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Hyperaldosteronism: how to discriminate among different disease forms?

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1 **Abstract**

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

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

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1 **Introduction**

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

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

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

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

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

3

4 **Diagnosis**

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

13

14 **Imaging techniques and steroid measurements**

15 Once PA is confirmed, patients should undergo contrast-enhanced computed
16 tomography (CT) scanning evaluation of the adrenal glands [5,12] as a first exam.
17 CT findings can vary, from bilateral hyperplasia, to unilateral hyperplasia, to
18 unilateral or bilateral micro and macro-nodules (diameter < 1 cm or > 1 cm).
19 Lesions bigger than 4 cm are suspicious for the rare but deadly adrenal carcinoma
20 [5]. CT displays several limitations: even using fine-cuts, CT may fail at identifying
21 micro-APAs; moreover, it does not provide information on the functional role of
22 the nodules, which can be especially crucial when bilateral lesions are observed
23 [13].

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

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

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

25

1 **Adrenal venous sampling**

2 Adrenal venous sampling, or AVS, is widely recognized as the gold standard
3 technique in the distinction of unilateral from bilateral PA [5,12]. In a review of
4 950 cases, imaging technique (either CT scanning or magnetic resonance)
5 resulted in a wrong diagnosis in around 38% of cases compared to AVS [20]. CT
6 scanning can suggest inappropriate adrenalectomy in patients with BAH and
7 unilateral nodule, or miss the opportunity of cure by adrenalectomy in cases of
8 unilateral PA and bilateral nodules (with only one having secretory activity) or
9 normal appearing adrenals and undetected micro-APA [5,20]. The main pitfall of
10 CT scanning is the lack of a proper identification of secretory activity of detected
11 nodules; different parameters have been suggested, such as densitometry and
12 contrast wash-out, but neither has been proven to provide definite answers in the
13 distinction between secreting lesions and incidentalomas [5,21]. Therefore, when
14 adrenalectomy is not contraindicated because of comorbidities, AVS should
15 always be suggested as the only reliable technique for PA subtype diagnosis
16 [5,22].

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

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

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

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

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

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

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

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

21

22 **Genetics of PA**

23 Familial hyperaldosteronism (FH) account for up to 5-6% of all PA cases [30,31].

24 The first form of FH to be discovered is also named glucocorticoid-remediable
25 aldosteronism (GRA, or FH-I), which is caused by the creation of a chimeric gene

1 from the fusion of the promoter region of the 11 β -hydroxylase gene *CYP11B1* with
2 the coding region of the aldosterone synthase gene, *CYP11B2* [32,33] (Table 2).
3 This determines a regulation of aldosterone production under the very active
4 *CYP11B1* (encoding 11 β -hydroxylase) promoter, which is regulated by ACTH.
5 Patients with GRA usually present hypertension in the first two decades of their
6 lives, and an increased risk of cerebral hemorrhage; moreover they show high
7 levels of hybrid steroids. Transmission is autosomal dominant, and the disease,
8 once diagnosed, can be easily managed by administration of low doses of
9 glucocorticoids, often associated with mineralocorticoid receptor blockers [34].
10 FH-II is clinically undistinguishable from sporadic PA, and it is diagnosed by the
11 familial pattern of the disease. Its genetics basis is still unknown, though a linkage
12 to chromosome 7p22 has been suggested [35,36]. It is conceivable that more
13 types of genetic alterations are responsible for this condition (Table 2).
14 FH-III is has been recently described by Choi in 2011 [37]. This disease is
15 characterized more frequently by very early onset of severe hypertension and
16 hyperaldosteronism that require bilateral adrenalectomy in most cases. FH-III is
17 determined by gain-of-function mutations of *KCNJ5* which *KCNJ5* encodes for the
18 potassium inwardly-rectifying channel Kir 3.4 [34] (Table 2); the mutations cause
19 the channel to lose its selectivity for potassium, allowing large quantities of
20 sodium to enter the cell, thus causing a membrane depolarization and the
21 activation of voltage-gated calcium channels, with calcium influx into the cell, and
22 activation of the cascade that results in aldosterone overproduction [38]. It should
23 be noted, that cases with more mild phenotypes have been described [34,39,40].
24 Primary aldosteronism with seizures and neurological abnormalities (PASNA) is
25 an extremely severe form of PA, caused by a germline mutation in *CACNA1D*, which

1 encodes a voltage-gated L-type calcium channel (Table 2); the mutations result in
2 channel activation at less depolarized potentials, which in turn causes calcium
3 influx and aldosterone production [41,42]. Only two cases have been diagnosed
4 so far: both patients were diagnosed at a very young age from healthy parents; it
5 is believed that their neurological impairment is so severe not to allow affected
6 individuals to reproduce. Therefore, this condition is considered not-familial
7 despite the genetic cause and is expected to be caused only by *de novo* mutations.
8 The latest discovery in familial forms is FH-IV, described by Scholl et al. in 2015
9 [43]: FH-IV is caused by a germline mutation in another voltage-gated calcium
10 channel gene, CACNA1H, highly expressed in the *zona glomerulosa* (Table 2). All
11 index cases had PA and severe hypertension diagnosed by the age of 10 years, but
12 without neurological abnormalities. Inheritance of FH-IV is autosomal dominant,
13 though with incomplete penetrance, as carrier parents did not show the same
14 severe hypertension as the index cases [44] and also normotensive individuals
15 with the mutation were observed.

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

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

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

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

9

10 **Conclusions**

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

15

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9

| 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) | $\frac{\text{Aldosterone/Cortisol}_{\text{non-dominant adrenal vein}}}{\text{Aldosterone/Cortisol}_{\text{peripheral vein}}}$ | Retro-inhibition of aldosterone secretion in the non-dominant adrenal gland | CLR < 1 and LI between 3 and 4 = unilateral PA |

10

11 **Table 1. Calculation and clinical use of AVS indices**

| | FH-I | FH-II | FH-III | FH-IV |
|---------------------------------|---------------------------|---------------------------|------------------------------------|-----------------------------|
| Gene | Hybrid <i>CYP11B1/B2</i> | Unknown Linkage at 7p22 | <i>KCNJ5</i> | <i>CACNA1H</i> |
| 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) |

1

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

7