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1 **Adrenal vein sampling in primary aldosteronism: towards a standardized protocol.**

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1 **Abstract**

2 Primary aldosteronism (PA) comprises subtypes that require different therapeutic strategies.
3 Adrenal vein sampling (AVS) is recognized by current Endocrine Society guidelines as the only
4 reliable means to perform the correct subtype diagnosis of PA. Unfortunately, despite being the
5 “gold standard” procedure, there is no a standardized procedure both in terms of performance and
6 interpretation criteria. This review addresses several questions that regard clinicians faced with
7 AVS. For each of these questions we will provide replies based on the available evidence as well as
8 opinions based on our experience. In particular we will discuss the most appropriate way of
9 preparing the patient, if it is possible to avoid AVS for some subgroups of patients, the use of
10 cosyntropin during the procedure, the most appropriate criteria for interpretation of cannulation and
11 lateralisation, the utility of contralateral “suppression” and finally the strategies to improve success
12 rates of AVS in centres with limited experience.

13

14 **Search strategy**

15 We searched in the Cochrane Library (1993-2014) and in MEDLINE (1959-2014). We used the
16 search terms "adrenal vein sampling" or "AVS" in combination with the terms "primary
17 aldosteronism" or "hyperaldosteronism". We selected mainly publications from the past 5 years, but
18 did not exclude important and highly referenced older publications. We also considered the
19 reference lists of articles and reviews identified by this strategy and selected the most relevant.
20 Review articles are cited to provide readers with details and references that are beyond the scope of
21 this Personal Views article.

22

23 **Introduction**

24 Primary aldosteronism (PA) is recognized as the most frequent form of secondary hypertension
25 accounting for 5% of cases of the general hypertensive population and for 10% of patients referred
26 to hypertension units (1). Diagnosis and treatment is of particular relevance since PA it is associated

1 with a higher risk of cardio- and cerebro-vascular events than essential hypertension (2-4). The
2 diagnosis of PA comprises three steps, screening, confirmatory/exclusion testing and subtype
3 diagnosis (5) (specific aspects are reviewed in ref 6-8).

4 This last step is of fundamental importance to allocate patients to correct management, unilateral
5 adrenalectomy for aldosterone-producing adenoma (APA) and unilateral adrenal hyperplasia
6 (UAH) and pharmacotherapy with mineralocorticoid receptor antagonists (MRA) for patients with
7 bilateral adrenal hyperplasia (BAH) (5). Subtype diagnosis comprises CT scanning and adrenal vein
8 sampling (AVS).

9 AVS is a demanding technique, requiring a skilled radiologist; however, for a procedure that is
10 considered the “gold standard” in PA subtype diagnosis there is a poor reproducibility of the
11 interpretation of the results between centres due to a lack of standardisation of protocols i.e.,
12 different criteria and protocols are used to define both cannulation of the adrenal veins and
13 lateralisation of aldosterone production (15,22). This lack of a standardized and widely accepted
14 AVS protocol used in all centres has meant that some clinicians are reluctant to perform AVS or to
15 refer patients to centres where this procedure is performed.

16 In this review we will address several questions that regard clinicians faced with PA subtype
17 diagnosis and in particular with AVS. For each of these questions we will provide replies based on
18 available evidence plus opinions based on our experience.

19

20 ***Subtype diagnosis***

21 First step in subtype diagnosis is the performance of a fine-cuts CT scanning with contrast of the
22 adrenals which is able to exclude the rare but often fatal aldosterone-producing carcinoma, and
23 provides anatomical description of the adrenal morphology and often venous drainage. APA are
24 usually less than 3 cm in diameter and microAPA often less than 1 cm in diameter. MicroAPAs are
25 not always detectable by CT scanning and sometimes considered part of bilateral nodular
26 hyperplasia. Similarly, in the case of bilateral nodularity, it is not possible to distinguish between a

1 non-functioning adrenal adenoma and an APA, and UAH is frequently undetectable by CT scanning
2 (5,11). Unfortunately, CT scanning does not provide information about the secretory activity of a
3 detected nodule and densitometry parameters (Hounsfield units) and contrast wash-out (12) has
4 proven inadequate to distinguish APA from non-secretory nodules (5). Magnetic resonance is
5 inferior to CT scanning because its spatial resolution is lower and therefore is a second choice
6 imaging technique (5).

7 Following the introduction of AVS in the management of subtype diagnosis of PA (13), many
8 studies demonstrated the lack of sensitivity and specificity of CT scanning for the detection of
9 APAs and its ability to distinguish them from non-secretory nodules (11,14-16). Furthermore, the
10 centralisation of CT scanning to a dedicated radiologist improves sensitivity but does not increase
11 specificity in the diagnosis of APA (11). Until now, a randomized controlled trial comparing
12 imaging based decision making versus AVS based decision making with clinical and biochemical
13 remission of PA as outcome is lacking. Furthermore, the analysis of some APA studies is hampered
14 by the fact that the removal of the adenoma is not succeeded by immunohistochemical evaluation
15 and demonstration of biochemical cure of hyperaldosteronism, including post-operative
16 demonstration of aldosterone suppressibility.

17 A systematic review of studies that compared imaging techniques and AVS for final PA subtype
18 diagnosis (using AVS as 'gold' standard) included a total of 950 patients (9). If only imaging was
19 considered, inappropriate adrenalectomy would have occurred in 15% of patients (where AVS
20 showed bilateral PA), inappropriate exclusion from adrenalectomy would have occurred in 19%
21 (where AVS showed unilateral PA) and adrenalectomy on the wrong side would have occurred in
22 4% (where AVS showed aldosterone secretion on the opposite side) (9).

23 For these reasons, when adrenalectomy is not contraindicated for concomitant comorbidities and is
24 desired by the patient, the distinction between unilateral and bilateral PA should be made by AVS
25 (5,17).

26

1 ***Key considerations before AVS***

2 AVS should be performed under no medication that can potentially interfere with the renin-
3 angiotensin system and more specifically that can stimulate renin secretion. For this reason, α 1-
4 adrenergic receptor blockers and long-acting calcium channel blockers (preferably non-
5 dihydropyridine, such as verapamil or diltiazem, although dihydropyridine can also be used), which
6 have no or minimal effects on renin secretion are the preferred drugs to manage blood pressure
7 before AVS (5,18). If needed, β -blockers, angiotensin converting enzyme inhibitors and angiotensin
8 II receptor blockers can also be used in most cases; in contrast, loop and thiazide diuretics,
9 amiloride and MRA should be stopped for at least 4-6 weeks before the sampling. A safe measure
10 to verify that the effect of the drugs on renin levels is lost is to measure renin or plasma renin
11 activity (PRA) and, if suppressed then AVS can be performed regardless of the time the drug has
12 been withdrawn. Potassium levels should also be corrected as much as possible (Panel 1). AVS is
13 best performed early in the morning after at least one hour of recumbency to avoid stimulation of
14 the renin-angiotensin system on aldosterone secretion thereby potentially reducing the gradient
15 between adrenal glands. If AVS is performed in the afternoon, similar attention should be taken and
16 cosyntropin stimulation used to allow correct detection of the cannulation.

17

18 ***AVS procedure***

19 AVS is performed via a percutaneous femoral vein approach and adrenal veins are commonly
20 sequentially catheterized under fluoroscopy (Figure 1). The correct position of the catheter is
21 verified by gentle injection of a small volume (no more than 3 mL) of contrast medium, and blood
22 is then collected by slow aspiration. Left adrenal vein cannulation is relatively easy to perform since
23 it generally merges with the inferior phrenic vein to generate a common trunk draining directly into
24 the left renal vein (19). The shorter and smaller right adrenal vein which usually drains directly into
25 the inferior vena cava may be difficult to locate and cannulate and to distinguish from other
26 adjacent small vessels including small hepatic vein branches (19). The difficulty of placing the

1 catheter tip within the right adrenal vein and the anatomy of the left adrenal vein that joins the
2 phrenic vein sometimes causes dilution of the blood: for this reason it is necessary to measure in
3 each sample both cortisol and aldosterone concentrations. Because cortisol is presumed to be
4 equally produced by both adrenals of a PA patient, the aldosterone measurement corrected by
5 cortisol levels in each sample corrects for dilution by non-adrenal blood. Furthermore, cortisol
6 levels are a measure of correct cannulation of the adrenal veins (Table 1).

7 The experience and the dedication of the interventional radiologist are fundamental for the success
8 of the procedure in that centres in which multiple radiologists perform AVS display much lower
9 success rates of cannulation and higher rates of complications than centres with a dedicated
10 radiologist (21-23).

11

12 **Is it possible to avoid AVS for some subgroups of patients?**

13 According to available guidelines, AVS should be performed in all patients with confirmed PA who
14 are candidates for adrenalectomy (5,17). Unfortunately, AVS is only available in specialized centres
15 and therefore, alternative methods that reduce the number of AVS procedures would be attractive
16 for clinicians. Some centres have observed that in young PA patients (<35-40 years), the presence
17 of a unilateral nodule (> 10 mm in diameter) and normal appearance of the contralateral adrenal
18 gland at CT scanning, is always associated with an APA (11,23) (Panel 2). This finding is in
19 agreement with the observation that non-functioning adrenal adenomas are very rare in young
20 subjects (24). However, even in young patients the occurrence of incidentalomas is not zero; in a
21 recent analysis relying on imaging alone in patients with age below 40, this had a specificity of 83%
22 and a sensitivity of 68% (25). Therefore, the risk of inappropriate adrenalectomy should always be
23 weighed against the rate of AVS complications in each case and each centre.

24 Other clinical characteristics have been associated with APA as opposed to BAH (higher
25 aldosterone and blood pressure levels, lower potassium levels, negative posture test) (23); however,
26 none of these criteria have proven to be specific enough to avoid performing AVS in an individual

1 patient (11,25,26). The [6β - 131 I]iodomethyl-19-norcholesterol (NP-59) scan performed under
2 dexamethasone suppression, has the potential advantage of correlating function with anatomical
3 findings. This technique is not available in all countries, is not sensitive enough to detect small
4 APA and is therefore rarely used (5). Recently, 11 C-metomidate positron emission tomography
5 (PET)-CT scanning has been evaluated for PA subtype diagnosis: the sensitivity (76%) and
6 specificity (87%) were not high enough to replace AVS (27).

7 The measurement of minor steroids (18-hydroxycorticosterone, 18OHB, 18-hydroxycortisol,
8 18OHF and 18-oxo-cortisol, 18oxoF) in serum has also been proposed to distinguish PA subtypes
9 (28-30). 18OHB is synthesized by aldosterone synthase from deoxycorticosterone as an
10 intermediate during aldosterone biosynthesis (31) and is secreted at a higher rate in APA than BAH
11 patients (28,29). 18OHF and 18oxoF, also known as “hybrid steroids”, are synthesized by
12 aldosterone synthase from 11-deoxycortisol (32,33); these two steroids are also present at higher
13 levels in APA than BAH (28,30), and at very high levels in some familial forms of PA (familial
14 hyperaldosteronism type 1 and some families with type 3) (34). Recently, 18OHB, 18OHF and
15 18oxoF have been measured in essential hypertensives and PA patients (35): despite their potential
16 use in PA diagnosis and its subtypes (for example patients with very high values were all APA and
17 with very low values were all essential hypertensives), such measurements need to be standardized
18 and verified in a large population of hypertensives (35). Interestingly, measurement of 18oxoF and
19 18OHB in the adrenal veins has been reported to be useful in subtype diagnosis of PA (36,37).

20 Before performing AVS, familial hyperaldosteronism types I and III (34) should be considered and
21 excluded at least in young PA patients and/or with a suggestive family history (34).

22

23 **Is it preferable to perform AVS with or without ACTH infusion?**

24 Cosyntropin [a synthetic derivative of the adrenocorticotrophic hormone (ACTH) that contains only
25 the first 24 of 39 amino acids of ACTH but retains full function, also called ACTH 1-24] infusion
26 was introduced to AVS to improve its reliability (14). Cosyntropin constant infusion (50 μ g/hour,

1 starting 30 minutes before the procedure) or bolus (usually 0.25mg = 10 IU) is used in many centres
2 to minimize aldosterone fluctuations induced by procedure-associated stress, to maximize the
3 cortisol gradient between the adrenal and peripheral veins and to maximize aldosterone production
4 from the APA if present (20) (Panel 3). In contrast, cosyntropin infusion carries the potential pitfall
5 of stimulating aldosterone secretion from the adrenal contralateral to the APA, resulting in a
6 reduction of the lateralisation index (LI) (Table 1) (38). A recent multicentric study compared the
7 role of both continuous cosyntropin infusion and bolus on the performance and interpretation of
8 AVS (39). Both cosyntropin infusion and bolus resulted in a significant increase in the selectivity
9 index (SI) (Table 1) whereas LI was not significantly affected. In most patients the diagnosis
10 reached with AVS was the same whether unstimulated or cosyntropin infusion or bolus results were
11 considered (39), when strict criteria for interpretation were used (see below). Although there are no
12 studies that have specifically compared the two protocols of cosyntropin stimulation, continuous
13 cosyntropin infusion is preferable to intravenous bolus because it avoids the fluctuation in
14 aldosterone concentration but does not cause the supraphysiological stimulation of the bolus that
15 may stimulate aldosterone production from the contralateral adrenal gland. In some centres, both
16 basal and cosyntropin stimulated AVS are performed in each patient. Cosyntropin stimulation is
17 necessary for those patients with a history of contrast allergy that require preparation with steroids
18 before the procedure and when the procedure is not performed in the early morning when the
19 ACTH secretion is maximal.

20

21 **Which adrenal/peripheral cortisol ratio is preferable to define successful cannulation?**

22 Selectivity index (SI) is defined as the ratio between the cortisol measured in the adrenal vein and in
23 a peripheral vein (often the inferior vena cava) (Table 1). SI measures the adequacy of adrenal vein
24 cannulation and therefore, has to be higher than 1 (Panel 4). However, there is no consensus on the
25 ideal SI, with cut-offs between 1.1 and 3 under basal conditions (16,40) with most authors using an
26 SI between 2 and 3 (41,42) and between 2 and 10 after cosyntropin stimulation (21) and most

1 commonly between 3 and 5 (15,43,44). In a study performed in the Torino and Brisbane units, the
2 reproducibility of the diagnosis between two AVS performed in the same patient under basal
3 conditions, using different criteria, was evaluated (42). AVS in each patient was repeated because it
4 was not considered successful the first time according to the unit criteria. The authors observed that
5 a $SI > 2.7$ was necessary to achieve the reproducibility between AVS in the same patient (42).
6 Evaluation of the procedures using $SI < 2$ resulted in discordant diagnoses in a high proportion of
7 cases and would result in the removal of the wrong adrenal in 14% of cases (42). The requirement
8 of a high SI for diagnostic reproducibility was also seen in a study using high doses of continuous
9 cosyntropin infusion (45). The use of a high SI for AVS interpretation is associated with a lower
10 proportion of procedures that achieve a diagnosis: however, an increase in procedure success may
11 be obtainable by other strategies (see below) rather than using lower SI cut-offs and thereby risking
12 an incorrect diagnosis and inappropriate therapy.

13
14 **Which aldosterone/cortisol ratio (adrenal to contralateral or adrenal to peripheral) is**
15 **preferable to define lateralisation?**

16 This is an important issue because the LI determines the final therapeutic decision for the patient,
17 surgical with unilateral adrenalectomy or pharmacological with MRA. The ideal LI (Table 1)
18 should be theoretically identified by evaluation of post-surgical improvement of blood pressure
19 levels and biochemical cure of PA after adrenalectomy (43): in such a study all dominant adrenals
20 (that is those with the higher aldosterone/cortisol ratio) should be removed regardless of the LI
21 value. This type of study is obviously not feasible for ethical reasons and therefore the question
22 remains unresolved. Most centres require $LI > 4$ to indicate unilateral adrenalectomy, but many
23 centres accept an LI between 3 and 4 and a few centres use a LI between 2 and 3 (Panel 4); finally
24 one centre does not use a LI but requires an ipsilateral ratio (ILR) > 2 (Table 1) together with a
25 contralateral ratio (CLR) < 1 (Table 1) to diagnose unilateral PA (14). It is conceivable that $LI > 4$
26 are definitively diagnostic of unilateral PA and $LI < 2$ are consistent with bilateral PA, with

1 intermediate values representing a grey zone between the two conditions: in such cases, other
2 clinical, biochemical and AVS findings (such as CLR and ILR) can be used to reach a therapeutic
3 decision in the single patient (46). Some authors evaluate absolute aldosterone levels, that if higher
4 than 1,400 ng/dL in one adrenal vein, suggest aldosterone hypersecretion from that side (17).
5 However, we recommend to always take into account the cortisol corrected ratios, because the
6 contralateral adrenal could also secrete very high aldosterone levels and a non-selective cannulation
7 would result in a false diagnosis of unilateral PA.

8

9 **Is contralateral “suppression” necessary?**

10 Contralateral suppression defines patients that on AVS show an aldosterone to cortisol ratio in the
11 non-dominant adrenal less than the ratio in the peripheral vein sample (Table 1). Some units have
12 suggested that contralateral suppression might identify the source of aldosterone hyperproduction
13 when only one adrenal vein is able to be accessed (43,46) (Panel 4): however, there is agreement
14 that the CLR cannot be relied on to predict unilateral PA by itself, since up to 30% of BAH patients
15 display CLR <1 (15). It is not known if the presence of CLR<1 should be a prerequisite for
16 recommending adrenalectomy since it has not been systematically evaluated in terms of outcome.
17 For instance, a few centres use it to define the diagnosis of unilateral PA together with an ipsilateral
18 ratio (ILR) >2 (Table 1) (16).

19 The concept of CL suppression does not reflect a complete suppression of aldosterone secretion in
20 the contralateral gland: in fact, the aldosterone levels measured from the contralateral adrenal gland
21 to an APA are usually higher than aldosterone levels in a peripheral vein. It should be noted that in
22 most cases peripheral blood is expected to have low aldosterone/cortisol ratios, given the longer
23 half-life of cortisol when compared to aldosterone. The fact that the aldosterone hyperproduction
24 from an APA is not sufficient to completely inhibit aldosterone production from the “normal”
25 adrenal cortex has been reinforced by the observation that the zona glomerulosa surrounding an
26 APA is often hyperplastic, containing nodules that may express aldosterone synthase (47). These

1 observations recall the findings that rats under long-term high sodium diets display a reduction in
2 the size of the zona glomerulosa, but always show nests of cells with high aldosterone synthase
3 expression levels (48). For this reason some authors have hypothesized that a proportion of PA
4 patients with unilateral adrenal disease are in reality affected by BAH in which a nodule
5 subsequently became dominant analogous to a multinodular thyroid goiter (49,50). However,
6 several studies have demonstrated recently that somatic mutations in different genes (*KCNJ5*,
7 *ATP1A1*, *ATP2B3* and *CACNA1D*) are present in APAs, but not in BAH patients (34,51-54). Some
8 authors observed an effect of the mutational status of the APA on LI (55) whereas in other cohort
9 this was not the case (56).

10

11 **How can success rates of AVS in centres with limited experience be improved?**

12 For AVS to be more widely used it is crucial that centres with non-expert radiologists improve the
13 rate of successful, and therefore diagnostic, procedures. The first and fundamental issue is the
14 training of one or at most two motivated radiologists who perform all the procedures: it has been
15 shown that centres having more radiologists performing AVS have much lower success rates than
16 centres with a single expert radiologist (15,16,19,20,22,42,44,57). AVS success increases with the
17 standardization of the protocol and with experience: wherever possible, procedures should be
18 concentrated in a single centre per geographical area to increase the number of AVS per year. It is
19 also important that all AVS results are discussed together by the specialists involved in the patient's
20 management (hypertension specialist/endocrinologist and radiologist) and that the clinician
21 consulted attends the radiological procedure (41). This feed-back and encouragement between
22 physicians clearly contributes to an increase in AVS success rate.

23 Centres that perform AVS should generate a standardized AVS report including all measurements, a
24 statement of final PA subtype diagnosis and a recommendation regarding treatment (Panel 5). The
25 use of a specified protocol in each centre is a fundamental requirement while waiting for a
26 universally accepted and standardized protocol to be used in all centres. A recent study showed that

1 the use of cosyntropin infusion resulted in a higher number of AVS with diagnostic results
2 compared to the unstimulated procedure (39). Therefore, AVS with cosyntropin infusion should be
3 considered together with or in alternative to the basal procedure in units with a low success rate or
4 limited experience. Finally, and more importantly, many studies have shown the fundamental role
5 of the rapid cortisol assay during AVS (41,58-60). Cortisol is measured immediately after sampling
6 from each site and results are given in a short time (less than 20 minutes) allowing an almost
7 immediate feed-back on the success of the procedure. Most units that employ the intraprocedural
8 cortisol assay, measure the hormone at the central laboratory of the hospital (41,59,61) usually
9 using an immunochemiluminometric assay (41,61); in the Torino unit, the use of a quick and
10 reliable cortisol immunofluorimetric assay method that can be performed inside the radiology room
11 using a benchtop analyzer and provides the radiologist with almost immediate information on the
12 correct positioning of the catheter tip for sampling, and reduces the timing and risk of confusion in
13 tube handling (58). All rapid cortisol measurements have the advantage of allowing the radiologist
14 further attempts at cannulation until cortisol measurements demonstrate sampling success. This has
15 an impact both on self-training of the radiologist and in reducing the number of unsuccessful
16 procedures (41,58-61).

17

18 **Unresolved issues**

19 Over the last decade many studies have helped clarify AVS protocol and interpretation; a number of
20 unresolved issues remain. One is the simultaneous as opposed to sequential adrenal vein
21 cannulation. Simultaneous cannulation has the potential advantage of avoiding oscillations of
22 aldosterone secretion during the procedure, and the disadvantage of making AVS even more
23 difficult and demanding. In our experience the variations in aldosterone and cortisol production
24 during the procedure are minimal if AVS is relatively rapid and uncomplicated. Another issue is the
25 interpretation of AVS in cases in which the APA co-secretes a variable amount of cortisol (62). The
26 simultaneous cosecretion of aldosterone and cortisol from the APA may cause a reduction of LI and

1 result in the diagnosis of bilateral PA. The presence of an APA that cosecretes cortisol has been
2 found to be rare in an Italian study (62) but more frequent in the Japanese population (63). It seems
3 that this occurrence is more frequent when the adenoma is large in size (64): for this reason we
4 suggest performing an overnight 1 mg dexamethasone suppression test in patients with a suspect
5 APA larger than 10 mm before AVS; if positive, the procedure should be performed during
6 cosyntropin infusion. Alternatively, plasma metanephrine rather than cortisol might be used to
7 correct for blood dilution during AVS (65).

8 Another issue is the interpretation of AVS with an aldosterone/cortisol ratio in both adrenal veins
9 that is lower than that measured in a peripheral vein: a recent study showed that repetition of the
10 procedure show half of the patients have a unilateral PA (66); it is not known if the performance of
11 the procedure under cosyntropin infusion could avoid repetition of the AVS.

12 Finally, it has been shown recently that it is possible to perform a super-selective cannulation of the
13 adrenal branches: this technique could be potentially applied during AVS in patients with adrenals
14 that cosecrete aldosterone and cortisol from different nodules, to distinguish BAH from bilateral
15 APAs, and in those (rare) patients who are candidates for partial adrenalectomy (67,68). At present
16 it is unknown if this technique is associated with a higher rate of complications than the usual
17 procedure.

18 AVS cut-offs are destined to be arbitrary and difficult to be prospectively tested in most cases.

19 Therefore, AVS consensus guidelines are necessary and should consider all relevant information
20 concerning a patient to obtain the best diagnosis and treatment.

21 Recently, a consensus statement was published by other authors that has some common
22 recommendations with our manuscript and some differences. The differences include the impact of
23 the stress on the final diagnosis, the importance of the simultaneous cannulation in unstimulated
24 procedures, the role of cosyntropin stimulation and the cut-off level of SI and LI (69).

25

26 **Conclusions**

1 Following the publication of the first Endocrine Society Guidelines for the diagnosis and treatment
2 of PA, it has become increasingly appreciated that PA is a frequent condition and therefore a
3 significantly large number of hypertensive subjects now undergo screening and
4 confirmatory/exclusion testing. AVS has undoubtedly been proven to be the only reliable means for
5 PA subtype diagnosis to direct patients to surgery or medical treatment. The training of dedicated
6 radiologists and the use of standardized protocols for the performance and interpretation of AVS,
7 including the use of higher SI and LI for interpretation of the results and the use of an
8 intraprocedural rapid cortisol assay, results in robust PA subtype diagnosis and may improve the
9 therapeutic management of this disease.

10 **Legend to figure 1.**

11 Schematic description of adrenal vein anatomy and adrenal vein sampling performance.

12

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15

16 **Contributors**

17 S.M., A.V. and P.M. performed the literature search and wrote the manuscript; D.R., F.V., C.G-S.

18 and M.R. critically revised the manuscript and provided suggestions and comments.

19

20 **Conflicts of interest**

21 SM, AV, DR, FV, MR, CG-S and PM have no conflict of interest.

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30 *Panel 1*

31 **Selection and preparation of the patients for AVS**

- 32 ● AVS only for patients with confirmed PA and after CT scan
- 33 ● Rule out familial hyperaldosteronism type I and III
- 34 ● Rule out subclinical hypercortisolism for APA > 10 mm
- 35 ● Have a defined standardized protocol
- 36 ● Withdraw interfering medication (especially diuretics and MRA)
- 37 and/or have PRA < 1 ng/ml/h (or PRC < 20 U/ml)
- 38 ● At least 1 h recumbency before beginning of the procedure
- 39 ● Measure cortisol and aldosterone in each sample
- 40 ● Take a sample for cortisol and aldosterone assay from a
- 41 peripheral vein every time an adrenal vein is sampled

42 *Panel 2*

43 **Patients that could avoid AVS**

- 44 ● If < 40 y.o., with unilateral APA > 10 mm and normal
- 45 contralateral adrenal
- 46 ● Positive ¹¹C-metomidate PET-CT and no AVS available
- 47 ● Severe clinical phenotype, including high aldosterone and
- 48 hypokalemia, unilateral lesions on CT, FH1 and 3
- 49 excluded and AVS unavailable on site or by referral
- 50
- 51

52 *Panel 3*

- 1 **Use of cosyntropin infusion**
 2 ●Increases SI and success rate of cannulation of the adrenal veins
 3 ●Does not change LI and final diagnosis
 4 ●Avoids potential fluctuation in aldosterone secretion
 5 ●Is necessary for the allergic patient undergoing steroid
 6 treatment before the procedure
 7 ●Is necessary when the procedure is not performed
 8 in the early morning
 9 ●If AVS is performed without cosyntropin, collect simultaneous
 10 peripheral samples in sequential AVS or perform
 11 simultaneous bilateral AVS

12
 13 *Panel 4*

- 14 **Role of SI, LI and CLR**
 15 ●SI expresses the correct cannulation of the adrenal veins
 16 ●SI should be > 3 under basal conditions
 17 and > 5 under cosyntropin
 18 ●AVS with SI <2 (basal) or <3 (cosyntropin) should
 19 be discarded as not diagnostic
 20 ●No LI have been validated in prospective trials
 21 ●LI > 4 are considered diagnostic for unilateral PA
 22 ●LI < 3 are considered diagnostic for bilateral PA
 23 ●For intermediate values, clinical and biochemical
 24 factors are used for final decision
 25 ●Contralateral suppression is defined by CLR<1
 26 ●Relevance for adrenalectomy indication unknown
 27 ●Useful for interpretation of suboptimal AVS studies

28
 29
 30 *Panel 5*

- 31 **Use of standardised AVS report**
 32 ●report aldosterone concentration, cortisol concentration and
 33 aldosterone/cortisol ratio for each collected sample
 34 ●the minimum data set comprises right adrenal vein, left adrenal
 35 vein, and inferior vena cava sample
 36 ●calculate and report SI including centre-specific cut-offs
 37 ●calculate and report LI including centre-specific cut-offs
 38 ●calculate and report CLR
 39 ●generate a statement regarding PA subtype and a
 40 recommendation regarding treatment

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AVS Indices	Measurement	Clinical significance	Suggested Cut-off
Selectivity Index (SI)	$\frac{\text{Cortisol}_{\text{adrenal vein}}}{\text{Cortisol}_{\text{peripheral vein}}}$	Adequacy of cannulation of the adrenal veins	Minimal requirement of SI>2 under basal conditions, SI>3 during cosyntropin (>3 and >5)

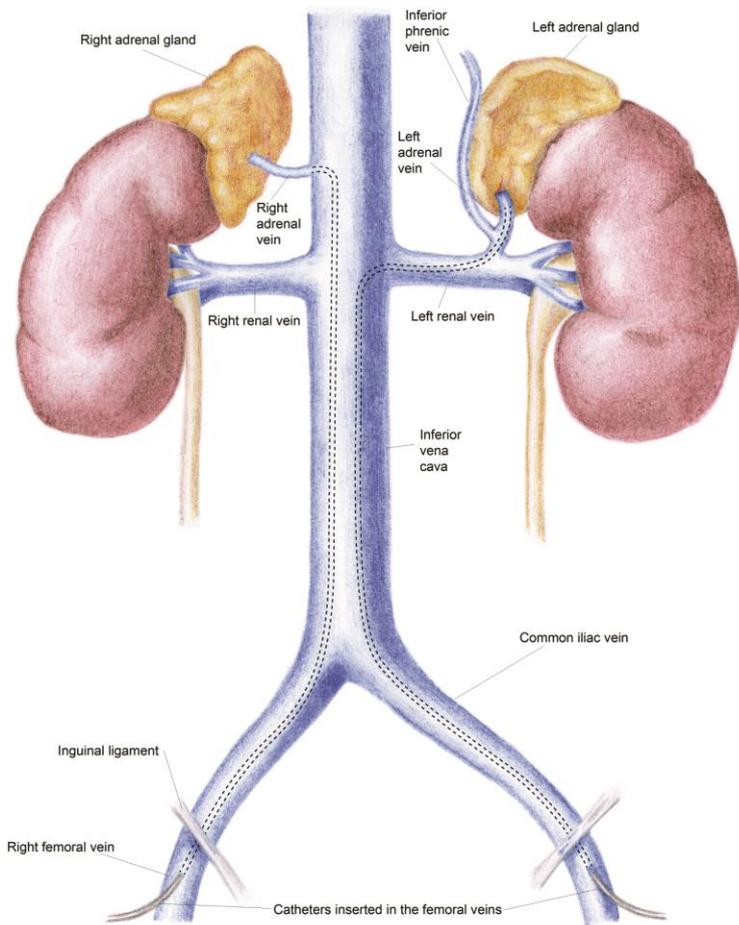
			respectively are preferable)
Lateralisation Index (LI)	$\frac{\text{Aldosterone/Cortisol}_{\text{adrenal vein}}}{\text{Aldosterone/Cortisol}_{\text{contralateral adrenal vein}}}$	Lateralisation of aldosterone production. To distinguish between unilateral and bilateral PA	LI > 4 indicates unilateral PA; LI < 3 indicates bilateral PA; 3 < LI < 4 is a grey zone
Contralateral ratio (CLR)	$\frac{\text{Aldosterone/Cortisol}_{\text{nondominant adrenal vein}}}{\text{Aldosterone/Cortisol}_{\text{peripheral vein}}}$	Inhibition of aldosterone secretion in the non-dominant adrenal gland	CLR < 1 confirms unilateral PA in the opposite side; can be used when the other adrenal vein is not cannulated or when LI is in the grey zone
Ipsilateral Ratio (ILR)	$\frac{\text{Aldosterone/Cortisol}_{\text{dominant adrenal vein}}}{\text{Aldosterone/Cortisol}_{\text{peripheral vein}}}$	Gradient between the dominant adrenal and the peripheral vein	ILR > 2 is required together with CLR < 1 in some centres to diagnose unilateral PA

1

2 **Table 1. AVS indices, definition and clinical significance and cut-offs in the clinical setting.**

3

4



1