Adrenal vein sampling for primary aldosteronism: the assessment of selectivity and lateralization of aldosterone excess baseline and after adrenocorticotropic hormone (ACTH) stimulation
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Background Adrenal vein sampling is crucial for identifying the primary aldosteronism subtypes, but the cutoff values for ascertaining selectivity of catheterization and lateralization of aldosterone secretion remain controversial.

Objectives To investigate the safety of adrenal vein sampling, the cutoff values for the selectivity and lateralization indexes, and the effect of adrenocorticotropic hormone stimulation on selectivity index and lateralization index performance.

Design We assessed the proportion of selective adrenal vein sampling at different selectivity index cutoff values in 151 consecutive patients with primary aldosteronism undergoing bilaterally simultaneous adrenal vein sampling. Aldosterone-producing adenoma was diagnosed on the basis of the evidence of primary aldosteronism and lateralized aldosterone secretion, adenoma at pathological examination, and normokalemia, and correction of primary aldosteronism and cure or improvement of hypertension at follow-up. In 44 patients with bilaterally selective adrenal vein sampling and unequivocal diagnosis of aldosterone-producing adenoma on the basis of all these criteria, we examined the cutoff values of the lateralization index for assessing the lateralization of aldosterone excess and the effect of adrenocorticotropic hormone stimulation on selectivity index and lateralization index.

Results Adrenal vein rupture occurred in one case (0.7%). Bilaterally selective adrenal vein sampling decreased steadily (from 79.9 to 40.2%) with increase in the selectivity index cutoffs from 1.1 to 5.0. Likewise, the proportion of correctly identified aldosterone-producing adenomas decreased (from 95.5 to 43.2%) with increase in lateralization index cutoffs from 1.125 to 5.0. Adrenocorticotropic hormone improved the assessment of selectivity but exerted a confounding effect on lateralization index.

Conclusion Adrenal vein sampling is safe; increasing the selectivity index cutoffs lowers the number of usable adrenal vein samplings; higher lateralization index cutoff values lead to missing a proportion of aldosterone-producing adenomas. The improved selectivity rate provided by adrenocorticotropic hormone stimulation should be weighed against the loss of correct lateralization.


Keywords: adrenal vein sampling, adrenocorticotropic hormone, aldosterone, aldosteronism, catheterization

Abbreviations: ACTH, adrenocorticotropic hormone; APA, aldosterone-producing adenoma; ARR, aldosterone to renin ratio; AUC, Area Under Curve; AVS, adrenal vein sampling; BAH, bilateral adrenal hyperplasia; CT, computerized tomography; GITS, gastrointestinal therapeutic system; IHA, idiopathic adrenal hyperplasia; IVC, inferior vena cava; LI, Lateralization Index; MR, magnetic resonance; PA, primary aldosteronism; PAC, plasma aldosterone concentration; PCC, plasma cortisol concentration; PRA, plasma renin activity; ROC, Receiver Operating Characteristic; SI, Selectivity Index

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Original article
The surgically curable subtypes of primary aldosteronism [3], such as aldosterone-producing adenoma (APA) [1,2,8–12] and primary unilateral hyperplasia [13].

The diagnosis of APA requires not only finding an adenoma, which could be a nonfunctioning one coexisting with bilateral adrenal hyperplasia (BAH), at pathological examination but also demonstrating a cure for primary aldosteronism at follow-up, as recently described in the Primary Aldosteronism Prevalence in hypertension study [14]. On the basis of these solid criteria, we diagnosed APA in a large collection of primary aldosteronism patients consecutively undergoing AVS. This allowed investigation of the performance of different cutoff values of the selectivity index and the lateralization index for determining the selectivity of AVS and the lateralization of aldosterone excess, respectively, using the diagnosis of APA as a referent. The results of this study are herein reported to provide a framework for proper use and interpretation of the AVS results.

Patients and methods

Among patients with an established diagnosis of primary aldosteronism, obtained as described elsewhere [14], we selected those who were to be admitted for AVS on the basis of the following criteria: lack of contraindications to general anesthesia and surgery and informed consent to eventually undergo laparoscopic adrenalectomy if lateralized aldosterone secretion was eventually identified. Written informed consent was obtained from each participating patient.

Adrenal vein sampling

At least 2 months before the date on which AVS was scheduled, the patients started a normal (100–300 mEq per day) Na+ intake. The patients were prepared from the pharmacological standpoint by withdrawing treatment with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, β-blockers, and direct vasodilators at least 15 days before AVS for the reasons discussed in depth elsewhere [4]. Mineralocorticoid receptor antagonists were withdrawn no less than 6 weeks before the test because these agents, as all diuretics, can stimulate aldosterone secretion from the unaffected side to a greater extent than from the APA side, at least in the considerable proportion of APA that are angiotensin II unresponsive [15]. When necessary, nifedipine gastrointestinal therapeutic system (GITS) and/or doxazosin was allowed to control blood pressure, because this treatment was found to have no effect on the plasma aldosterone and cortisol secretion rate under the study conditions [14]. To avoid the blunting of aldosterone secretion, hypokalemia, if present, was systematically corrected with oral or intravenous potassium ion supplementation before AVS [4].

Sequential AVS was performed by three experienced interventional radiologists for the first 104 AVS, as reported [1]. We thereafter introduced bilateral simultaneous AVS to investigate the usefulness of ACTH stimulation [5]. All these later AVS studies were performed by the same interventional radiologist. Briefly, the procedure was carried out after a 3-h rest in the supine position between 8:00 a.m. and 12:00 p.m. After placing a catheter in each adrenal vein, blood was simultaneously obtained from the infra-renal inferior vena cava (IVC) and from both sides by gravity or, if necessary, gentle negative pressure for measuring the plasma aldosterone concentration (PAC) and the plasma cortisol concentration (PCC). The tip of the catheter was left in place in the left side, whereas it was withdrawn from the right adrenal vein in the IVC to avoid the risk of thrombosis. A high dose [250 µg as an intravenous (i.v.) bolus] of 1–24 synthetic ACTH (Synacthen, Novartis, Origgio (VA) - Italy) was given to maximally stimulate aldosterone and cortisol secretion; blood sampling was repeated with identical modalities after 30 min. This ACTH dose was chosen because in a pilot study we found that it provided a similar stimulation of cortisol at 30 min in the IVC to the continuous infusion of a lower 5 µg/h dose [2], in keeping with the results obtained in peripheral blood with a lower dose [16]; a much lower dose (250 pg as a bolus i.v. followed by 0.5 pg/min infusion) [7,8] did not elicit any cortisol response in our hands.

Measurements of plasma renin activity (PRA), PAC, and PCC were done as described [1]; the intraassay and interassay coefficients of variation of our assay for PAC and PCC were both less than 5.6% and the cross-reactivity of the antibody against aldosterone for the other adrenal steroids was less than 0.001%.

Adrenal vein sampling derived indexes

The selectivity index was calculated as follows:

\[
PCC_{\text{side}} / PCC_{\text{IVC}}
\]

where PCC\text{side} and PCC\text{IVC} are the PCC in blood from each adrenal vein and in IVC, respectively.

The lateralization index was calculated as follows:

\[
PAC_{\text{dominant}} / PCC_{\text{dominant}} / PAC_{\text{nondominant}} / PCC_{\text{nondominant}}
\]

where PAC\text{dominant} and PAC\text{nondominant} are PAC on the side with higher and lower aldosterone secretions, respectively, and PCC\text{dominant} and PCC\text{nondominant} are PCC on the side with higher and lower aldosterone secretions, respectively.

Diagnosis of aldosterone-producing adenoma

For a diagnosis of APA, all the following criteria had to be satisfied: first, biochemical evidence of primary aldosteronism; second, lateralization of aldosterone secretion at AVS (see later); third, evidence of adenoma at pathological examination; and fourth, demonstration of correction...
of hypokalemia, if present, and cure or improvement of hypertension at follow-up at least 120 days after adrenalectomy [14]. Cure was defined as a systolic blood pressure less than 140 mmHg and/or a diastolic blood pressure less than 90 mmHg without medications; improvement was defined as a systolic and/or a diastolic blood pressure less than 140/90 mmHg, respectively, on the same or reduced number of medications and/or a reduced number of defined daily doses. Patients with biochemical evidence of primary aldosteronism, but without conclusive evidence for a lateralized aldosterone excess, were presumed to have idiopathic hyperaldosteronism (IHA).

**Indications for adrenalectomy**

On the basis of the receiver operating characteristic (ROC) curve analysis of the results of a large study of AVS patients [1], a lateralization index value greater than 2.0 was held to indicate lateralization of aldosterone secretion, provided that AVS was bilaterally selective. The decision to undertake laparoscopic adrenalectomy, however, was based on not only the AVS results but also the overall clinical assessment of each patient, including the diameter and features of the adrenal mass at computed tomography and/or MR, the resistance of hypertension and/or hypokalemia to pharmacological treatment and the occurrence of intolerable side effects of mineralocorticoid receptor antagonist treatment. Thus, adrenalectomy was performed in nine of the 44 patients (20.5%), despite a lateralization index of less than 2.0. In these patients, an adenoma was found at pathological examination; moreover, the follow-up data after adrenalectomy demonstrated cure of hypertension and primary aldosteronism, thus, conclusively confirming the diagnosis of APA.

**Statistical analysis**

Results are expressed as mean ± SEM or median and range when appropriate. For serum K⁺, PRA, and PAC, data were log transformed to attain a Gaussian distribution, as verified by Kolmogorov–Smirnov test. Baseline and post-adrenalectomy values were compared with paired t-test; for serum K⁺, Wilcoxon nonparametric test was used (SPSS for Windows statistical package, version 15.1, SPSS, Chicago, Illinois, USA). A P value less than 0.05 was considered statistically significant.

To determine the optimal lateralization index cutoff, for example, the value furnishing the best trade-off of sensitivity and false positive rate in ROC analysis was used and a sensitivity–false positive rate versus criterion value plot was built [MedCalc software (version 9.3.0.0, MedCalc Software, Mariakerke, Belgium)]. The unequivocally defined APA group served as a referent in this analysis; the control group comprised the cases with presumed IHA, because performing AVS in a group of patients without primary aldosteronism is regarded as unethical.

| Table 1 Clinical features of the 151 patients with primary aldosteronism who underwent adrenal vein sampling |
|------------------|------------------|
| **Age (years)** | 51 ± 1 |
| **Gender (men : women) (%)** | 83 : 67 (55/45) |
| **Systolic blood pressure (mmHg)** | 167 ± 2 |
| **Diastolic blood pressure (mmHg)** | 102 ± 1 |
| **K⁺ (mEq/l)** | 3.4 (3.2 – 3.7) |
| **s-aldosterone (ng/dl)** | 21.4 (14.3 – 27.3) |
| **s-PRA (ng/ml/h)** | 0.43 (0.22 – 0.78) |
| **capto-aldosterone (ng/ml)** | 15.1 (10.8 – 23.3) |
| **capto-PRA (ng/ml/h)** | 0.82 (0.32 – 1.28) |
| **s-ARR (ng/dl)/(ng/ml/h)** | 80.4 ± 8.0 |
| **capto-ARR (ng/dl)/(ng/ml/h)** | 45.4 ± 6.2 |

**Results**

**Patients and diagnosis**

Between 1988 and 2006, we performed a total of 199 AVS in 151 white patients whose main anthropometric data are shown in Table 1. At the time of AVS, arterial hypertension was controlled; for example, systolic and/or diastolic blood pressure was less than 140/90 mmHg roughly 87% of the patients with the aforementioned medications.

Adrenal vein rupture occurred only in one patient, as described [1], thus evidencing a complication rate of 0.7%. Figure 1 shows the flowchart of the study: briefly, we examined the impact of different cutoff values of the selectivity index in 151 consecutive patients, of which 104 [1] and 21 [5,17,18] were previously described in studies having different purposes. The number of catheterizations exceeded the number of patients because, in two patients, the procedure was repeated a few years after the first AVS for clinical reasons and in 46 cases in which the ACTH stimulation was undertaken, catheterization was performed twice on the right side to avoid thrombosis, which might occur when the catheter is left in the right adrenal vein during the 30 min required for the protocol, at the catheter’s tip.

As the procedure (sequential versus bilateral simultaneous) for AVS and the number of radiologists performing the AVS have changed since 2001, we analyzed our dataset first as a whole and then after splitting it according to 3-year periods.

Of the 151 patients, 53 underwent adrenalectomy because of an APA. Owing to the aforementioned tight criteria for diagnosing APA [14], we could not include nine patients in the analysis of the effect of different cutoff values of the lateralization index for various reasons including lack of bilaterally selective AVS results (eight patients) and lack of response to adrenalectomy (one patient). In 44 patients with conclusive diagnosis of APA (Table 2), the outcome of
surgery included decrease in blood pressure and normalization of serum K\(^+\), PAC, and ARR both at baseline and after dynamic stimulation with captopril.

### Effect of different cutoff values of the selectivity index
To adjust for the differences in the number of performing radiologists and AVS protocol, results were analyzed as a function of the time when AVS were performed (Fig. 2). This analysis showed that after the initial decrease due to the small number of AVS performed between 1988 and 1991, there was no change in the proportion of bilaterally selective AVS results over time, and between AVS performed before 2001 using the

![Flowchart of the study. The number and features of the patients who were investigated for the different endpoints are shown. All 199 AVS studies were examined to determine the effect of the selectivity index cutoff on the proportion of bilaterally selective AVS results. Only the patients with baseline data and data after adrenocorticotropic hormone AVS were examined to determine the impact of adrenocorticotropic hormone on the selectivity index. Only the patients fulfilling the tight criteria for the diagnosis of APA were analyzed to determine the impact of different lateralization index cutoffs for assessing the correct lateralization of aldosterone excess to the APA side. Of these patients, those with baseline data and data obtained after adrenocorticotropic hormone stimulation and bilaterally selective AVS results were used to determine the effect of adrenocorticotropic hormone on the lateralization index.

### Table 2  Effect of adrenalectomy on the clinical and biochemical picture of primary aldosteronism in 44 patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before</th>
<th>After</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>167 ± 5</td>
<td>138 ± 4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>102 ± 2</td>
<td>84 ± 2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>K(^+) (mEq/l)</td>
<td>3.3 (3.1–3.5)</td>
<td>4.3 (4.0–4.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>s-aldosterone (ng/dl)</td>
<td>23.7 (15.7–37.3)</td>
<td>5.8 (3.8–11.9)</td>
<td>0.009</td>
</tr>
<tr>
<td>s-PRA (ng/ml/h)</td>
<td>0.36 (0.20–0.65)</td>
<td>0.90 (0.57–2.47)</td>
<td>NS</td>
</tr>
<tr>
<td>capto-aldosterone (ng/dl)</td>
<td>16.7 (11.9–25.3)</td>
<td>3.7 (1.8–8.8)</td>
<td>0.007</td>
</tr>
<tr>
<td>capto-PRA (ng/ml/h)</td>
<td>0.42 (0.22–1.05)</td>
<td>1.35 (0.95–3.45)</td>
<td>NS</td>
</tr>
<tr>
<td>s-ARR (ng/dl)/(ng/ml/h)</td>
<td>62.09 ± 22.03</td>
<td>8.23 ± 1.56</td>
<td>0.008</td>
</tr>
<tr>
<td>capto-ARR (ng/dl)/(ng/ml/h)</td>
<td>19.34 ± 9.65</td>
<td>3.59 ± 1.24</td>
<td>NS</td>
</tr>
</tbody>
</table>

capto-aldosterone, post-captopril plasma aldosterone; capto-ARR, post-captopril aldosterone/renin (PRA) ratio; capto-PRA, post-captopril plasma renin activity; K\(^+\), serum potassium level; s-aldosterone, supine plasma aldosterone; s-ARR, supine aldosterone/renin (PRA) ratio; s-PRA, supine plasma renin activity; NS, not significant. Data are mean ± SEM, except for K\(^+\), s-aldosterone, s-PRA, capto-aldosterone, and capto-PRA, for which median (range) are shown. Normal values for s-aldosterone is 0.8–15.0 ng/dl, s-PRA is 0.51–2.64 ng/ml/h, s-ARR is <25.96, and capto-ARR is <29.91.
sequential technique and afterward using the bilaterally simultaneous technique.

To evaluate the impact of different selectivity index cutoff values on the proportion of clinically usable AVS studies, we next plotted the percentage of bilaterally selective AVS results as a function of the different cutoff values (Fig. 3a).

At any selectivity index cutoff value overall, the proportion of selective adrenal vein catheterization decreased markedly on both sides with increase in the selectivity index from 1.10 to 5.0 (from 79.9 to 40.2%).

**Effect of different cutoff values of the lateralization index**

According to the STARD recommendations [19, 20], assessment of a diagnostic test requires availability of a ‘gold standard’ as a referent. Therefore, the impact of different cutoff values of the lateralization index was examined in only 44 patients with unequivocally confirmed APA on the basis of the aforementioned strict criteria. The diagnosis of APA in all these cases was confirmed beyond doubt by demonstration of adenoma at pathological examination, normalization of systolic and
diastolic blood pressures and, serum K$^+$, and decrease in PAC and ARR. The plot of the percentage of lateralized AVS as a function of different lateralization index cutoff values (Fig. 3b) shows that the proportion of correctly identified APAs decreased sharply (from 95.5 to 43.2%) with increase in the cutoff values from 1.125 to 5.0.

The ROC curve analysis and sensitivity–specificity versus criterion value plot yielded an optimal cutoff value of 1.98 (Fig. 4). With this relatively low cutoff, the low sensitivity and specificity achieved were due to the fact that 20.5% of the patients with APA, who were cured by an adrenalectomy performed for the reasons specified above, showed a lateralization index less than 2.0. In all these patients, pathological examination showed an adenoma and, compared with preadrenalectomy values, systolic ($P=0.001$) and diastolic ($P=0.011$) blood pressures decreased, serum K$^+$ increased ($P=0.019$), and PAC and the ARR decreased, albeit the latter not significantly. Therefore, these results indicate that some patients with APA can be overlooked even with this low cutoff.

Effect of adrenocorticotropic hormone stimulation on adrenal vein sampling results

To assess the effect of ACTH on the selectivity index, we analyzed the data of the 46 AVS performed both under basal conditions and after ACTH stimulation in a pairwise comparison. Results show that regardless of the selectivity index cutoff value chosen, the proportion of bilaterally selective AVS studies increased significantly ($P<0.001$) after ACTH stimulation (Fig. 5a).

When the effect of ACTH stimulation on the lateralization index was examined in the 24 patients who had a conclusive diagnosis of APA and bilaterally selective AVS results both under baseline conditions and after ACTH, the proportion of correctly identified APAs decreased markedly at any cutoff value (panel B). Of these patients, ACTH stimulation increased the lateralization index to the APA side in five (20.8%), lowered it in 14 (58.4%), and inverted it in a further five (20.8%).

Discussion

This study shows that with a complication rate of 0.7%, AVS is a safe procedure for diagnosing surgically curable forms of primary aldosteronism in experienced hands. Moreover, it discloses some important findings on the use of AVS concerning the performance of the test under baseline conditions and after ACTH stimulation [3].

Overall, the results show that the use of increasingly restrictive criteria for the selectivity index leads to a marked decrease in the number of AVS studies that can be regarded as bilaterally selective (Fig. 3a). Likewise, adoption of a high cutoff for the lateralization index results in exclusion of a fairly large number of patients, who can be cured, from adrenalectomy (Fig. 3b).

Assessment of selectivity of catheterization at baseline and after adrenocorticotropic hormone stimulation

Considering all 199 AVS studies, the use of more restrictive cutoff values for the selectivity index determines a steep decrease in the number of AVS studies that can be regarded as bilaterally selective and therefore usable for determining the lateralization of aldosterone excess
Adoption of the less-restrictive cutoffs permitted use of roughly 80% of the studies for diagnostic purposes. Conversely, with the more stringent cutoff value of 5.0, only about 40% of the AVS were bilaterally selective and could therefore be used for diagnosis. The impact of the selectivity index cutoff was more striking under baseline conditions, because after exclusion of the data obtained after ACTH stimulation, the decline in the proportion of bilaterally selective studies was steeper (not shown). Thus, when AVS is performed without ACTH stimulation, the use of progressively higher cutoff values of the selectivity index implies exposing a large proportion of primary aldosteronism patients to the (minimal) risks associated with performing AVS with a tapering chance of gathering diagnostically useful data from this test.

The low cutoff value of the selectivity index of 1.1 that emerged from the ROC curve analysis is remarkably similar to that previously found in a smaller series [1], but might appear, at first sight, a low one. Anatomical and pathophysiological considerations, however, might explain why. On the right side the adrenal vein commonly shares egress in the IVC with accessory hepatic veins. Hence, samples collected from the adrenal vein comprise an admixture of blood with a high cortisol (and aldosterone) concentration coming from the adrenal gland and blood coming from the liver that carries cortisol and aldosterone at a concentration much lower than that even in the systemic circulation. Therefore, a high selectivity index cannot conceivably be expected from this side, unless cortisol secretion is stimulated. On the left side, the greater length of the left adrenal vein and its drainage into the left renal vein makes successful selective catheterization almost a rule. This vein, however, also receives blood (from phrenic and renal capsular veins), which does not have a high concentration of adrenocortical hormones. Thus, on both sides, the value of the selectivity index can be substantially lowered by these blood supplies even with selective catheterization of the adrenal veins. These considerations justify, in our view, the adoption of less tight cutoffs for assessing selectivity under baseline conditions, in keeping with previous studies [1].

These conclusions do not apply to AVS studies performed during or after ACTH stimulation: the selectivity index increases significantly after ACTH stimulation and moreover the proportion of bilaterally selective studies decreases only slightly with increase in the cutoffs from 1.25 to 5.0 (Fig. 5a). Thus, owing to the consistent stimulation of cortisol secretion on both sides, ACTH effectively enhances the ascertainment of the selectivity and, for selectivity index greater than 2.0, the choice of the cutoff value is much less critical after ACTH stimulation than under baseline conditions. These results can account for the divergent choices of optimal selectivity index cutoffs across centers that use baseline [1] or ACTH-stimulated data [2,8,9]. As discussed later, however, the use of ACTH should be weighed against the loss of lateralization.

**Does the adrenal vein sampling performance change over time?**

One might argue that, regardless of the cutoffs used, the proportion of selective studies can vary depending on the radiologist’s learning curve and experience, the method used for AVS, for example, sequential or bilaterally simultaneous, the accuracy of cortisol assays, and the possibility of an extemporaneous assay of the hormone during AVS [21]. Comparison of the AVS studies performed at our institution before 2001 by three radiologists using the sequential technique with those performed thereafter by a single ‘dedicated’ radiologist using the bilaterally simultaneous technique, however, showed no consistent differences in the proportion of bilaterally selective studies (Fig. 2). Thus, increased experience and use of bilateral catheterization does not seem to be associated with a marked increase of success rate in achieving both selective catheterization and correct lateralization.

**Assessment of lateralization of aldosterone excess**

The second most important finding of this study entails the demonstration that when bilaterally selective AVS blood has been obtained, adoption of high cutoff values for assessing lateralization leads to the exclusion of a substantial proportion of surgically curable forms of primary aldosteronism from adrenalectomy. We could examine a relatively large number of APA that were diagnosed with the most stringent criteria ever used: we used not only all the tests that are being utilized for making the diagnosis preoperatively but also the pathological and postoperative data to document APA and cure of hyperaldosteronism and arterial hypertension postadrenalectomy [14]. A ROC curve analysis applied to this series of APA showed that the optimal cutoff value for the lateralization index was 1.98, which is remarkably close to that reported previously [1]. Moreover, by using the most popular cutoff value of 4.0 [2], less than 50% of the APA could be diagnosed on either side. It is, however, important to underline that under baseline conditions, about one fifth of these patients conclusively diagnosed with APA had a lateralization index below the relatively low threshold of 2.0 previously found with a ROC curve analysis in a much smaller dataset of patients with primary aldosteronism [1].

**Effect of adrenocorticotropin hormone on adrenal vein sampling performance**

The results of the ACTH stimulation test during AVS also disclose some novel data that deserve comment. Smaller studies [5,17,18] in which blood was collected with bilaterally simultaneous AVS before and after ACTH showed that administration of a large dose (250 μg) of 1–24 synthetic ACTH as an i.v. bolus did not improve
the lateralization of aldosterone secretion to the APA side, whereas the lowest ACTH dose (250 µg as a bolus followed by a 0.5 µg/min infusion) did not elicit any cortisol response. Our present expanded series not only confirms our conclusions concerning failure of the high ACTH dose to enhance lateralization but also shows that by increasing cortisol secretion bilaterally, ACTH stimulation enhances the ascertainment of the selectivity of AVS on both sides (Fig. 5a) as mentioned above. This conclusion is fully consistent with that of a smaller study in which the rate of successful bilateral cannulation doubled after ACTH stimulation (250 µg as a bolus followed by a continuous infusion of 10 µg/min) compared with baseline [22].

Notwithstanding this improved detection of selectivity, the effect of ACTH on correct lateralization of aldosterone secretion was disappointing (Fig. 5b); an increased lateralization to the correct side was seen only in about one fifth of the cases at the cost of a decrease (in roughly 60%) and even inversion (in about one fifth) of the correct lateralization.

Therefore, overall these results warrant two conclusions: first, the response of APA in vivo to ACTH stimulation is heterogeneous because of the existence of ACTH-responsive and ACTH-nonresponsive APA; second, the use of ACTH, although facilitating the improvement in selectivity of adrenal vein catheterization, does not increase the overall diagnostic accuracy of AVS, at least with the maximal stimulation protocol that was used in this study. Whether the same conclusion applies to the smaller doses and different protocols of ACTH stimulation that have been used previously [2,7–10] remains to be investigated. We, however, recently showed no effect whatsoever on either cortisol secretion and selectivity index or lateralization index of the very low ‘physiological’ dose of ACTH originally described by Doppman and Gill [7], indicating that this dose is ineffective in eliciting any adrenocortical hormone response [17].

Is adrenal vein sampling the ‘gold standard’ for diagnosing aldosterone-producing adenoma?

The fact that using a lateralization index cutoff of 2.0 some APAs were not identified may suggest that AVS is rather insensitive and inaccurate in identifying APA. This issue deserves, in our view, further prospective investigation; nevertheless, the present findings suggest that adrenalectomy could be proposed for some patients with a lateralization index below such a cutoff, particularly in the presence of resistance of hypertension and/or hypokalemia to pharmacological treatment, evidence of excess target organ damage [23], occurrence of intolerable side effects with mineralocorticoid receptor antagonist treatment, and a diameter and/or features of the adrenal mass at computed tomography and/or magnetic resonance that might suggest malignancy [24,25].

Limitations of the study

Our results cannot provide any useful information for identification of IHA because, according to the STARD recommendations [19,20], the performance of any diagnostic test should be examined using a ‘gold standard’, for example a conclusive diagnosis as a reference. Hence, because of the absence of accepted criteria for diagnosing IHA, examination of the lateralization index performance in the patients with presumed IHA would have been pure academic exercise. Moreover, the lack of lateralization of aldosterone secretion at AVS is applied to exclude APA and thereby diagnose IHA. Therefore, examining the usefulness of AVS for diagnosing IHA, which cannot be diagnosed with methods other than AVS, would inevitably result in a tautological (‘circular reasoning’) bias. Instead, restriction of our analysis to the patients with APA furnished solid evidence that a low lateralization index cutoff should be used to avoid denying the chance of long-term cure to a substantial proportion of patients with APA.

Conclusions

The present study shows that AVS can be safely performed in most patients who are plausible candidates for adrenalectomy. Moreover, these results can provide a framework for the proper use and interpretation of AVS. They show that: first, under baseline conditions use of more stringent criteria for ascertainment of selectivity of AVS results in discarding a good deal of studies and, therefore, in exposure of a large proportion of primary aldosteronism patients to the risks of AVS without any gain from the diagnostic standpoint; second, likewise, adoption of tight criteria for assessing lateralization of aldosterone excess translates into exclusion of a large proportion of patients who have surgically curable primary aldosteronism from curative adrenalectomy; third, the use of ACTH stimulation increases the chances of getting bilaterally selective AVS studies at the price of a confounding outcome on lateralization.

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