Adrenocorticotropic Hormone Stimulation During Adrenal Vein Sampling for Identifying Surgically Curable Subtypes of Primary Aldosteronism: Comparison of 3 Different Protocols

Teresa M. Seccia, Diego Miotto, Renzo De Toni, Gisella Pitter, Franco Mantero, Achille C. Pessina, Gian Paolo Rossi

Abstract—Adrenocorticotropic hormone administration was proposed to overcome the biases associated with pulsatile aldosterone secretion during adrenal venous sampling, but the usefulness of different protocols of stimulation was never systematically assessed. We, therefore, compared the effects of a high dose (HD; 250 μg IV as a bolus), a very low dose (VLD; 250 pg IV), and an intermediate dose (ID; 50 μg/h) of adrenocorticotropic hormone on the selectivity index (SI) and the lateralization index in primary aldosteronism patients, using the diagnosis of aldosterone-producing adenoma, based on pathology and follow-up data, as a reference. The HD (n=47) significantly increased plasma cortisol concentration in infrarenal inferior vena cava (+79%) and the SI on both sides (SI\textsubscript{RIGHT} +113% and SI\textsubscript{LEFT} +131%), as compared with baseline values. The ID (n=14) also markedly increased both plasma cortisol concentration inferior vena cava (+93%) and the SI (SI\textsubscript{RIGHT} +690% and SI\textsubscript{LEFT} +410%); the very low dose (n=6) had no effect on either the plasma cortisol concentration or SI. In the patients with unilateral aldosterone-producing adenoma, the increase of selectivity with the HD and ID was counterbalanced by a confounding effect on the correct identification of the aldosterone-producing adenoma side, which was attributed to the wrong side in 3.0% and 12.5% with HD and ID, respectively. In conclusion, the HD and the ID, but not the very low dose, adrenocorticotropic hormone stimulation protocol facilitated the ascertainment of selectivity of adrenal vein catheterization. However, this favorable effect was overridden by a confounding effect on the identification of lateralized aldosterone excess to the aldosterone-producing adenoma side. Hence, we do not recommend adrenocorticotropic hormone stimulation. (Hypertension. 2009;53:00-00.)

Key Words: aldosteronism ■ adrenal venous sampling ■ adenoma ■ ACTH ■ cortisol

Adrenal vein sampling (AVS) is considered the gold standard for diagnosing the surgically curable subtypes of primary aldosteronism. However, the pulsatile nature of aldosterone secretion, which can even be augmented in patients with hyperaldosteronism under the stressful condition occurring during AVS, can create artificial gradients between the sides, particularly when the sequential blood sampling technique is used, and, therefore, hamper the diagnostic accuracy of AVS.

To overcome these potential biases, in 1979, Weinberger et al introduced the stimulation with adrenocorticotropic hormone (ACTH), which was thereafter widely used, even without conclusive evidence for its advantages. Recent studies with bilaterally simultaneous AVS where baseline and post-ACTH AVS data were compared head to head indicated that a maximal (250 μg as an IV bolus) ACTH dose facilitates the ascertainment of selectivity of catheterization during AVS. They also suggested, however, that this dose can confound the identification of the side harboring the aldosterone-producing adenoma (APA). Nonetheless, if other doses and protocols of ACTH administration had similar effects or whether they might improve the diagnostic accuracy of AVS remained to be determined.

This study was, therefore, set up to prospectively compare the effects of a very low dose (VLD), an intermediate dose (ID), and a maximal ACTH dose administered during AVS on the ratio between plasma cortisol concentration (PCC) and aldosterone concentration (PAC) in the right or left adrenal vein and the infrarenal inferior vena cava (IVC), herein termed selectivity index (SI), and the ratio of plasma aldosterone concentration (PAC):PCC on the side with the higher ratio over the contralateral PAC to PCC, herein defined as the lateralization index (LI).

Methods

Patient Selection
Among the patients referred for suspected primary aldosteronism (PA) at the Specialized Centre for Hypertension in the Department of
Clinical and Experimental Medicine of the University Medical School of Padua from 2001 to 2007, those who had unequivocal biochemical evidence of PA,11 no contraindications to surgery under general anesthesia, and were willing to have laparoscopic adrenalectomy were offered AVS and recruited for this study. Exclusion criteria were composed of coexisting heart failure or renal insufficiency.

The patients were prepared from the pharmacological standpoint by stopping diuretics, β-blockers, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers ≥2 weeks before and after stimulation with synthetic ACTH (Synacthen, Novartis), as described.9 AVS was undertaken after correction of hypokalemia, if present, with oral or intravenous potassium ion supplementation. All of the procedures followed the institutional guidelines, adhered to the principles of the Declaration of Helsinki, and were approved by the institutional review committee. An informed written consent was obtained from each participant.

### AVS Procedure

AVS was performed with bilateral simultaneous catheterization, eg, by using 1 catheter for each adrenal vein before and after stimulation with synthetic ACTH (Synacthen, Novartis), as described.9 The following protocols of ACTH administration were used: (1) a high dose (HD; 250 μg as an IV bolus; n = 47); (2) an ID (100 μg ACTH as a priming dose followed by 50 μg/h of infusion; n = 14); and (3) a VLD (250 μg ACTH as a priming IV bolus followed by 0.5 μg/min of infusion; n = 6).

All of the AVS studies were performed by the same experienced radiologist (D.M.) with the same methodology, as described.11 Blood samples for the measurements of PAC and PCC were obtained from the infrarenal IVC and the right and left adrenal veins by gravity at baseline (t₀) and again 30 minutes after ACTH stimulation (t₃₀). On the left side, the catheter remained in the adrenal vein during the entire procedure. On the right side, it was withdrawn from the vein after obtaining the t₀ sample to avoid the risk of thrombosis; it was thereafter repositioned in the vein to collect the blood sampling at t₃₀. Because of these differences, the effects of ACTH on the AVS results of the left and right sides were analyzed separately to avoid any bias associated with the repositioning.

### Definitions

The SI was calculated as the ratio between PCC in the right or left adrenal vein and the infrarenal IVC.9 The LI was assessed as the ratio of PAC/PCC on the side with the higher ratio over the contralateral PAC to PCC, as described.10 The cutoff value for ascertaining selectivity of 1.1 was established previously using a receiver operator characteristic curve analysis.10,12 The fact that the right adrenal vein shares egress in the IVC with hepatic accessory veins carrying blood with a much lower PCC concentration in ~11% of the patients12 justifies the selection of this seemingly low value.10,12 The LI cutoff for diagnosing lateralization was similarly determined using the diagnosis of APA as the referent.12 For the reasons detailed previously,11 the LI was calculated only when SI values at t₀ were ≥1.1 on both sides.

### Diagnostic Criteria

Plasma renin activity, aldosterone, cortisol, and ACTH were assayed, as described.9 The diagnosis of APA was based on the “4 corners” approach11 that required all of the following: (1) biochemical diagnosis of PA; (2) lateralization of aldosterone secretion of AVS; (3) evidence of adrenocortical adenoma at imaging, including computed tomography or magnetic resonance and/or pathology; and (4) follow-up demonstration of correction of the hyperaldosteronism and an unequivocal fall of blood pressure.14

### Statistical Analysis

Results are expressed as mean±SEM or median and range, as appropriate. Because of the skewed distribution of PAC and PCC, values were examined after achievement of a Gaussian distribution with log transformation. ANOVA and Bonferroni’s posthoc test were used to compare variables across the groups; a paired t-test was used to compare baseline with ACTH-stimulated log-transformed variables. Statistical significance was defined as P<0.05 (2 sided).

### Calculation of Power

A 2-group t test with a 0.05 2-sided significance level had a 94% power to detect a difference between HD and ID means of 55, assuming that the common SD was 50, given sample sizes of 47 and 14, respectively (nQuery Advisor 6.0, Statistical Solutions). With similar assumptions, the study had a 77% power to detect a difference between HD and VLD means with sample sizes of 47 and 14, respectively (nQuery Advisor 6.0, Statistical Solutions). With similar assumptions, the study had a 77% power to detect a difference between HD and VLD means with sample sizes of 47 and 6, respectively. With these sample sizes, the power to detect a difference between t₀ and t₃₀ values using a paired t test was >99% for the HD versus ID comparison and >90% for the HD versus VLD comparison.

### Results

#### Clinical Features of Patients

Sixty-seven consecutive consenting patients (33 men and 34 women; mean age: 51 ± 12 years) met the inclusion criteria and were recruited. Their main demographic and clinical features are presented in Table 1. Variable differences between HD versus ID and HD versus VLD were compared with paired t tests, and P<0.05 was considered significant...
characteristics (Table 1) were evidence of suppressed PRA and elevated PAC, which translated in a prominent elevation of the aldosterone:renin ratio. In computed tomography, the median maximum diameter of the APA was <20 mm.

**AVS Procedure**

No adverse effects occurred during AVS. In 4 of the 67 patients, right adrenal vein cannulation was unfeasible at t₀; therefore, they were excluded from analyses concerning the post-ACTH data.

**Baseline AVS Results and Effect of Adrenalectomy**

At baseline, AVS was selective in 98% and 77% of the cases on the left and on the right, respectively. Thirty-eight patients (60%) with a lateralized aldosterone secretion were presumed to harbor an APA. Of them, 35 patients underwent laparoscopic adrenalectomy and could be followed-up. All showed complete correction of the hyperaldosteronism at follow-up; moreover, hypertension was cured in 33%, markedly improved in 53%, and mildly improved in 13% by the criteria defined previously.¹⁴ Three patients refused adrenalectomy despite having initially consented to it; they were not used to analyze the diagnostic accuracy of t₀ and t₃₀ AVS data.

**Effects of the Different ACTH Protocols**

Overall, after ACTH stimulation, 100% and 82% of the AVS studies were selective on the left and on the right, respectively. The HD and the ID markedly stimulated cortisol secretion from both sides, as evidenced by an increase of the SI bilaterally (SIRIGHT +113%, from baseline P<0.001; SILeft +131%, P<0.001 with HD; SIRIGHT +690%, P<0.01; SILeft +410%, P<0.001 with the ID; Figure 1A and 1B). Hence, both doses significantly increased PCC in the infrarenal IVC (PCCİVC; +79%, P<0.001; +93%, P<0.001, respectively, from t₀ values). Although the percentage increase from baseline of PCC in the IVC did not differ between the HD and the ID; Figure 2A), the increase of the SI was significantly greater with the ID than with the HD on both the right and the left (Figure 1).

Compared to t₀ values both the HD (+87%; P<0.0001) and the ID (+129%; P<0.001) significantly increased PACİVC, without significant differences between the 2 doses (Figure 2B). By contrast, the VLD did not significantly increase the SI on both sides (Figure 1A and 1B), the PCCİVC (Figure 2A) and the PACİVC (Figure 2B).

**ACTH Doses for the Identification of Lateralized Aldosterone Secretion**

To assess the outcome of the ACTH administration protocols on the identification of the APA side, we used APA diagnosed with tight criteria as referent.¹¹ Because the VLD did not affect PCCİVC and PACİVC appreciably, this analysis was confined to the HD and ID protocols. We found that neither dose increased the LI (Figure 3); the HD actually lowered it significantly, likely because the larger sample size in this group decreased the spread of the values and, thus, enhanced the statistical power.

Overall, after ACTH, the proportion of correctly lateralized AVS studies was 60.6% and 50.0% with the HD or the ID, respectively (Table 2). These rates correspond with a fall of 22.4% and 33.0% of correctly lateralized AVS studies as compared with baseline AVS results. After the HD, aldosterone excess was lateralized to the wrong side in 3.0% and not lateralized in 36.4% of the APA, which would have led to misdiagnosing APA as idiopathic hyperaldosteronism in more cases (+19.4%), as compared with t₀ AVS results.

Because some centers use a cutoff for the LI of 5,⁸ we investigated whether this cutoff would alter the results obtained with a cutoff of 2. We found that, with an LI cutoff of 5, the proportion of AVS studies correctly identified at t₀ was markedly lower (53.2%) than with a cutoff of 2 (83.0%; Table 3). Moreover, after the HD, this rate fell by 14.3% (to 39.4%); therefore, the missed lateralization rate increased by the same percentage to 60.6%.

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**Figure 1.** Effects of the 3 ACTH protocols on the SIRIGHT (A) and SILeft (B) sides. An increase of the SI was evident bilaterally after both the HD and the ID as a result of the marked stimulation of cortisol secretion from both sides. No changes were found after the VLD. The value under baseline conditions for each ACTH dose is set to 100%, and therefore the black bars show the percent change from baseline.
Although AVS represents the gold standard for identifying the surgically curable subtypes of PA, uncertainties on the optimal protocol for maximizing its diagnostic performance remain. We prospectively investigated the usefulness of 3 popular protocols of ACTH administration using bilaterally simultaneous AVS to avoid the potential bias associated with the different timing of blood sampling between sides.

**Effect of ACTH Stimulation Protocols on Cortisol Secretion and SI**

The HD (250 μg as IV bolus) was initially used based on the hypothesis that it could maximally stimulate hormone secretion, minimize the confounding effects of stress and pulsatile hormone secretion, thus enhancing the identification of surgically curable PA subtypes. We found that this ACTH dose markedly stimulates cortisol secretion and, therefore, facilitates the ascertainment of selectivity of adrenal vein catheterization, thus supporting previous results. The ID, a 10-fold lower dose, has also been extensively used at some centers but its advantages have never been shown thus far. In our hands, this dose, given as a bolus followed by a continuous infusion, elicited results quite similar to those of the HD: it increased PCC in the adrenal vein and, to a lesser extent, in the IVC blood, where the changes of adrenal hormone concentration reflect the contribution of both adrenal glands (Figure 2). Hence, it enhanced the SI. These results confirm findings of a smaller study with the HD and extend to the ID the conclusion that ACTH stimulation facilitates the ascertainment of selective adrenal vein catheterization during AVS. The ID, given as a priming dose followed by a continuous infusion, increased the SI on both sides to a significantly greater extent than the HD, indicating that it induces a more sustained cortisol response, at least within the 30-minute time span of this study.

Much lower doses of ACTH have also been contended to enhance the diagnostic accuracy of AVS because APA would be exquisitely sensitive to ACTH. An overstimulation of the “healthy” gland might, therefore, blunt the aldosterone gradient between the APA and the contralateral side.

**Table 2. Proportion of Correctly and Misclassified Lateralization Rates and Changes From Baseline (t0) After ACTH Stimulation (t30) of AVS Protocols in Patients With Unilateral APA**

<table>
<thead>
<tr>
<th>Lateralization</th>
<th>LI Cutoff = 2</th>
<th>LI Cutoff = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HD ACTH, %</td>
<td>ID ACTH, %</td>
</tr>
<tr>
<td>Lateralization to the correct side</td>
<td>61 (−22)</td>
<td>50 (−33)</td>
</tr>
<tr>
<td>Lateralization to the wrong side</td>
<td>3 (+3)</td>
<td>12 (+12)</td>
</tr>
<tr>
<td>Missed lateralization</td>
<td>36 (+19)</td>
<td>37 (−20)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentage changes for t30 from t0. ... indicates no data.

**Figure 2. A, Effects of the 3 different ACTH protocols on PCCIVC.** The HD and the ID similarly increased (P value not significant, for comparison) PCCIVC significantly vs t0 values. The VLD induced no significant change. B, Effects of the 3 ACTH doses on PACIVC. The HD and ID, but not the VLD, induced a significant increase of PACIVC.

**Figure 3.** Effects of the high and intermediate ACTH protocols on identification of the lateralized aldosterone secretion. On average, neither protocol increased the LI but rather lowered it, although the decrease was significant only with HD.
milliion-fold lower than the HD, on cortisol and aldosterone secretion, was, therefore, investigated to test this hypothesis. We found that the VLD had no appreciable effect on PCC in the IVC and adrenal vein blood. Hence, under the moderately stressful conditions occurring during AVS, this dose of ACTH stimulation does not improve the selectivity of AVS.

**ACTH Stimulation Protocols and Aldosterone Secretion**

The VLD induced no changes in PAC in the infrarenal IVC blood, thus supporting the conclusion that this dose is too low to induce any measurable change of the adrenocortical hormone secretion during AVS. The changes of $\text{PAC}_{\text{IVC}}$ with the HD or the ID protocol paralleled those of $\text{PCC}_{\text{IVC}}$ and did not differ significantly between doses. Thus, unlike the VLD, these doses equipotently stimulate adrenocortical hormone secretion.

**ACTH Doses for the Identification of Lateralized Aldosterone Secretion**

The adoption of strict predefined diagnostic criteria based on surgery, pathology, and follow-up data permitted us to conclusively diagnose unilateral forms of excess aldosterone secretion,\(^{11}\) which entailed APA in 82% and multiple unilateral adrenocortical micronodules in 1 of our patients. The hyperaldosteronism was cured postadrenalectomy in all; moreover, all exhibited a clear-cut fall of blood pressure and/or medications postadrenalectomy. Hence, the adrenalectomy side could be used as a referent to determine whether the HD and ID protocols improved the identification of surgically curable PA. On average, both the HD and the ID protocols induced a decrease, rather than the anticipated increase of the LI (Figure 3), thus confirming results of previous smaller studies,\(^{9,10}\) as well as the heterogeneous nature of the APA responsiveness to ACTH in vivo.\(^9\)

The LI value depends by definition on the secretion of aldosterone from the APA side and from the contralateral adrenal cortex. Moreover, contralateral suppression was contended to be a distinctive feature of APA,\(^9\) this notwithstanding the fact that in situ hybridization studies evidenced persistent aldosterone synthesis even in the adrenal cortex surrounding the APA.\(^{20}\) Therefore, it was worth examining the aldosterone secretion response to ACTH of the contralateral gland as well. After we found a paradoxical blunting of lateralization with the HD,\(^9\) we hypothesized that this HD could trigger a response not only of the gland harboring the presumably “more ACTH-sensitive” APA but also of the “less sensitive” contralateral gland. An ACTH dose within the “physiological range” could be devoid of such undesirable action and might, therefore, be useful for revealing an APA in the patients without lateralization at a baseline. Our results with either the VLD or the ID do not lend any support to this contention. Instead, they suggest that ACTH stimulation can lead to erroneous results in terms of ascertaining lateralization of aldosterone excess in a substantial rate of cases (Table 2), regardless of the choice of the cutoff value for the SI. The molecular heterogeneity of APA in terms of steroidogenic enzymes and regulatory factor gene expression revealed by whole transcriptome studies,\(^{21}\) rather than the ACTH dose or protocol used to stimulate the adrenal secretion, could account for the variable effects of ACTH on the LI.

**Clinical Implications of These Results**

From the practical standpoint, the administration of ACTH during AVS with the HD or the ID protocol facilitates the ascertainment of selectivity but translates into the failure to identify correctly the APA side in 3.0% and 12.5%, respectively. Furthermore, misclassification of the APA as idiopathic hyperaldosteronism occurred in more than one third of the cases with both doses (Table 2). Thus, ACTH stimulation can be more confusing than useful for identifying the surgically curable subtype of PA.

Using the popular cutoff value of 5 for the LI, lateralization to the APA side was seen in less AVS studies than with a cutoff of 2; moreover, a greater proportion was misdiagnosed as having idiopathic hyperaldosteronism with the HD and with the ID (Table 2). Thus, the diagnostic accuracy of the AVS studies markedly fell with either dose, leading to the preclusion of curative adrenalectomy in a fairly large rate of PA patients.

**Limitations of the Study**

The use of simultaneous bilateral AVS was necessary in this study to avoid a time-related bias in the evaluation of the ACTH effect. Because most centers do not use simultaneous bilateral sampling procedures, it might be argued that the present results do not apply to studies using a single-catheter approach. However, this seems unlikely, because at experienced centers, the time difference between the right and left adrenal vein catheterization is usually only few minutes, eg, well within the 30-minute time span of the ACTH effect on hormone secretion that we documented in this study.

**Conclusions**

A high and an intermediate ACTH dose stimulated cortisol secretion from the adrenal with APA and from the contralateral one, thus improving the ascertainment of the selectivity of adrenal vein catheterization during AVS. By contrast, the lowest previously proposed dose\(^5,7\) had no appreciable effect on cortisol secretion and, therefore, was not helpful for confirming the selectivity of AVS. The improvement in the ascertainment of selectivity occurring with both the high and the intermediate ACTH doses was overridden by the confounding effect on identification of lateralized aldosterone.
excess. Therefore, we do not recommend using systematic ACTH stimulation.

**Perspectives**
Further investigation should be devoted to determine whether the HD given as a bolus plus a continuous infusion would produce identical results to the ID. Moreover, whether other stimulatory tests can provide better results remains a goal for future research.

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**Disclosures**
None.

**References**
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