spironolactone, mg for mg, so that higher doses are required. However, its greater selectivity permits this increase to adequate dosage, evidenced by renin rising into the mid-normal range, without pro-oestrogenic side-effects. At present in Australia, it is available under favourable pricing arrangements only for patients with heart failure following recent myocardial infarction.

Patience is required as the full effect of each dose level takes weeks or months to be achieved. These medications correct hypokalaemia quickly in all but the most severe cases of PA, and potassium supplements should therefore usually be ceased when they are commenced, and, if not ceased, then progressively reduced (watching potassium levels) as the aldosterone antagonist has its full effect. Overtreatment with these agents can cause volume contraction with pre-renal failure, rising creatinine concentrations and potentially life-threatening hyperkalaemia.[34] They should therefore be used with great caution in patients with existing renal impairment, and electrolytes and creatinine should be regularly checked.

The extent to which renin levels become 'unsuppressed' is a useful indication of the degree of blockade of aldosterone effect induced by any given treatment dose. While normalisation of potassium and blood pressure levels have been traditionally regarded as evidence of adequate treatment, it could be argued (given that aldosterone excess is now known to induce adverse cardiovascular effects independently of its effects on blood pressure) that the goal should be 'complete' reversal of excessive aldosterone action. In this way, normalisation of renin levels may be more reassuring than simply correcting hypertension and hyperkalaemia since it indicates that the dose of mineralocorticoid antagonist is adequate to correct the sodium/volume expansion. Given that adverse cardiovascular effects of aldosterone excess appear to be dependent on sodium balance, normal renin levels would imply correction of sodium overload as well. In some patients, however, the degree to which doses can be increased in order to achieve normalisation of renin levels will be limited by tolerability. Even at spironolactone doses of 12.5–25 mg daily, side effects (such as gynaecomastia in males and menstrual irregularity in females) are not uncommon. As with the risk of hyperkalaemia and uraemia, these side effects are dose-dependent.

Assessment of renin levels is also helpful in guiding changes in management for patients in whom hypertension has not yet become optimally controlled following introduction of mineralocorticoid antagonist treatment. If renin levels have already become 'unsuppressed', for example, persisting hypertension is best treated by adding in or increasing the dose of other antihypertensive medications. It must be remembered that renin suppression can persist for long periods after 'cure' of unilateral PA by adrenalectomy, if PA was longstanding. It may therefore also take some time for 'complete' blockade of the mineralocorticoid receptor to result in renin becoming 'unsuppressed'.

Laboratory Assessment During Glucocorticoid Treatment of FH-I

Most patients with FH-I are able to maintain control of hypertension on doses of dexamethasone as low as 0.125–0.25 mg (or prednisolone 2.5–5 mg).[88,94] These low doses are associated with only partial suppression of cortisol, consistent with a lower total glucocorticoid level and therefore a lower risk of side effects. As expected, hybrid gene expression appears to be only partially suppressed at these doses, as evidenced by suppressed PRA, elevated ARR and elevated urinary 18-oxo-cortisol concentrations and tight correlation of circadian aldosterone with cortisol (rather than PRA) concentrations.[94] Clearly, then, it is not necessary to normalise urinary 18-oxo-cortisol levels and completely abolish ACTH-regulated aldosterone overproduction in order to achieve control of hypertension. A reasonable approach is to use the lowest dose of glucocorticoid treatment required to maintain normotension, as assessed by clinic, home and ambulatory blood pressure monitoring, and by periodic (for example, yearly) echocardiographic assessments of left ventricular mass index and diastolic function. As discussed in the previous section, however, this may not fully address the potential to develop blood pressure-independent adverse cardiovascular effects of aldosterone excess and an argument could be made for using a combined approach (by adding a mineralocorticoid antagonist in low dose) to optimise cardiovascular protection. Further work in this area would help to address this issue.

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