Effect of Adrenocorticotropic Hormone Stimulation During Adrenal Vein Sampling in Primary Aldosteronism

Silvia Monticone, Fumitoshi Satoh, Gilberta Giacchetti, Andrea Viola, Ryo Morimoto, Masataka Kudo, Yoshitsugu Iwakura, Yoshikiyo Ono, Federica Turchi, Enrico Paci, Franco Veglio, Marco Boscaro, William Rainey, Sadayoshi Ito, Paolo Mulatero

Abstract—Adrenal vein sampling (AVS) is fundamental for subtype diagnosis in patients with primary aldosteronism. AVS protocols vary between centers, especially for diagnostic indices and for use of adrenocorticotropic hormone (ACTH) stimulation. We investigated the role of both continuous ACTH infusion and bolus on the performance and interpretation of AVS in a sample of 76 patients with confirmed primary aldosteronism. In 36 primary aldosteronism patients, AVS was performed both under basal conditions and after continuous ACTH infusion, and in 40 primary aldosteronism patients, AVS was performed both under basal conditions and after ACTH IV bolus. Both ACTH protocols determined an increase in the rate of successful cannulation of the adrenal veins. Both ACTH infusion and bolus determined a significant increase in selectivity index for the right adrenal vein and ACTH bolus for the left adrenal vein. Lateralization index was not significantly different after continuous ACTH infusion and IV bolus. In 88% and 78% of the patients, the diagnosis obtained was the same before and after ACTH infusion and IV bolus, respectively. However, the reproducibility of the diagnosis was reduced using less stringent criteria for successful cannulation of the adrenal veins. This study shows that ACTH use during AVS may be of help for centers with lower success rates, because a successful adrenal cannulation is more easily obtained with this protocol; moreover, this technique performs at least as well as the unstimulated strategy and in some cases may be even better. Stringent criteria for cannulation should be used to have a high consistency of the diagnosis.

Key Words: primary aldosteronism • endocrine hypertension • aldosterone • aldosterone-producing adenoma • adrenal vein sampling

Diagnosis of primary aldosteronism (PA), the most frequent cause of secondary hypertension, requires 3 steps, screening, confirmation, and subtype differentiation.1,2 The last step is fundamental, because some subtypes (aldosterone-producing adenoma [APA] and unilateral adrenal hyperplasia [BAH]) benefit from adrenalectomy, and others (bilateral adrenal hyperplasia [BAHI]) should be treated pharmacologically with mineralocorticoid receptor antagonists.1,2 Subtype diagnosis requires computed tomography (CT) scanning and adrenal vein sampling (AVS). If adrenalectomy is considered, the latter procedure is an indispensable part of disease lateralization, because CT scanning has been demonstrated to be unreliable in terms of sensitivity and specificity.1,4 However, AVS is a complex procedure, requiring a skilled and dedicated radiologist and a standardized protocol.1,2,7,8 Unfortu-
dilution. LI is the ratio of the cortisol-corrected aldosterone levels between the dominant and nondominant adrenal glands.

One of the most important issues in the AVS procedure is the ACTH stimulation: cosyntropin infusion or bolus is used in some centers to minimize stress-induced fluctuations in aldosterone secretion in nonsimultaneous AVS, to maximize the gradient in cortisol from the adrenal vein to the inferior vena cava, and to maximize aldosterone secretion from an APA. However, in some cases ACTH administration may result in the stimulation of aldosterone production in the gland contralateral (CL) to an APA, thus reducing the gradient of aldosterone production. A recent study showed that a bolus of high-dose ACTH can result in incorrect lateralization of aldosterone secretion. However, in this article the authors did not investigate the role of continuous cosyntropin infusion (without bolus) and interpreted the results using SI that has been shown to be unreliable in subsequent studies. The aim of our study was to investigate the role of both continuous cosyntropin infusion (in patients from the Torino and Ancona units) and bolus (in patients from the Sendai unit) on the performance and interpretation of AVS in a large sample of 76 PA patients.

Materials and Methods

Patient Selection

The study was carried out in 3 referral centers: (1) the Division of Internal Medicine and Hypertension Unit, University of Torino; (2) the Division of Endocrinology, University of Ancona; and (3) the Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Medicine. Patients were enrolled following written, informed consent and approval of the study protocol by the local ethics committees. In all 3 of the units, patients were studied after all of the antihypertensive drugs were withdrawn ≥3 weeks before screening (≥6 weeks for diuretics and ≥8 weeks before for spironolactone and eplerenone).

Patients who, for clinical reasons, could not be left untreated were allowed to take an α1-blocker (doxazosin) and/or a calcium channel blocker (verapamil or amlodipine) and maintained on this same therapy for screening and the period between the screening and the final subtype diagnosis. During AVS, blood samples were collected by passive gravity flow or by gentle aspiration, especially when a microcatheter was used.

SI was defined as cortisol_{adrenal vein}/cortisol_{peripheral vein} and LI as aldosterone/cortisol_{adrenal vein}/aldosterone/cortisol_{CL adrenal vein}. CL suppression was defined as aldosterone/cortisol_{dominant adrenal vein}/aldosterone/cortisol_{peripheral vein} < 1.

Torino

Sixteen consecutive PA patients who underwent AVS in the University of Torino Hypertension Unit were selected. PA patients were selected as described previously. Briefly, patients were screened using the ARR and confirmed with an IV saline load CT scanning with fine cuts (2.5 mm) of the adrenal with contrast was performed in all of the PA patients. Adrenal vein cannulation, performed in all of the patients with a positive saline load test, was considered successful if the adrenal vein/inferior vena cava cortisol gradient was ≥1.1. The study was considered to show lateralization when the aldosterone/cortisol ratio from 1 adrenal was ≥2 times the ratio from the other adrenal gland. As for the Torino Unit, AVS was performed between 08:00 AM and 11:00 AM both in basal conditions and after continuous cosyntropin infusion, started 30 minutes before sampling.

Ancona

Twenty consecutive PA patients who underwent AVS in the unit at the University of Ancona were selected. PA patients were selected as described previously. Briefly, patients were screened using the ARR and confirmed with an IV saline load CT scanning with fine cuts (2.5 mm) of the adrenal with contrast was performed in all of the PA patients. Adrenal vein cannulation, performed in all of the patients with a positive saline load test, was considered successful if the adrenal vein/inferior vena cava cortisol gradient was ≥1.1. The study was considered to show lateralization when the aldosterone/cortisol ratio from 1 adrenal was ≥2 times the ratio from the other adrenal gland. As for the Torino Unit, AVS was performed between 08:00 AM and 11:00 AM both in basal conditions and after continuous cosyntropin infusion, started 30 minutes before sampling.

Sendai

The diagnosis for PA was established after a positive screening test with ARR measurement, by captopril test as described. Dexamethasone suppression tests were performed in all of the patients to exclude PA patients with cortisol-producing adenomas before AVS. Forty consecutive PA cases underwent AVS at Tohoku University Hospital, following the protocol described previously. Bilateral adrenal veins were simultaneously catheterized in all of the patients. After baseline samples were simultaneously obtained from both adrenal veins, a second set of blood samples was collected from the same sites 15 minutes after IV bolus injection of 0.25 mg (10 IU) of ACTH. Successful adrenal venous cannulation was based on an AVS cortisol level that was >5-fold compared with that in the iliac vein sample after ACTH stimulation. The study was considered to show lateralization when the aldosterone/cortisol ratio from 1 adrenal was ≥2.6 times the ratio from the other adrenal gland.

Results

Clinical and biochemical parameters of patients participating to the study are described in Table 1. Overall, the patients cover the typical phenotypic spectrum of PA patients with a higher prevalence of grade 3 and resistant hypertension and a proportion of hypokalemic patients of 49%. Patients from the Torino unit tended to display a more severe phenotype in terms of blood pressure, number of antihypertensive drugs, and aldosterone levels but not potassium levels compared with other units, in particular, the Sendai unit (Table 1).

For the evaluation of the SI and of the LI in basal condition we defined the criteria as follows: (1) strict criteria if SI was >3.0 and LI was >4.0; (2) intermediate if SI was >2.0 and LI was >3.0; and (3) permissive criteria if SI was >1.1 and LI was >2.0. For post-ACTH evaluation we defined the criteria as strict if SI was >4.0 and LI was >4.0 and intermediate if SI was >2.0 and LI was >3.0. Permissive criteria for this condition was not defined, because we are not aware of units using SI <2.0 after ACTH infusion.

Effect of ACTH on Cortisol and Aldosterone Secretion

ACTH infusion and bolus determined a significant and quantitatively similar increase in peripheral cortisol and aldosterone levels (Figure 1). In particular, continuous ACTH infusion increased peripheral cortisol levels (median [25th–75th percentile]) from 13.9 μg/dL (10.0–18.3) to 25.4 (19.1–29.1; P < 0.001), and peripheral aldosterone levels from 26.5 ng/dL
(12.6–42.0) to 40.2 (26.2–67.9; \(P=0.006\)). ACTH IV bolus caused an increase of peripheral cortisol levels from 7.5 \(\mu g/dL\) (4.6–11.0) to 14.3 (11.9–16.4; \(P=0.001\)), and peripheral aldosterone levels from 12.5 ng/dL (8.7–19.4) to 21.7 (15.7–33.1; \(P=0.001\)).

### Effect of Continuous IV ACTH Infusion on Success Rate of Adrenal Vein Cannulation

In basal conditions, left adrenal vein was cannulated with a higher success rate compared to the right adrenal vein, independent of the criteria used for the SI (Table 2). ACTH infusion caused an increase of the success rate of cannulation of both adrenal veins, in particular the right adrenal vein (from 53% to 72%). This effect was evident in both Torino (+13%) and Ancona units (+25%), with a larger increase in the latter. This difference is probably because of a lower success rate in basal conditions in the Ancona unit, possibly related to a shorter experience of the radiologist in this unit.

Interestingly, even using intermediate criteria, we observed a significant increase in the rate of cannulation of both adrenal veins (from 69% to 92%), and again the increase was more evident for the right adrenal vein (from 72% to 92%). It should be noted that the term “cannulation” is used when a certain SI is achieved. However, in some cases the catheter tip may not be in the adrenal vein but close to it, especially for the post-ACTH measurements.

### Effect of IV Bolus of ACTH on Success Rate at Adrenal Vein Cannulation

Using the ACTH bolus IV infusion, in the Sendai unit, the effect of the success rate at cannulation was even more evident than for the other units: all of the patients were successfully cannulated after ACTH, even using strict criteria, compared with less than a half of the patients in basal conditions (Table 3). It should be noted that, in this unit, the use of permissive criteria to interpret the AVS findings in basal conditions would result in successful cannulation in all of the patients, as much as obtained after ACTH bolus using strict criteria for interpretation of AVS findings.

### Effect of ACTH on Selectivity and Lateralization Indices

Both ACTH infusion and bolus determined a significant increase in SI for the right adrenal vein, from 3.2 (1.2–16.0) to 9.9
(2.5–24.1; \( P = 0.03 \)), and from 3.6 (2.6–5.5) to 51.6 (39.0 to 67.4; \( P = 0.001 \)), respectively (Figure 2A). SI for the left adrenal vein increased significantly after ACTH bolus from 3.1 (2.5–5.0) to 52.3 (38.1–65.8; \( P = 0.001 \)) but not after ACTH infusion, from 6.4 (2.8–14.9) to 12.5 (4.5–18.2; \( P = 0.1 \); Figure 2A and Table S1, available in the online-only Data Supplement). We hypothesize that this difference results from either a greater cortisol stimulation by bolus ACTH injection compared with ACTH infusion or the potential dilution of left adrenal vein blood when sampling is performed in the common trunk originating from the union with the inferior phrenic vein. LI was not significantly different after continuous ACTH infusion and after IV bolus (Figure 2B and Table S1).

### Effect of Continuous ACTH Infusion on Final Diagnosis

Seventeen (47%) of 36 AVSs were successful under both basal and post-ACTH conditions using strict criteria. Fifteen (88%) of 17 had the same diagnosis before and after ACTH (Table 4). Eighteen (72%) of 25 had the same diagnosis before and after ACTH. In 3 cases a diagnosis of APA became a diagnosis of BAH after ACTH, and in 3 cases a BAH became an APA after ACTH. However, in the other 3 patients with SI satisfying intermediate but not strict criteria, only 5 had the same diagnosis before and after ACTH. In the other 3 cases, 2 BAHs became APAs and 1 APA became BAH after ACTH. In the 4 patients having SI between 1.1 and 2.0 in basal conditions, none had the diagnosis confirmed after ACTH (2 BAHs became APAs and 2 APAs became BAHs).

Interestingly, in 24 (92%) of 26 cases in which post-ACTH was successful with both criteria, the diagnosis reached was the same. In the 2 cases with different diagnosis, this was because of an LI between 3 and 4 and not because of the different SI.

### Effect of IV ACTH Bolus on Final Diagnosis

Eighteen (45%) of 40 AVSs were successful under both basal and post-ACTH conditions using restrictive criteria (Table 5). Fourteen (78%) of 18 had the same diagnosis before and after ACTH. In all 4 of the cases, the difference was because of a diagnosis of APA becoming a diagnosis of BAH after ACTH infusion. In 2 cases, patients were adrenalectomized, confirming the diagnosis of BAH.

<table>
<thead>
<tr>
<th>Basal AVS</th>
<th>Strict (SI &gt;3.0)</th>
<th>Intermediate (SI &gt;2.0)</th>
<th>Permissive (SI &gt;1.1)</th>
<th>Unsuccessful (SI &lt;3.0/2.0/1.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAV cannulated, n (%)</td>
<td>28 (78)</td>
<td>31 (86)</td>
<td>34 (94)</td>
<td>8/5/2 (22/14/6)</td>
</tr>
<tr>
<td>RAV cannulated, n (%)</td>
<td>15/13 (94/65)</td>
<td>15/16 (94/80)</td>
<td>16/18 (100/90)</td>
<td></td>
</tr>
<tr>
<td>Both AV, n (%)</td>
<td>19 (53)</td>
<td>26 (72)</td>
<td>29 (81)</td>
<td>17/10/7 (47/28/19)</td>
</tr>
<tr>
<td>LAV cannulated, n (%)</td>
<td>31 (86)</td>
<td>33 (92)</td>
<td></td>
<td>5/3 (14/8)</td>
</tr>
<tr>
<td>RAV cannulated, n (%)</td>
<td>16/15 (100/75)</td>
<td>16/17 (100/85)</td>
<td></td>
<td>10/3 (28/8)</td>
</tr>
<tr>
<td>Both AV, n (%)</td>
<td>26 (72)</td>
<td>33 (92)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ACTH indicates adrenocorticotrophic hormone; AV, adrenal vein; RAV, right adrenal vein; LAV, left adrenal vein; AVS, adrenal vein sampling; SI, selectivity index. Data are expressed as total No. and percentage after subdivision into Torino and Ancona units.
Thirty-two (80%) of 40 AVSs were successful under both basal and post-ACTH conditions using intermediate criteria. Twenty-six (81%) of 32 had the same diagnosis before and after ACTH. Using basal permissive criteria and post-ACTH intermediate criteria, all of the AVSs were successful. However, only 26 (65%) of 40 displayed the same diagnosis before and after ACTH. In 1 case, the diagnosis of BAH became a diagnosis of APA after ACTH, whereas in all of the other 13 cases the change in the diagnosis was from an APA to a BAH.

Interestingly, in 25 (96%) of 26 cases in which post-ACTH was successful with both criteria, the diagnosis reached was the same. In the unique case with a different diagnosis, this was because of an LI between 3 and 4 and not because of the different SI.

It should be noted that basal permissive criteria allowed the diagnosis of 5 cases of APA but also would caused the adrenalectomy in 7 cases of BAH. Interestingly, in 3 of 7 of these BAH cases, the diagnosis was confirmed by histology with immunohistochemical staining of steroidogenic enzyme and postsurgical clinical evaluation.17

**CL Suppression in Patients With Diagnosis of APA**

CL suppression, when AVS is performed under basal conditions, is considered by some authors as a necessary indicator for adrenalectomy.5 Therefore, we also considered the presence of CL suppression in patients with diagnosis of APA according to different criteria (Table S2). We observed that most patients with a concordant diagnosis of APA obtained both under basal and post-ACTH had CL suppression, as reported by others.6 Under basal conditions, more patients with a diagnosis of APA were less likely to exhibit CL suppression, and this was even more evident if permissive criteria were applied. However, the differences between groups were not statistically significant.

**Discussion**

AVS is considered the most reliable approach to distinguish unilateral from bilateral forms of PA. In fact, imaging techniques of the adrenal glands have been shown to be unreliable because of lack of sensitivity for unilateral micro-APAs and unilateral adrenal hyperplasia and lack of specificity for nonsecreting adrenal nodules.7 For this reason, recent Endocrine Society1 and Japan Endocrine Society20 guidelines indicated that, when adrenalectomy is considered in a PA patient, unilateral forms have to be identified by AVS.1,20 A recent study showed some promising findings for the use of the 11C-metomidate positron-emission tomography-CT imaging to localize adrenal APA.21 However, the sensitivity and specificity are still not high enough to be considered a valuable alternative to AVS in PA subtype differentiation.

PA subtype differentiation is fundamental, because unilateral PA is treated by adrenalectomy, whereas bilateral forms are treated with mineralocorticoid receptor antagonists. Unfortunately, there is no agreement on AVS protocols and interpretation of the procedure. This may cause confusion in the final diagnosis and limit the diffusion of this technique. One of the controversies on AVS protocol is the use of ACTH.

### Table 4. Effect of Continuous ACTH Infusion on Final Diagnosis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Basal Strict Cr. +</th>
<th>Basal Intermediate Cr. +</th>
<th>Basal Permissive Cr. +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successful cannulation, n/N (%)</td>
<td>17/36 (47)</td>
<td>25/36 (69)</td>
<td>29/36 (81)</td>
</tr>
<tr>
<td>Diagnosis concordance, n/N (%)</td>
<td>15/17 (88)</td>
<td>18/25 (72)</td>
<td>18/29 (62)</td>
</tr>
<tr>
<td>Diagnosis changes</td>
<td>1 APA → BAH</td>
<td>3 APA → BAH</td>
<td>5 APA → BAH</td>
</tr>
<tr>
<td></td>
<td>1 BAH → APA</td>
<td>4 BAH → APA</td>
<td>6 BAH → APA</td>
</tr>
</tbody>
</table>

ACTH indicates adrenocorticotropic hormone; BAH, bilateral adrenal hyperplasia; APA, aldosterone-producing adenoma; Cr., criteria.
stimulation during the procedure. ACTH infusion could theoretically be of help in reducing fluctuation of aldosterone and cortisol production during nonsimultaneous sampling but also nonsynchronous fluctuation of these hormones during simultaneous AVS and to maximize aldosterone production from an APA. Furthermore, ACTH stimulation is necessary for those patients who require steroid prophylaxis because of a history of allergic reactions to contrast and for procedures performed in the afternoon, when cortisol production is lower and a demonstration of successful cannulation more difficult. We have shown in the present study that ACTH use during AVS may be of help for clinicians: centers with lower success rates under basal conditions should consider performing AVS after ACTH stimulation, because a successful adrenal cannulation is more easily obtained with this protocol. Overall, the success rate at cannulation after ACTH was 87% compared with 49% obtained in basal conditions. Moreover, our data show that this technique performs at least as well as the unstimulated strategy and in some cases may be even better. This finding is in disagreement with a previous report that raised concerns about the potential negative effects of ACTH infusion, resulting in a misleading subtype diagnosis. Surprisingly, we did not observe an increase in LI after ACTH, resulting in a misleading subtype diagnosis. We underline the extreme difficulty of finding the ideal cutoff that allows discrimination between unilateral and bilateral PAs. In fact, this cutoff could only be obtained by removing every single dominant adrenal in PA patients, regardless of the level of SI and LI, and re-evaluating the postsurgery outcomes. Such a study, which could be ethically challenging, would also be hampered by the fact that a consistent number of BAH patients also display blood pressure reduction and sometimes a cure for hypertension and hypokalemia after unilateral adrenalectomy, as shown recently by Sukor et al.

**Perspectives**

Endocrine Society Guidelines clearly stated the importance of AVS to determine subtype diagnosis of PA and the necessity to perform this evaluation for all patients for whom the adrenalectomy is considered. However, the lack of standardization for AVS protocols created confusion for clinicians both in terms of performance and interpretation of the

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Successful cannulation, n/N (%)</td>
<td>18/40 (45)</td>
<td>32/40 (80)</td>
<td>40/40 (100)</td>
</tr>
<tr>
<td>Diagnosis concordance, n/N (%)</td>
<td>14/18 (78)</td>
<td>26/32 (81)</td>
<td>26/40 (65)</td>
</tr>
<tr>
<td>Diagnosis changes</td>
<td>4 APA → BAH</td>
<td>5 APA → BAH</td>
<td>13 APA → BAH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 BAH → APA</td>
</tr>
</tbody>
</table>

ACTH indicates adrenocorticotropic hormone; BAH, bilateral adrenal hyperplasia; APA, aldosterone-producing adenoma; Cr., criteria.
AVS results. The present study demonstrated that cosyntropin infusion may be of help for those centers with a low rate of cannulation and perform at least as well as the unstimulated protocol for final diagnosis of PA subtypes. Furthermore, we have shown that strict criteria for selectivity and lateralization indices are of primary importance to ensure diagnostic reproducibility. Future guidelines should consider establishing widely accepted protocols for AVS performance and interpretation to more easily compare diagnostic results and to allow the diffusion of this technique to a larger number of centers.

Disclosures
None.

References
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Running Title: ACTH and adrenal vein sampling

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Supplemental Table S1. Basal and post-ACTH SI and LI

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Torino</th>
<th>Ancona</th>
<th>Sendai</th>
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<tr>
<td>Basal SI LAV</td>
<td>15.8 (1.1-44.6)</td>
<td>9.1 (0.7-34.7)</td>
<td>5.9 (1.6-36)</td>
</tr>
<tr>
<td>Basal SI RAV</td>
<td>26.2 (0.9-89)</td>
<td>3.5 (0.8-24.2)</td>
<td>6.5 (1.6-43.7)</td>
</tr>
<tr>
<td>Basal LI</td>
<td>5.6 (1.2-21.5)</td>
<td>2.7 (1-5.7)</td>
<td>15.1 (1.1-75.4)</td>
</tr>
<tr>
<td>Post-ACTH SI LAV</td>
<td>21.4 (4.7-73.9)</td>
<td>10.8 (0.9-43.5)</td>
<td>56.3 (18.3-127.3)</td>
</tr>
<tr>
<td>Post-ACTH SI RAV</td>
<td>23.7 (2.2-62)</td>
<td>9.2 (0.9-37.1)</td>
<td>56.3 (7.8-128.5)</td>
</tr>
<tr>
<td>Post-ACTH LI</td>
<td>5.6 (1.3-15.4)</td>
<td>3.8 (1-9.6)</td>
<td>10.3 (1-59.7)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (min-max).

Supplemental Table S2. Contralateral suppression in APA patients

<table>
<thead>
<tr>
<th>basal AVS</th>
<th>strict (SI&gt;3)</th>
<th>intermediate (SI&gt;2)</th>
<th>permissive (SI&gt;1.1)</th>
<th>concordant diagnosis of APA (strict criteria)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APA with CL suppression (%)</td>
<td>76%</td>
<td>77%</td>
<td>62%</td>
<td>88%</td>
</tr>
</tbody>
</table>

| post-ACTH AVS      | strict (SI>4) | intermediate (SI>2) | 93% | 89% | 94% |
| APA with CL suppression (%) (continuous/bolus) | (92%/94%) | (83%/95%) | |

Concordant diagnosis is intended when a diagnosis of APA is made both under basal and post-ACTH conditions, using strict criteria.