Adrenal Venous Sampling
Where Do We Stand?

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KEYWORDS

• Aldosterone • Diagnosis • Hyperaldosteronism • Adrenal vein sampling

KEY POINTS

- The high diagnostic accuracy, minimal rate of complications, and better outcome with adrenal venous sampling-guided adrenalectomy support the guidelines recommendation that adrenal venous sampling should serve as the gold standard diagnostic test for the subtyping of primary aldosteronism.
- With some exceptions, adrenal venous sampling should be used systematically before referring a patient to the surgeon.
- As for all invasive diagnostic tests appropriate training of the interventionists, a tight collaboration with experienced endocrinologists and hypertensiologists is needed.
- Adherence to the suggestions that are herein summarized for the interpretation of the test will make the best clinical use of adrenal venous sampling for the doctor and for the patient.

INTRODUCTION

Primary aldosteronism (PA) is the most common endocrine form of hypertension and carries an increased risk of damage to the target organs of hypertension with ensuing cardiorenal complications.[1–3] Accordingly, early identification of affected patients followed by early institution of a specific treatment is a key step for prevention of cardiovascular events and reversal of damage.

Selection of the most appropriate treatment for patients with PA requires the distinction between bilateral and unilateral forms of PA. The former comprise adrenal hyperplasia (also known as idiopathic hyperaldosteronism), which requires a target

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medical therapy, whereas the latter, which are optimally treated with unilateral adrenalectomy, mainly entail aldosterone-producing adenoma (APA) and, less commonly, unilateral adrenal hyperplasia.[4]

To the aim of distinguishing unilateral from bilateral causes, all current clinical practice guidelines recommend adrenal vein sampling (AVS) to measure the plasma aldosterone concentration (PAC) and also a marker of selectivity, usually the plasma cortisol concentration (PCC), in adrenal vein blood.[5,6]

Unfortunately, AVS is not so widely available as it should be: for example, even in Europe there are entire countries where no center can offer AVS. Moreover, AVS is underused even at major referral centers as shown, by the Adrenal Vein sampling International Study (AVIS-1), a survey of AVS use worldwide.[7] The far from optimal clinical use of AVS translates into worse outcomes, as shown in the recently published AVIS-2 Study,[8] which provided evidence of a better outcome when adrenalectomy was AVS guided than when it was not.

Hence, the question, "Where do we stand now with AVS?" is timely and appropriate, and is addressed in this review.

WHY IS ADRENAL VEIN SAMPLING UNDERUSED?

AVS is a straightforward diagnostic test, but notwithstanding this, it is underused owing to a number of misconceptions, including the idea that AVS is technically challenging, invasive, risky, and, furthermore, that it is not always necessary, despite abundant evidence to the contrary.[7] Furthermore, a lack of accepted standards for the performance of AVS and of established criteria for interpretation of its results, contributes to prevent appropriate use of AVS in many patients with PA. The publication of a severely biased[9] randomized clinical trial[10] added further fuel to worsen this already worrying scenario. As a result of this study, far too many patients with PA are denied curative adrenalectomy, because lateralized aldosterone excess could not be convincingly demonstrated or, even worse, undergo adrenalectomy without such demonstration, which might translate into removal of the nonculprit adrenal gland.[11,12]

The largest registry of patients with PA submitted to AVS over 15 years in 19 referral centers showed that AVS was successful in 80.1% of all cases and allowed identification of unilateral PA in only 45.5%.[8] Moreover, adrenalectomy was performed in about 42% of all patients and cured arterial hypertension in roughly 20% of them, 2-fold more frequently in women than men ($P<.001$). When AVS-guided, surgery provided a higher rate of cure of hypertension than when not AVS-guided (40% vs. 30%; $P = .027$). Compared with surgical cases, patients treated medically needed more antihypertensive medications ($P<.001$) and exhibited a higher rate of persistent hypokalemia requiring potassium supplementation (4.9% vs. 2.3%; $P<.01$). Hence, the low rate of adrenalectomy and cure of hypertension indicates suboptimal AVS use in patients with PA seeking surgical cure. Although this suboptimal use is related to issues in patient selection, technical success, and AVS data interpretation, the better outcome of AVS-guided adrenalectomy calls for actions to improve the diagnostic use of this test.[8]

SELECTION OF THE PATIENTS FOR ADRENAL VEIN SAMPLING

Before considering AVS, it is mandatory to have reached an unequivocal biochemical diagnosis of PA, because this test aims at identifying the surgically curable cases of PA (**Fig. 1**).

An exception to this rule is patients with drug-resistant hypertension, who often have concealed PA. These patients are on multiple antihypertensive drugs, which renders very difficult, and sometimes even impossible, to reach a conclusive biochemical

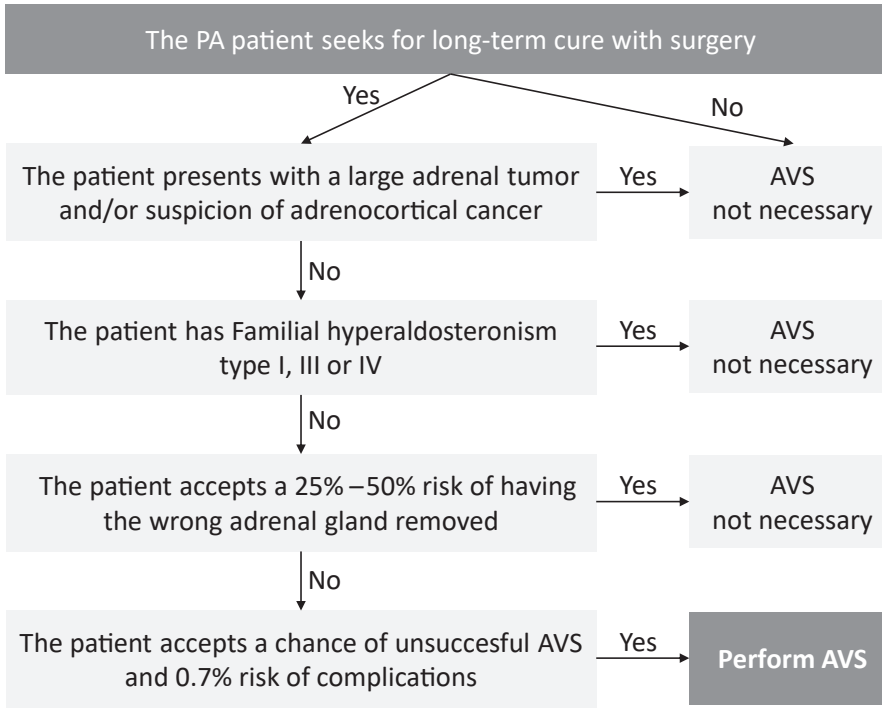

Fig. 1. Flow chart showing the algorithm to be used to select the patients for AVS.

diagnosis of PA. In these patients with very high cardiovascular risk, unilateral laparoscopic adrenalectomy often results in control of the high blood pressure values. If renin is not upregulated, AVS can be in performed in these patients, and demonstration of a lateralized aldosterone excess in the authors' experience is feasible.

Another exception entails the patients with germ-line mutations causing bilateral adrenal hyperplasia, for example, those with familial hyperaldosteronism (**Box 1**),[13] who should not undergo AVS, because they are unlikely to have a unilateral form of PA.

Finally, AVS is not indicated when either the patient prefers life-long medical treatment with a mineralocorticoid receptor (MR) antagonist, or the risks of surgery outweigh its benefits because of the patient's age or risk of general anesthesia and surgery itself, or when surgery is already mandated by the radiologic features, and/or size of the adrenal mass suspicious of adrenocortical carcinoma.

It has been contended that AVS might be skipped in young patients (eg, <35 years of age)[11] with a florid PA phenotype (as evidenced by spontaneous hypokalemia,

Box 1
Conditions for which AVS is not indicated in the patients with PA

1. Patients who are not candidate to general anesthesia and/or surgery;
2. Patients who prefer medical treatment;
3. Patients with familial hyperaldosteronism owing to germline mutations;
4. Patients with large adrenal tumors suggesting adrenocortical carcinoma.

undetectable renin, high plasma aldosterone concentration, and a clear-cut unilateral cortical adenoma with a contralateral normal adrenal on computed adrenal imaging),[14] because nonfunctioning adrenocortical adenomas ("incidentaloma") are infrequent in young people. However, the evidence supporting this proposition is weak and limited to only 6 patients, who fulfilled these criteria in a retrospective study.[11]

In summary, without AVS it is impossible to exclude (i) bilateral aldosterone secretion and (ii) unilateral aldosterone excess from a small adrenal computed tomography (CT)-invisible APA in the adrenal gland contralateral to a CT-detectable adrenal mass.

UNRELIABILITY OF IMAGING

It is intuitive, and also proved by overwhelming evidence, that imaging (with CT scans and MRI) cannot identify unilateral aldosteronism. Accordingly, all available evidences concur in demonstrating the poor accuracy of imaging in localizing the source of aldosterone excess. Furthermore, aldosterone-producing microadenoma and most cases of idiopathic hyperaldosteronism are, by definition, CT- and MR-undetectable. Therefore, in line with the global experience, to distinguish between unilateral and bilateral aldosterone excess, the US Endocrine Society and the Japan Endocrine Society guidelines recommend that AVS be offered to all patients with an unequivocal diagnosis of PA who want to pursue surgical cure.[5,6] For a more in-depth discussion of the reasons why AVS cannot be skipped the reader is referred to recent papers published elsewhere.[15,16]

OUTCOME OF ADRENAL VEIN SAMPLING-GUIDED ADRENALECTOMY

Nearly all patients who undergo AVS-guided adrenalectomy benefitted from surgery, as evidenced by correction of the hyperaldosteronism and improved blood pressure control; even if hypertension was not cured, drug treatment could be tapered and long-term regression of left ventricular hypertrophy occurred.[2] However, when proposing AVS the clinician should discuss with the patient realistic expectations of surgical outcomes.

Because surgical treatment is simply not feasible in the large proportion of the hypertensive patient population (6% to $\geq$11%) that has unilateral PA, a selection policy must be implemented in countries where health resources are limited. To this end, it is worth considering that some preoperative characteristics are associated with cure of hypertension after adrenalectomy, such as young age, shorter duration of hypertension (eg, <5 years), fewer antihypertensive medications (eg, $\leq$2), higher preoperative blood pressure and normal renal function, a body mass index of 25 kg/m^2 or less, female sex, lack of a family history of hypertension,[17–22] and no evidence of vascular remodeling.[17]

Therefore, in most public health care systems, priority should be given to (i) young patients, and particularly women as shown in the AVIS-2 Study,[8] who are the most likely to be cured and to gain the most in life-years off-treatment and (ii) patients with resistant hypertension (or antihypertensive drug intolerance), whose absolute risk of cardiovascular complications is the highest.

PREPARATION OF THE PATIENT FOR ADRENAL VEIN SAMPLING

The success of AVS requires an interdisciplinary team made of experienced hypertensiologists and/or endocrinologists and well-trained interventionists. Careful preparation of the patient for the procedure and standardization of the conditions for its performance are also essential steps. These recommendations are based on knowledge the factors that influence technical success and accuracy (**Box 2**).[18]

Box 2
Summary of recommendations on how to perform AVS

1. Prerequisites for performing AVS
   - Prior correction of hypokalemia
   - Prior adjustment of antihypertensive medications
   - A multidisciplinary team in centers with extensive expertise
   - One hour of supine rest
   - Only if the success rate in achieving cannulation is low and the rapid cortisol assay is not available use cosyntropin stimulation
2. Preparation of the patient and preferred techniques for AVS
   - Prevent and treat emotional and pain-related stress, because it can increase the SI, but lowers the LI
   - The bilaterally simultaneous sampling should be the method of choice because it minimizes the time-related variability owing to the pulsatile pattern of secretion of cortisol and aldosterone, which can generate variability in hormone concentrations in the adrenal vein blood.
3. Pharmacologic stimulation
   - Cosyntropin stimulation during AVS facilitates the assessment of selective adrenal vein catheterization; however, it confounds or even invert lateralization because it decreases the relative secretion index and there is no conclusive evidence that cosyntropin stimulation leads to a better outcome than unstimulated AVS
   - Metoclopramide does not increase the SI or the LI. However, it helps in unmasking factitious contralateral suppression in non-APA cases

Hypokalemia, if present on the day of admission or during the previous days, should be corrected with oral or intravenous potassium supplements before AVS, because it decreases aldosterone secretion, thus potentially masking a unilateral aldosterone excess.[18]

Complete withdrawal of antihypertensive treatment is neither indicated nor necessary, but careful adjustment of the antihypertensive agents before and during AVS is important. Peripheral alpha1-adrenergic receptor blockers (eg, doxazosin mesylate) and/or the long-acting dihydropyridine or nondihydropyridine calcium-channel blockers (verapamil or diltiazem) are recommended, because these agents negligibly affect renin secretion. Even in stage 3, and/or drug-resistant hypertensive patients, who need multiple agents, including angiotensin I converting enzyme inhibitors, angiotensin receptor blockers, diuretics, and beta-adrenergic blockers, AVS can provide accurate diagnostic information, as long as renin is suppressed.[18] Nonsuppressed renin can stimulate aldosterone secretion from the unaffected side, thus minimizing the lateralization. As MR antagonists have the potential to increase renin, it is generally held that these drugs should be withdrawn for at least 6 weeks, and amiloride should be held for at least 2 weeks before AVS. The clinical risk of this withdrawal is probably small, even in patients with resistant hypertension,[19] but this is outweighed by the long-term benefits if a lateralized aldosterone excess is demonstrated. However, even though the issue of whether to withdraw MR antagonists and for how long has not been investigated prospectively, an anecdotal report,[20] and a retrospective study,[21] would suggest the possibility of using MRA as long as renin is not increased.

The use of plasma renin measurement to decide whether to perform AVS without withdrawing, or downtitrating, the MR antagonists is based on the premise that the finding of low renin is evidence for unlikely stimulation of the contralateral adrenal cortex at a level sufficient to confuse interpretation of lateralization. Nonetheless, in the presence of elevated renin (eg, a direct renin of >10 mIU/L) or hypokalemia, the results of AVS should be considered with caution because they are probably not valid.

TIME OF ADRENAL VEIN SAMPLING

To avoid false-negative results owing to diurnal fluctuation in adrenocorticotropic hormone having a more variable effect on many APAs than on the contralateral adrenal, AVS is best performed in the morning.

Some centers conduct AVS in outpatients, in which case time should be allowed for the patient to rest supine for 1 hour before AVS.[22,23] However, at the authors' institution we prefer to perform AVS during a 1-day hospital stay and to recommend 3 days of rest at home, because adrenal vein rupture (discussed elsewhere in this article) can occur suddenly, even after 24 hours, and is best managed in experienced hands in the hospital.

ASSESSMENT OF SUCCESSFUL CATHETERIZATION

In the early years of AVS when CT was not available, retrograde injection of contrast medium in the adrenal vein was used to confirm the success of catheterization with venography and to visualize the abnormal vascular tree of an APA.[24] This procedure carries an increased risk of adrenal vein rupture and contributed to create the fame of risky procedure for AVS.[7,22] Even though the injection of a small amount of dye with a gentle pressure is still commonly used to confirm the correct positioning of the catheter's tip inside the adrenal vein, with improved imaging techniques adrenal venography is no longer needed, and must be avoided.[25]

On the left side, the tip of the catheter should be placed distal to the orifice of the left inferior phrenic vein. On the right side, the right adrenal vein draining into the accessory hepatic vein should be identified beforehand on a CT scan and the location of the tip of the catheter in the right adrenal vein, instead of hepatic venous tributaries, should be confirmed by gentle injection of a very small amount of contrast just before blood sampling. The intraprocedural rapid cortisol assay during AVS,[26] (discussed elsewhere in this article) can be of help in this undertaking.

INTERPRETATION OF ADRENAL VEIN SAMPLING RESULTS

Selective catheterization of the left adrenal vein is achieved in almost 100% of the cases if the catheter's tip is positioned either at, or distal to, the orifice of the left inferior phrenic vein to avoid a dilution effect. Selective catherization is much more difficult on the right side because of the small size and short length of the right adrenal vein and because it drains directly into the inferior vena cava at various angles,[27] or directly into a small accessory hepatic vein.[22,28]

In about 10% of patients, the ascertainment of selectivity, and thus the success rate of AVS, can be affected by dilution from accessory vein blood flow.[29] Prior knowledge of the right adrenal vein anatomy can facilitate catheterization in difficult cases; hence, we recommend that a contrast-enhanced multidetector CT scan be performed before AVS to identify the right adrenal vein and delineate its anatomy, including its position in relationship to surrounding structures and the presence of an accessory hepatic vein.[18] This can improve the rate of success by guiding selective cannulation of the right adrenal vein instead of the common trunk composed of the accessory hepatic vein and the right adrenal vein, and also to locate the adrenal vein.[23] In case of anatomic variants, this recommendation is of paramount importance for success, as we recently reported.[30]

A particularly well-trained radiologist can also achieve subselective catheterization, which can allow the identification of the culprit nodule within the adrenal cortex, and thus guide the undertaking of spare adrenalectomy.[27] This approach is, however,

confined to 1 center in Japan and remains to be validated in terms of improved outcomes.

INTRAPROCEDURAL RAPID CORTISOL ASSAY

Given that judgment of the achieved selectivity is possible only retrospectively when the hormonal data are available, a semiquantitative PCC measurement during AVS, which has the advantage of providing immediate feedback to the interventionist on whether selective blood sampling from each adrenal vein was accomplished or failed, has been proposed.[31] If selective catheterization failed, further attempts could be undertaken before removing the catheters, thus, avoiding the need for rescheduling the procedure.[31–33]

Thus far, this approach, which can improve the success rate during the interventionist's learning curve, was feasible only at centers where PCC could be measured rapidly, which implies a suitable logistic organization and a dedicated laboratory technician. However, the recent development of a quick cortisol assay, which needs neither a hardware nor an on-site technician, has made this strategy possible for the assessment of selectivity in a wider range of centers. Of note, a randomized study carried out in Japanese centers with a low level of experience in performing AVS showed an improved rate of success[26]; however, it remains to be determined if the same enhancement can be obtained in centers with a higher level of expertise in performing AVS. To answer this question, an international randomized prospective study, the Intra-Procedural cortisol Assay During Adrenal (I-Padua) vein sampling study, has been planned and is going.[34]

MINIMIZING STRESS DURING ADRENAL VEIN SAMPLING

Emotional and pain-related stress activates the hypothalamic pituitary adrenal axis, with ensuing adrenocorticotropic hormone-driven release of cortisol and aldosterone from both adrenals. The latter can be a major confounder of AVS results, particularly if bilaterally simultaneous blood sampling cannot be performed.[35] The latter technique was shown to provide better results in the AVIS-2 Study,[8] but owing to a number of misconceptions is not followed everywhere.

More cortisol than aldosterone is released during stress, which most patients experience when starting AVS; however, the cortisol can wane rapidly over about 15 minutes, and lateralization to the "culprit" adrenal can be obscured. A study that investigated the effect of stress on the selectivity index (SI) (**Table 1**) showed that a stress reaction increased the SI on both sides at the beginning of the procedure and influenced also the lateralization index (LI) values when using sequential AVS sampling.[35] Hence at the authors' institution, we systematically use the bilaterally simultaneous technique.

We also undertake precautions to minimize stress by explaining the procedure to the patient, providing reassurance by the doctor and nurses, administering benzodiazepines and local anesthesia before venipuncture and during AVS, and having the patient to rest quietly for at least 15 minutes before the blood sampling in a friendly environment with psychological assistance. Unfortunately, some of these measures were systematically used in only 5% of the centers participating in the AVIS Study.[7] Measures aimed at overriding stress effects by maximally stimulating cortisol release from both adrenals with cosyntropin are discussed elsewhere in this article.

In summary, the available evidence indicates that (i) a stress reaction can affect both the SI and LI, (ii) stress minimization measures should be taken when starting AVS (see **Box 2**), and (iii) a bilaterally simultaneous technique should be preferred.

Table 1
Indexes currently used for the interpretation of AVS results

Index	Formula	Interpretation
Selectivity Index (SI)	PCC_{side}/PCC_{IVC}[a]	Values above the cut-off confirm that the sample was obtained in the adrenal vein.
Relative secretion index (RASI)	$\frac{(PAC_{side}/PCC_{side})}{(PAC_{IVC}/PCC_{IVC})}$	Estimates aldosterone secretion of each adrenal gland relative to cortisol.
Contralateral suppression index (CLSI)	$\frac{(PAC_{non\ dominant}/PCC_{non\ dominant})}{(PAC_{IVC}/PCC_{IVC})}$	Values below the cut-off (generally 1.00) indicate suppression of aldosterone secretion in the non dominant gland.
Lateralization Index (LI)	$\frac{(PAC_{dominant}/PCC_{dominant})}{(PAC_{non\ dominant}/PCC_{non\ dominant})}$	Values above the cut-off indicate lateralized aldosterone excess.

At the author's institution androstenedione is also used for calculation of the SI and also for calculation of the other indexes, instead of PCC.

Abbreviations: Dominant, side with higher plasma aldosterone concentration; IVC, inferior vena cava; non dominant, contralateral side; PAC, plasma aldosterone concentration; PCC, plasma cortisol concentration.

[a] Peripheral venous blood can also be used.

BILATERAL SIMULTANEOUS CATHETERIZATION SHOULD BE THE PREFERRED METHOD

Owing to the pulsatile secretion of aldosterone, there are chances of creating artificial gradients between sides when the blood sampling is performed sequentially, that is, at different times, particularly if the interventionist is not proficient and fast enough. The untoward effect of the stress reaction occurring when starting AVS on assessment of lateralization can also worsen these problems. Undoubtedly, the time-dependent bias affecting AVS results can be avoided with bilaterally simultaneous AVS.[36]

These issues were clarified in a recent study where bilaterally simultaneous AVS was systematically used at time −15, that is, when starting the procedure, and again 15 minutes later and sequential AVS was then simulated by combining the samples obtained on each side at the different time points.[36] The simultaneously obtained samples at time 0 provided a more accurate identification of lateralization than those obtained sequentially; moreover, the chances of creating factitious lateralization to the last sampled side, regardless of its being right or left, were higher with the sequential technique, likely because of the waning of the aforementioned stress reaction.

Notwithstanding this factor, the AVIS study showed that only one-third of centers used the bilaterally simultaneous technique with no stimulation, whereas almost two-thirds still used the sequential technique with cosyntropin stimulation (discussed elsewhere in this article).[7] Similar results were obtained in the AVIS-2 Study.[8]

These findings could be anticipated, because (i) when cortisol secretion is maximally stimulated the time difference between blood sampling from one side and the other becomes less relevant, in the assessment of selectivity, and (ii) the bilateral simultaneity of blood sampling is crucial when AVS is performed without cosyntropin stimulation.

PHARMACOLOGIC STIMULATION DURING ADRENAL VEIN SAMPLING

Stimulation with a continuous cosyntropin infusion (50 μg/h started 30 min before sampling) or a bolus (250 μg) during AVS was introduced in 1979 and remains popular

at many centers,[7,37] for 3 main reasons: (i) enhancing the PCC gradient between the adrenal vein and the inferior vena cava, thus increasing the SI values and physicians' confidence of successful sampling; (ii) decreasing stress-induced fluctuations in cortisol and aldosterone secretion during sequential AVS; (iii) and increasing aldosterone secretion from APA.

In 2012 the AVIS study showed that the major referral centers were almost equally split into those that used and those that did not use cosyntropin stimulation[7]; moreover, centers that systematically used cosyntropin reported use of higher SI cutoffs values than centers that used baseline (unstimulated) values.

A detailed discussion of the pros and cons for using cosyntropin was recently published elsewhere,[38] and is summarized here. In brief, there are no doubts that cosyntropin increased the rate of AVS studies judged to be selective, but this increase comes at the expense of decreased accuracy of the LI as repeatedly shown[39–41] and recently confirmed in the AVIS-2 Study,[8] and explained at the mechanistic level.[42]

In summary, in keeping with the theoretic premises, the bulk of the data indicate that cosyntropin increases the SI and, by doing so, facilitates the ascertainment of selective catheterization. To date, given the lack of conclusive evidence for the superiority of using pharmacologic stimulation with cosyntropin to determine lateralization of aldosterone excess, the recommendation needs to be limited to the following: (i) each center should use a consistent protocol, (ii) if cosyntropin stimulation is used, then higher SI and LI values are necessary, and (iii) if cosyntropin stimulation is not used, then bilateral simultaneous AVS should be performed.

As regards other stimuli, metoclopramide was used in only 2 studies[42,43] that reported that it did not increase the success of AVS or the LI to the culprit side. However, metoclopramide can be useful to unmask the factitious suppression of aldosterone production in the nondominant side in patients without APA.[44] A further study investigating the use of clarithromycin, which can blunt aldosterone secretion specifically from APA harboring the KCNJ5 mutation during AVS is ongoing.[45]

HOW TO INTERPRET ADRENAL VEIN SAMPLING RESULTS

A number of indexes have been developed for establishing whether catheterization was successful and for a proper interpretation of the results.[46] They easily can be calculated from the hormonal values measured in AVS-derived blood (see **Table 1**).

The SI (see **Table 1**) is the most popular technique to confirm the success of AVS,[43] based on the theoretic assumption that the hormone of interest is exclusively made in the adrenal cortex and, is not overproduced in the culprit adrenal. Therefore, the finding of a concentration gradient between a blood sample in a vein supposedly draining the adrenal cortex, and the inferior vena cava or a peripheral vein, indicates the correct placement of the catheter's tip into the adrenal vein. To this end, the most widely used hormone is cortisol, given its high rate of production, although attempts to use also chromogranin A,[47] epinephrine, metanephrine,[48] and other steroids have been made.

According to studies with liquid chromatography and tandem mass spectrometry that measured all steroids released by the human adrenal cortex, the step-up between the adrenal vein and the inferior vena cava or a peripheral vein blood would be greater for some steroids other than cortisol, suggesting that they can be better markers for selectivity.[49] A recent study that examined the performance of 17α hydroxyprogesterone and androstenedione in a number of AVS studies judged to be nonselective with cortisol-based SI, showed that this hypothesis was in fact correct[50]: 17α hydroxyprogesterone and androstenedione showed a 1.6-fold and 12-fold

higher step-up than cortisol, respectively, thus allowing to rescue 43% and 72%, respectively, for diagnostic purposes. Hence, at the authors' institution we measure systematically androstenedione, besides cortisol, to confirm selectivity of AVS.

Although use of the SI is straightforward, the AVIS Study showed that even some major international referral centers continue to analyze their results using the absolute hormonal values without a prior assessment of the selectivity and correction for the degree of sample dilution, a practice that is not evidence-based and should be discouraged because the absolute hormonal values are markedly affected by the degree of proximity of the catheter's tip to the adrenal cortex. Of further concern, even in those centers that used the SI to assess selectivity, there was considerable variability in the SI cutoff values used.[7]

Some general considerations can, however, be made: the cutoffs are lower at centers that perform AVS with no pharmacologic stimulation than at those that use cosyntropin stimulation, an expected finding given that cosyntropin increases the cortisol release and therefore the gradient between adrenal vein and inferior vena cava blood.

Based on the experience gained and the results of the AVIS Study, the suggestion was made to use of a SI cutoff of 2.0 or greater for AVS performed under unstimulated conditions, and 3.0 or greater for AVS performed during cosyntropin stimulation (**Box 3**).[18] However, the choice of the SI cutoff is center-dependent and determined based on the accuracy of the laboratory in measuring these hormones.

To validate the findings and select the optimal cutoffs, it should be acknowledged that the only solid diagnosis that can be used as reference is that of APA. Studies that used the AVS results to validate the own AVS-based diagnosis and justify the choice of their cutoff values should be disregarded because they did not comply with the rules set by the Standards for Reporting of Diagnostic Accuracy committee.[51,52]

There is no doubt that (i) the higher the cutoff chosen to establish selectivity, the lower the proportion of AVS studies that could be defined as bilaterally selective and vice versa, as demonstrated conclusively in the AVIS-2 study[8]; (ii) too restrictive criteria lead to exclude a proportion of successful studies from diagnostic use; and (iii) conversely, too permissive SI cutoffs could limit the diagnostic accuracy of AVS.

Moreover, as the SI increases, so does the confidence of the interpretation. In some cases where unilateral aldosterone production is extremely high, a low SI can give the

Box 3
Interpretation of AVS

Assessment of selectivity
- Successful AVS should be determined by calculating the SI.
- AVS studies that are not bilaterally successful should not be used to establish lateralization.
- The cutoff value for the SI should be 2.0 or greater under unstimulated conditions and 3.0 or greater during cosyntropin stimulation.
- Where possible, the rapid intraprocedural cortisol measurement confers the advantage of drawing a repeat blood sample after catheter repositioning in case of unsuccessful initial catheterization.

Assessment of lateralization
- Lateralization of aldosterone secretion should be determined by the LI and not by absolute hormonal values.
- For assessing lateralization, there is no compelling evidence for the use of cosyntropin stimulation in terms of outcome.
- Most centers used LI between 2.0 and 4.0 under unstimulated conditions and between 2.6 and 4.0 during cosyntropin stimulation

correct interpretation, but, in cases when the overproduction is modest, wrong conclusions are possible.

A trade-off between too restrictive and too permissive cutoffs is needed, and it has also to be considered that the cutoffs depend on the accuracy (within-assay coefficient of variation) with which the hormone used to assess selectivity can be measured. For example, if this is greater than or around 10%, lower cutoffs such as 1.1 are not feasible, whereas if the assay coefficient of variation is less than 10%, an SI cutoff of 1.1 may be reasonable.[18,46]

A recent analysis of the large AVIS-2 database showed that use of less restrictive cutoffs for both the SI and the LI allowed to refer for surgery a much large proportion of the patients with PA, as discussed later. More important, more liberal cutoffs were not associated with a worse clinical outcome so that a greater number of patients could gain a clear-cut benefit.[53]

In summary, verification of bilateral selectivity is a prerequisite to the use of the data for diagnostic purposes, which implies that AVS studies that are not bilaterally successful must not be used to establish lateralization. Nonetheless, recognizing that many studies do not turn out to be bilaterally selective and/or can provide equivocal results, we refer the reader to what has been discussed in greater depth as regards how to make the diagnosis of unilateral PA in challenging cases.[54]

ASSESSMENT OF LATERALIZATION

The LI is the index to be used for assessment of lateralization of aldosterone excess (see **Table 1**). Because of the inevitable dilution of the samples by nonadrenal blood, PCC and/or androstenedione are used for correction of the adrenal aldosterone levels.

There are several caveats in assessing the accuracy of AVS criteria using outcome after adrenalectomy as a reference. Ideally, outcome should be defined using postoperative normalization of plasma aldosterone and renin to determine curative adrenalectomy.[2,55] In contrast, blood pressure normalizes in about 45% of biochemically cured patients, but it may not decrease if the patient has concomitant essential hypertension, chronic kidney disease, and/or vascular remodeling,[17,56] or it may decrease for reasons unrelated to biochemical cure of PA, such as the Hawthorne effect, lifestyle changes, and incident left ventricular systolic dysfunction, for example, owing to myocardial infarction.

In 2012 most of the referral centers performed AVS with sequential sampling and cosyntropin stimulation,[7] which is not the optimal way to perform it, for the reasons discussed elsewhere in this article.

Evidence for optimal diagnostic accuracy of LI cutoffs should come from prospective studies in patients undergoing unilateral adrenalectomy regardless of the AVS results, and in which the different LI are thereafter linked with postoperative biochemical cure of hyperaldosteronism rather than only high blood pressure, which is a composite phenotype. Unfortunately, the only such prospective randomized controlled studies available was underpowered to provide any conclusive information.[10]

In the AVIS study, referral centers used arbitrarily chosen LI cut-offs values that ranged between 2.0 and 4.0;[7] undoubtedly the choice of more restrictive (higher) cutoffs allows selection of a population with a higher chance of being cured with adrenalectomy, but precludes the chances of cure to several potentially curable patients.

The recent AVIS-2 study assessed the rate of patients deemed to have unilateral disease by LI cut-off definitions ranging from 2.0 to 5.0, in concert with SI cut-off definitions of 1.4, 2.0, and 3.0 for unstimulated measurements, and 5.0 for postcosyntropin values. It showed that, among bilaterally successful studies, the rate of

lateralization dropped significantly with adoption of higher LI cut-off definitions and with each unit increase in LI cut-off definition. With commonly used biochemical definitions under unstimulated and cosyntropin-stimulated conditions, that is, an SI of 2.0 or greater with an LI of 3.0 or greater, and an SI of 5.0 or greater with an LI of 4.0 or greater, the proportion of patients deemed to have unilateral disease was about 40% and 37%, respectively (P = NS). It increased significantly to 56% (P<.001) with less stringent cutoff values of unstimulated SI and/or LI, that is, an SI of 1.4 or greater combined with a cut-off of 2.0 or greater for lateralization. Importantly, the rate of patients submitted to adrenalectomy was lower with application of more stringent definitions for unilateral disease, and decreased to a nadir of less than 25% with an SI of 3.0 or greater and an LI greater than 4.0. However, the proportion of patients referred for adrenalectomy among those with AVS evidence of unilateral disease was higher with more restrictive criteria for lateralization, suggesting that physicians' confidence in results that meet the stricter definitions was higher.[53]

SAFETY AND THE MANAGEMENT OF COMPLICATIONS

AVS should be performed in specialized referral centers with sufficient throughput and expertise. However, the limited number of such specialized centers may result in missed opportunities for optimal surgical management in many patients who have no access to AVS. Hence, appropriate training programs and certification of proficiency in performing AVS for radiologists should be implemented.

The only major complication of AVS is adrenal vein rupture with subsequent intraglandular and/or retroperitoneal hematoma.[18,57] Only occasionally these complications are curative if they occur in the adrenal gland harboring the APA. Clinically, adrenal vein rupture is characterized by acute onset of persistent lumbar pain during or after catheterization, which increases in intensity and requires analgesics over 24 to 48 hours. Under these circumstances a CT scan or MRI is necessary to confirm the diagnosis, and careful monitoring of vital signs should be undertaken. The complication usually resolves with conservative treatment in few days and does not carry sequelae, except that, if on the APA side, it can render subsequent laparoscopic adrenalectomy more difficult owing to extensive retroperitoneal adhesions.

Although early studies suggested a wide range of rates of adrenal vein rupture, in the AVIS Study the rate of this complication was only 0.6%,[7] with avoidance of routine adrenal venography and minimization of the injection volume for anatomic confirmation of the adrenal vein catheterization. Complications are more common at the right than left adrenal vein, because of the anatomic diversity and complexity; they do not depend on the methods of catheterization, that is, sequential, or bilaterally simultaneous, and the use of cosyntropin stimulation,[7] but they differ significantly, even among major referral centers, indicating that the expertise of the radiologist and the experience of each center are key issues inasmuch as adrenal vein rupture was inversely related to the number of AVS performed by each radiologist and the number of AVS performed per centers.

SUMMARY

The high diagnostic accuracy, minimal rate of complications, and better outcome with AVS-guided adrenalectomy support the guidelines recommendation that AVS should serve as the golden standard diagnostic test for the subtyping of PA. With some exceptions, AVS should be systematically used before referring a patient to the surgeon. As for all invasive diagnostic tests appropriate training of the interventionists, a tight collaboration with experienced endocrinologists and hypertensiologists, and

adherence to the suggestions that are herein summarized for the interpretation of the test will make the best clinical use of AVS for the doctor and for the patient.

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