Hyperaldosteronism: How to Discriminate Among Different Disease Forms?

Hyperaldosteronism: how to discriminate among different disease forms?

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Short title: subtype diagnosis of primary aldosteronism

Key words: primary aldosteronism, aldosterone producing adenoma, adrenal vein sampling, secondary hypertension

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Authors disclosure/conflict of interest: the authors have nothing to disclose

Source of Funding: None

Word count: abstract: 112; manuscript: 2221 (excluded references and tables); number of tables: 2.

Abstract

Primary aldosteronism (PA), characterized by the inappropriate and excessive adrenal secretion of aldosterone, is the most common cause of secondary hypertension; PA has been shown to increase cardio- and cerebro-vascular risks in comparison with essential hypertension. PA is a multi-faceted disease, which comprises unilateral forms, benefitting from surgical treatment, and bilateral forms, which are best managed medically; PA is more frequently sporadic but in some cases displays a familial transmission pattern. For these reasons, it is important to diagnose PA early on and correctly distinguish and manage its different forms.

In this review, we analyze the different forms of PA, with attention on the diagnostic pathway and the genetics of the disease.

Introduction

Primary aldosteronism (PA) is defined as a syndrome, characterized by an inappropriate secretion of aldosterone, independent of the renin-angiotensin system and of sodium homeostasis. PA prevalence increases with blood pressure severity reaching up to 20% in patients with resistant hypertension [1,2]. Recent studies have also uncovered wider effects of aldosterone that go beyond BP, and affect a number of different target organs, in particular determining higher cardio- and cerebro-vascular risks in PA patients as compared to essential hypertensives with a similar risk profile [3,4].

Therefore, early detection of PA, is fundamental to best manage short and long-term complications of this condition.

The two most common forms of PA are the aldosterone-producing adenoma (APA), which accounts for 30-35% of all cases, and the bilateral adrenal hyperplasia (BAH), the most common PA subtype accounting for 65-70% of PA patients [5]. The therapeutic approaches are different depending from the subtypes, surgical for unilateral PA forms and medical treatment with mineralocorticoid receptors (MR) antagonists for BAH [5]. It should be noted that a relevant proportion of patients with unilateral PA, previously considered affectd by APA, display a moltinodular unilateral adrenal hyperplasia with one or more nodules expressing CYP11B2 with immunohistochemistry evaluation [6,7].

These two forms of PA, both sporadic, together comprise the vast majority of all PA cases; less common, but nonetheless important in the diagnostic pathway, are the four familial forms. Recognition of these is important not only in the treatment of patient, but also in the screening of first-grade family members.

In this manuscript we will review the subtype diagnosis of sporadic and familial PA.

Diagnosis

Diagnosis of PA is made by a screening test using the aldosterone to renin (or plasma renin activity ratio) [5]. Population with increased risk of PA that should be screened and the relationships and characteristics of different aldosterone and renin assay are described elsewhere [8-10]. Confirmation of the suspicion of PA is made by one of four confirmatory/exclusion tests: the intravenous saline loading test, the oral saline loading test, the fludrocortisone-suppression test and the captopril challenge test. Characteristics and performance of the different confirmatory tests are discussed elsewhere [5,11].

Imaging techniques and steroid measurements

Once PA is confirmed, patients should undergo contrast-enhanced computed tomography (CT) scanning evaluation of the adrenal glands [5,12] as a first exam. CT findings can vary, from bilateral hyperplasia, to unilateral hyperplasia, to unilateral or bilateral micro and macro-nodules (diameter < 1 cm or > 1 cm). Lesions bigger than 4 cm are suspicious for the rare but deadly adrenal carcinoma [5]. CT displays several limitations: even using fine-cuts, CT may fail at identifying micro-APAs; moreover, it does not provide information on the functional role of the nodules, which can be especially crucial when bilateral lesions are observed [13].

Other imaging techniques are available: the Endocrine Society approves magnetic resonance as an alternative to contrast CT, though it admittedly provides a lower quality of space resolution, and lower specificity [5].

Among functional imaging, ^{131}I-6β-iodomethyl-19-norcholesterol scintiscan has been abandoned; a more recent technique is the ^{11}C-metomidate positron emission tomography (PET)-CT scan, that could be considered an alternative to AVS when this is unsuccessful, but that display a lower sensitivity and specificity that makes it not suitable to substitute AVS routinely [14]. One of the limits of metomidate is its low selectivity for CYP11B1 over CYP11B2. For this reason, a Japanese group has tested a new, more CYP11B2-specific tracer, ^{18}F-CDP2230. However, this new technique needs to be tested in a prospective trial in comparison with AVS [15].

An additional instrument in the diagnosis of APA versus BAH has been suggested with the measurement of the so-called "hybrid" steroids 18-oxocortisol (18oxoF) and 18-hydroxycortisol (18OHF), that are produced in large amount in a consistent proportion of APA compared to BAH and incidentalomas [16,17]. Recently, through a more complete steroid profiling using liquid chromatography - tandem mass spectrometry (LC-MS/MS) to simultaneously measure 7 different adrenal steroids, it was possible to distinguish APA from BAH in a consistent proportion of cases and to identify those APA with a specific somatic genetic alteration [18,19]. If this results will be confirmed in large prospective studies, steroid profiling by LC-MS/MS promise to be a preliminary test in order to identify PA patients with high probability of unilateral PA, thus reducing significantly the requirement for AVS procedures.

Adrenal venous sampling

Adrenal venous sampling, or AVS, is widely recognized as the gold standard technique in the distinction of unilateral from bilateral PA [5,12]. In a review of 950 cases, imaging technique (either CT scanning or magnetic resomnance) resulted in a wrong diagnosis in around 38% of cases compared to AVS [20]. CT scanning can suggest inappropriate adrenalectomy in patients with BAH and unilateral nodule, or miss the opportunity of cure by adrenalectomy in cases of unilateral PA and bilateral nodules (with only one having secretory activity) or normal appearing adrenals and undetected micro-APA [5,20]. The main pitfall of CT scanning is the lack of a proper identification of secretory activity of detected nodules; different parameters have been suggested, such as densitometry and contrast wash-out, but neither has been proven to provide definite answers in the distinction between secreting lesions and incidentalomas [5,21]. Therefore, when adrenalectomy is not contraindicated because of comorbidities, AVS should always be suggested as the only reliable technique for PA subtype diagnosis [5,22].

AVS is a complicated procedure, which not only requires an expert operator, but also an adequate preparation of the patient. In the 4-6 weeks leading to the procedure, it is suggested to treat hypertension with alpha-blockers and non-dyhidropyridine calcium channel blockers, as they display the least influence on the renin-angiotensin system; if these drugs are not sufficient to reach the desired blood pressure control, beta-blockers, ACE-inhibitors and angiotensin-receptors blockers can be considered, while drugs such as thiazides, loop diuretics, amiloride and mineralocorticoid receptors antagonists should be avoided since they affect AVS results and interpretation. Potassium levels should be kept within

normal range [22]. AVS can be performed both under basal or during cosyntropin infusion with similar results [23]. Pro and cons of the two procedures are described elsewhere [22].

During AVS a catheter is inserted percutaneously in a femoral vein and, through the use of contrast medium and fluoroscopy, the adrenal veins are singled out, and blood is gently drawn. Procedure techniques can be challenging, and an expert operator is required, as success rates can vary between 44% and 96%, according to the radiologist expertise [22,24,25].

Left adrenal vein cannulation is relatively easy, as the vein joins the inferior phrenic vein to create a common vessel that drains into the left renal vein. Right adrenal vein, on the other hand, creates a very sharp angle in its emergency directly into the inferior vena cava, which can be challenging to cannulate even for an expert radiologist [24,25]. To avoid potential errors determined by blood dilution during adrenal veins cannulations, aldosterone values are always "corrected" by cortisol since it is considered that this hormone is equally produced from the two adrenals in PA patients [5,22].

Cortisol measurement is also used to determine the correct cannulation of the adrenal veins. The two most important parameters measured during AVS are are the selectivity index (SI), which is the ratio between cortisol in the adrenal vein and cortisol in a peripheral vein (Table 1): this provides information about the adequacy of cannulation of the adrenal vein; and the lateralization index (LI) which determine the presence or not of a lateralization of aldosterone secretion. It is calculated as the ratio between cortisol-corrected aldosterone levels in one adrenal compare to the contralateral. A result of LI> 4 is diagnostic for unilateral

PA (Table 1). A LI <3 is diagnostic for BAH. For LI between 3 and 4 other clinical parameters should be taken into account for final decision [5,21,26].

Another AVS parameter is the contralateral ration, that is the cortisol-corrected aldosterone ratio from the non-dominant adrenal vein in comparison with the peripheral vein (Table 1). A recent study has demonstrated that a contralateral suppression (i.e. a contralateral ratio <1) is not necessary to obtain cure or significant improvement of blood pressure levels after adrenalectomy [27].

AVS requires an experienced radiologist with an expertise in endovascular procedures, in order to minimize risks, most commonly the rupture of an adrenal vein during the procedure. The multi-centric study AVIS showed an inverse correlation between the experience of the radiologist and numbers of procedures done and the chance of complications [28]. The most serious complication of AVS is adrenal hemorrhage. A retrospective study analyzed 24 cases of adrenal hemorrhage in patients who underwent AVS in 6 different referral centers; of these, the majority involved the right adrenal, coherent with the difficulty in the right adrenal vein cannulation and were more frequent in older patients [29]. Interestingly, among all the analyzed cases, only one needed long-term corticosteroid replacement therapy for adrenal insufficiency. All hemorrhages were minor, and controlled by medical therapy without the need for new surgery or blood transfusions [29].

Genetics of PA

Familial hyperaldosteronism (FH) account for up to 5-6% of all PA cases [30,31]. The first form of FH to be discovered is also named glucocortidoid-remediable aldosteronism (GRA, or FH-I), which is caused by the creation of a chimeric gene

from the fusion of the promoter region of the 11β-hydroxylase gene *CYP11B1* with the coding region of the aldosterone synthase gene, *CYP11B2* [32,33] (Table 2). This determines a regulation of aldosterone production under the very active CYP11B1 (encoding 11β-hydroxylase) promoter, which is regulated by ACTH. Patients with GRA usually present hypertension in the first two decades of their lives, and an increased risk of cerebral hemorrhage; moreover they show high levels of hybrid steroids. Transmission is autosomal dominant, and the disease, once diagnosed, can be easily managed by administration of low doses of glucocorticoids, often associated with mineralocorticoid receptor blockers [34].

FH-II is clinically undistinguishable from sporadic PA, and it is diagnosed by the familial pattern of the disease. Its genetics basis is still unknown, though a linkage to chromosome 7p22 has been suggested [35,36]. It is conceivable that more types of genetic alterations are responsible for this condition (Table 2).

FH-III is has been recently described by Choi in 2011 [37]. This disease is characterized more frequently by very early onset of severe hypertension and hyperaldosteronism that require bilateral adrenalectomy in most cases. FH-III is determined by gain-of-function mutations of *KCNJ5* which KCNJ5 encodes for the potassium inwardly-rectifying channel Kir 3.4 [34] (Table 2); the mutations cause the channel to lose its selectivity for potassium, allowing large quantities of sodium to enter the cell, thus causing a membrane depolarization and the activation of voltage-gated calcium channels, with calcium influx into the cell, and activation of the cascade that results in aldosterone overproduction [38]. It should be noted, that cases with more mild phenotypes have been described [34,39,40].

Primary aldosteronism with seizures and neurological abnormalities (PASNA) is an extremely severe form of PA, caused by a germline mutation in *CACNA1D*, which

encodes a voltage-gated L-type calcium channel (Table 2); the mutations result in channel activation at less depolarized potentials, which in turn causes calcium influx and aldosterone production [41,42]. Only two cases have been diagnosed so far: both patients were diagnosed at a very young age from healthy parents; it is believed that their neurological impairment is so severe not to allow affected individuals to reproduce. Therefore, this condition is considered not-familial despite the genetic cause and is expected to be caused only by *de novo* mutations. The latest discovery in familial forms is FH-IV, described by Scholl et al. in 2015 [43]: FH-IV is caused by a germline mutation in another voltage-gated calcium channel gene, CACNA1H, highly expressed in the *zona glomerulosa* (Table 2). All index cases had PA and severe hypertension diagnosed by the age of 10 years, but without neurological abnormalities. Inheritance of FH-IV is autosomal dominant, though with incomplete penetrance, as carrier parents did not show the same severe hypertension as the index cases [44] and also normotensive individuals with the mutation were observed.

Intriguingly, mutations in KCNJ5 and CACNA1D were also described as somatic mutations in sporadic APAs [42,45]. Other somatic mutations in genes responsible for aldosterone overproduction (*ATP1A1* and *ATP2B3*) or involved in cell proliferation (*CTNNB1*) have also been described but without evidence of similar alterations responsible for familial forms [46,47].

PA patients with APA carrying mutations display in some cases specific steroid profiles that may help clinicians in the decision making process of PA subtype diagnosis [18].

Genetic basis for sporadic BAH are less understood for the difficulty of studying adrenal glands from this patients. In some cases, KCNJ5 mutations have been described, not associated to FH-III [48].

Heterozygous germline mutations in the armadillo repeat containing 5 gene (ARMC5) have been shown in patients with hypercortisolism due to sporadic primary bilateral macronodular adrenal hyperplasia [49] and were also described in observed in African-American PA patients [50] but not in a cohort of Caucasian BAH patients [51].

Conclusions

PA is a multi-faceted disease, which can lead to severe cardio- and cerebro-vascular complications in affected patients. Different subtypes of disease benefit from different treatment options, and should therefore be carefully distinguished, in order to ensure the best management for the patients.

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AVS Indices	Measurement	Clinical use	Cut-offs
Selectivity index (SI)	$\frac{\text{Cortisol}_{\text{adrenal vein}}}{\text{Cortisol}_{\text{peripheral vein}}}$	Adequacy of cannulation of the adrenal veins	SI> 3 = adrenal vein correctly cannulated
Lateralisation index (LI)	$\frac{\text{Aldosterone/Cortisol}_{\text{adrenal vein}}}{\text{Aldosterone/Cortisol}_{\text{contralateral adrenal vein}}}$	Differentiate unilateral from bilateral PA	LI > 4 = unilateral PA LI <3 = bilateral PA
Contra-lateral ratio (CLR)	$\frac{\text{Aldosterone/Cortisol}_{\text{nondominant adrenal vein}}}{\text{Aldosterone/ Cortisol}_{\text{peripheral vein}}}$	Retro-inhibition of aldosterone secretion in the non-dominant adrenal gland	CLR <1 and LI between 3 and 4 = unilateral PA

Table 1. Calculation and clinical use of AVS indices

	FH-I	FH-II	FH-III	FH-IV
Gene	Hybrid *CYP11B1/B2*	Unknown Linkage at 7p22	*KCNJ5*	*CACNA1H*
Transmission	AD	AD	AD	AD Incomplete penetrance
Severity of hypertension	Normotension to resistant	Normotension to resistant	Grade II to resistant hypertension	Normotension to resistant

u-18oxoF u-18OHF	Elevated	Not elevated	Mildly to extremely elevated	Normal
Aldosterone response to dexamethasone	Complete suppression	Partial reduction or no change	Paradoxical increase (in 1 family)	Suppression in 1 patient
Adrenal CT scanning/Adrenal pathology	Normal adrenal glands	BAH or APA	Bilateral hyperplasia or normal adrenals	Marked zona glomerulosa hyperplasia (in 1 patient)

Table 2. Familial forms of primary aldosteronism. FH-I = Familial Hyperaldosteronism type 1; FH-II = Familial Hyperaldosteronism type II; FH-III = Familial Hyperaldosteronism type III; FH-IV = Familial Hyperaldosteronism type IV; AD = autosomal dominant; CCBs = Calcium Channel Blockers; BAH = Bilateral Adrenal Hyperplasia; APA = Aldosterone Producing Adenoma