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

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Abstract

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

Search strategy

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

Introduction

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

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

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

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

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

Subtype diagnosis

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

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

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

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

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

Key considerations before AVS

AVS should be performed under no medication that can potentially interfere with the renin-angiotensin system and more specifically that can stimulate renin secretion. For this reason, α1-adrenergic receptor blockers and long-acting calcium channel blockers (preferably non-dihydropyridine, such as verapamil or diltiazem, although dihydropyridine can also be used), which have no or minimal effects on renin secretion are the preferred drugs to manage blood pressure before AVS (5,18). If needed, β-blockers, angiotensin converting enzyme inhibitors and angiotensin II receptor blockers can also be used in most cases; in contrast, loop and thiazide diuretics, amiloride and MRA should be stopped for at least 4-6 weeks before the sampling. A safe measure to verify that the effect of the drugs on renin levels is lost is to measure renin or plasma renin activity (PRA) and, if suppressed then AVS can be performed regardless of the time the drug has been withdrawn. Potassium levels should also be corrected as much as possible (Panel 1). AVS is best performed early in the morning after at least one hour of recumbency to avoid stimulation of the renin-angiotensin system on aldosterone secretion thereby potentially reducing the gradient between adrenal glands. If AVS is performed in the afternoon, similar attention should be taken and cosyntropin stimulation used to allow correct detection of the cannulation.

AVS procedure

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

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

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

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

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

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

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

The measurement of minor steroids (18-hydroxycorticosterone, 18OHB, 18-hydroxycortisol, 18OHF and 18-oxo-cortisol, 18oxoF) in serum has also been proposed to distinguish PA subtypes (28-30). 18OHB is synthesized by aldosterone synthase from deoxycorticosterone as an intermediate during aldosterone biosynthesis (31) and is secreted at a higher rate in APA than BAH patients (28,29). 18OHF and 18oxoF, also known as "hybrid steroids", are synthesized by aldosterone synthase from 11-deoxycortisol (32,33); these two steroids are also present at higher levels in APA than BAH (28,30), and at very high levels in some familial forms of PA (familial hyperaldosteronism type 1 and some families with type 3) (34). Recently, 18OHB, 18OHF and 18oxoF have been measured in essential hypertensives and PA patients (35): despite their potential use in PA diagnosis and its subtypes (for example patients with very high values were all APA and with very low values were all essential hypertensives), such measurements need to be standardized and verified in a large population of hypertensives (35). Interestingly, measurement of 18oxoF and 18OHB in the adrenal veins has been reported to be useful in subtype diagnosis of PA (36,37). Before performing AVS, familial hyperaldosteronism types I and III (34) should be considered and excluded at least in young PA patients and/or with a suggestive family history (34).

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

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

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

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

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

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

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

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

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

Is contralateral "suppression" necessary?

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

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

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

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

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

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

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

Unresolved issues

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

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

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

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

AVS cut-offs are destined to be arbitrary and difficult to be prospectively tested in most cases. Therefore, AVS consensus guidelines are necessary and should consider all relevant information concerning a patient to obtain the best diagnosis and treatment.

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

Conclusions

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

Legend to figure 1.

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

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S.M., A.V. and P.M. performed the literature search and wrote the manuscript; D.R., F.V., C.G-S. and M.R. critically revised the manuscript and provided suggestions and comments.

Conflicts of interest

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

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Panel 1

Selection and preparation of the patients for AVS

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

Panel 2

Patients that could avoid AVS

- If <40 y.o., with unilateral APA>10mm and normal contralateral adrenal
- Positive ^{11}C-metomidate PET-CT and no AVS available
- Severe clinical phenotype, including high aldosterone and hypokalemia, unilateral lesions on CT, FH1 and 3 excluded and AVS unavailable on site or by referral

Panel 3

Use of cosyntropin infusion

- Increases SI and success rate of cannulation of the adrenal veins
- Does not change LI and final diagnosis
- Avoids potential fluctuation in aldosterone secretion
- Is necessary for the allergic patient undergoing steroid treatment before the procedure
- Is necessary when the procedure is not performed in the early morning
- If AVS is perfomed without cosyntropin, collect simultaneous peripheral samples in sequential AVS or perform simultaneous bilateral AVS

Panel 4

Role of SI, LI and CLR

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

Panel 5

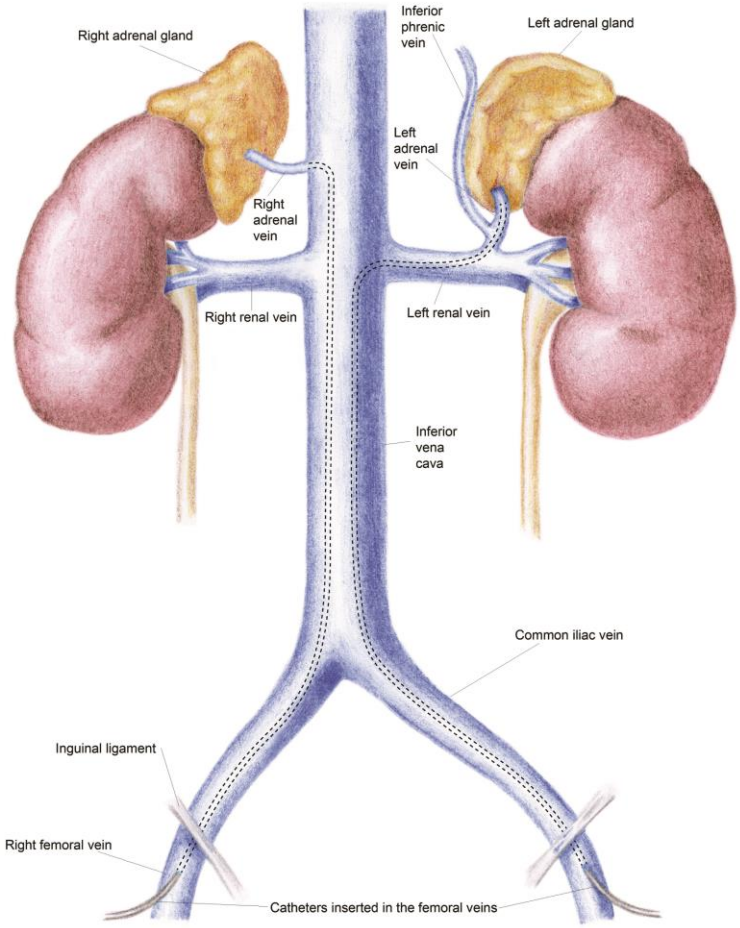
Use of standardised AVS report

- report aldosterone concentration, cortisol concentration and aldosterone/cortisol ratio for each collected sample
- the minimum data set comprises right adrenal vein, left adrenal vein, and inferior vena cava sample
- calculate and report SI including centre-specific cut-offs
- calculate and report LI including centre-specific cut-offs
- calculate and report CLR
- generate a statement regarding PA subtype and a recommendation regarding treatment

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

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


Right adrenal gland
Inferior phrenic vein
Left adrenal gland
Left adrenal vein
Right adrenal vein
Right renal vein
Left renal vein
Inferior vena cava
Common iliac vein
Inguinal ligament
Right femoral vein
Catheters inserted in the femoral veins

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