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

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Abstract

Adrenal vein sampling (AVS) is fundamental for subtype diagnosis in patients with primary aldosteronism (PA). AVS protocols vary between centers, especially for diagnostic indexes and for use of 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 PA. In thirty-six PA patients, AVS was performed both under basal conditions and after continuous ACTH infusion, and in 40 PA patients, AVS was performed both under basal conditions and after ACTH i.v. 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. Lateralisation index was not significantly different after continuous ACTH infusion and i.v. bolus. In 88% and 78% of the patients the diagnosis obtained was the same before and after ACTH infusion and i.v. 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

Introduction

Diagnosis of primary aldosteronism (PA), the most frequent cause of secondary hypertension, requires three steps: screening, confirmation and subtype differentiation (1,2). The last step is fundamental as some subtypes (aldosterone-producing adenoma, APA and unilateral adrenal hyperplasia, UAH) benefit from adrenalectomy and others (bilateral adrenal hyperplasia, BAH) should be treated pharmacologically with mineralocorticoid receptor (MR) antagonists (1,2). Subtype diagnosis requires 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 (1-6). However, AVS is a complex procedure, requiring a skilled and dedicated radiologist and a standardized protocol (1,2,7,8). Unfortunately, protocols for AVS are vary between centers, both in terms of procedure (bilaterally simultaneous or sequential), stimulation (ACTH bolus, continuous cosynthropin infusion or unstimulated) as well as in the interpretation of the selectivity index (SI) and lateralisation index (LI) (3). The SI measures the adequacy of the cannulation of the adrenal veins (AV) and is the ratio between cortisol levels in the adrenal veins (AV) and in the inferior vena cava (IVC). Because of the small size of the adrenal veins blood sampled at the time of AVS is often obtained near the orifice of the vein and may be diluted with other blood. The contaminating blood introduces an error in the measurement of AV aldosterone levels, which most often occurs in the case of the right adrenal vein. The simultaneous measurement of cortisol concentrations allows correction for this dilution. LI is the ratio of the cortisol-corrected aldosterone levels between the dominant and non-dominant adrenal gland.

One of the most important issues in the AVS procedure is the ACTH stimulation: cosynthropin infusion or bolus is used in some centers to minimise stress induced fluctuations in aldosterone secretion in non simultaneous AVS, to maximise the gradient in cortisol from the AV to the IVC and to maximise aldosterone secretion from an APA (9). However, in some cases ACTH administration may result in the stimulation of aldosterone production in the gland contralateral 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 lateralisation of aldosterone secretion (10). However, in this manuscript the authors did not investigate the role of continuous cosynthropin infusion (without bolus) and interpreted the results using SI that has been shown to be unreliable in subsequent studies (11-13).

The aim of our study was to investigate the role of both continuous cosynthropin 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.

Patient selection

The study was carried out in three referral centers: (1) the Division of Internal Medicine and Hypertension Unit, University of Torino, Italy (2) the Division of Endocrinology, University of Ancona, Italy and (3) Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan. Patients were enrolled after written informed consent and approval of the study protocol by the local ethics committees. In all three Units, patients were studied after all antihypertensive drugs were withdrawn at least 3 weeks before screening (at least 6 weeks before for diuretics and at least 8 weeks before for spironolactone and eplerenone). Patients that, for clinical reasons, could not be left untreated were allowed to take an α_1-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 $\text{cortisol}_{\text{adrenal vein}}/\text{cortisol}_{\text{peripheral vein}}$ and LI as $\text{aldosterone/cortisol}_{\text{adrenal vein}}/\text{aldosterone/cortisol}_{\text{contralateral adrenal vein}}$. Contralateral (CL) suppression was defined as $\text{aldosterone/cortisol}_{\text{non-dominant adrenal vein}}/\ \text{aldosterone/cortisol}_{\text{peripheral vein}} <1$.

Torino

Sixteen consecutive PA patients who underwent AVS in the Hypertension Unit at the University of Torino were selected. PA patients were selected as previously described (11). Briefly, patients were screened using the ARR and confirmed with an intravenous saline load (14). CT scanning with fine cuts (2.5 mm) of the adrenal with contrast was performed in all PA patients. Adrenal vein cannulation, performed in all patients with a positive saline load test, was considered successful if the SI was > 2. The AVS was considered to show lateralisation when the LI was > 4 or if it was > 3 together with an aldosterone/cortisol in the contralateral vein lower than that in the peripheral vein. All AVS procedures were performed between 08:00 h and 11:00 h, to minimize the chance that "poor" adrenal/peripheral cortisol gradients could be due to a low cortisol secretory rates from the adrenals as might be expected in the afternoon. Diagnosis of APA was confirmed after surgery, by pathology, blood pressure outcome and normal suppressibility of aldosterone after post-operative intravenous saline loading (4). Hormonal assays were performed as described previously (11). AVS was performed both in basal conditions and after continuous cosynthropin infusion, started 30 minutes before sampling (9).

Ancona

Twenty consecutive PA patients who underwent AVS in the Unit at the University of Ancona were selected. PA patients were selected as previously described (15). Briefly, patients were screened using the ARR and confirmed with an intravenous saline load (15). CT scanning with fine cuts (2.5 mm) of the adrenal with contrast was performed in all PA patients. Adrenal vein cannulation, performed in all patients with a positive saline load test, was considered successful if the adrenal vein/IVC cortisol gradient was at least 1.1 (16). The study was considered to show lateralisation when the aldosterone/cortisol ratio from one adrenal was at least 2 times the ratio from the other adrenal gland (16). As for the Torino Unit, AVS was performed between 08:00 h and 11:00 h both in basal conditions and after continuous cosynthropin infusion, started 30 minutes before sampling (9).

Sendai

The diagnosis for PA was established after a positive screening test with ARR measurement, by captopril test as described (17,18) Dexamethasone suppression tests were performed in all patients to exclude PA patients with cortisol-producing adenomas before AVS (18). Forty consecutive PA cases underwent AVS at Tohoku University Hospital, following the protocol described previously (18). Bilateral adrenal veins were simultaneously catheterized in all patients. After baseline samples were simultaneously obtained from both adrenal veins, a second set of blood samples was collected from the same sites 15 min after iv bolus injection of 0.25 mg (10 IU) of ACTH (18). Successful adrenal venous cannulation was based on an AVS cortisol level that was greater than 5-fold compared with that in the iliac vein sample after ACTH stimulation (18). The study was considered to show lateralisation when the aldosterone/cortisol ratio from one adrenal was at least 2.6 times the ratio from the other adrenal gland (18,19).

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 to 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: strict criteria if SI>3 and LI >4; intermediate if SI>2 and LI>3 and permissive criteria if SI >1.1 and LI > 2; for post-ACTH evaluation we defined the criteria as strict if SI>4 and LI>4 and intermediate if SI>2 and LI>3. Permissive criteria for this condition was not defined since we are not aware of units using SI<2 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 from 13.9 μg/dL [10-18.3] to 25.4 [19.1-29.1] ($p<0.001$), and peripheral aldosterone levels from 26.5 ng/dL [12.6-42] to 40.2 [26.2-67.9] ($p=0.006$). ACTH i.v. bolus caused an increase of peripheral cortisol levels from 7.5 μg/dL [4.6-11] 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 i.v. ACTH infusion on success rate of adrenal veins cannulation.

In basal conditions LAV was cannulated with a higher success rate compared to the RAV, independently 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 RAV (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 due to 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 RAV (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 i.v. bolus of ACTH on success rate at adrenal veins cannulation.

Using the ACTH bolus i.v. infusion, in the Sendai unit, the effect of the success rate at cannulation was even more evident than for the other units: all patients were successfully cannulated after

ACTH, even using strict criteria, compared to 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 patients, as much as obtained after ACTH bolus using strict criteria for interpretation of AVS findings.

Effect of ACTH on selectivity and lateralisation indexes

Both ACTH infusion and bolus determined a significant increase in SI for the RAV, from 3.2 [1.2-16] to 9.9 [2.5-24.1] ($p=0.03$), and from 3.6 [2.6-5.5] to 51.6 [39-67.4], $p<0.001$, respectively (Figure 2A). SI for the LAV increased significantly after ACTH bolus from 3.1 [2.5-5] 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 Supplemental Table 1, please see http://hyper.ahajournals.org). We hypothesize that this difference results from either a greater cortisol stimulation by bolus ACTH injection compared to ACTH infusion or the potential dilution of LAV 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 i.v. bolus (Figure 2B and Supplemental Table 1, please see http://hyper.ahajournals.org).

Effect of continuous ACTH infusion on final diagnosis

Seventeen out of thirty-six (47%) AVS were successful under both basal and post-ACTH conditions using strict criteria. Fifteen out of seventeen (88%) had the same diagnosis before and after ACTH (Table 4). In 1 case a diagnosis of BAH became a diagnosis of APA after ACTH infusion and in 1 case a diagnosis of APA became a diagnosis of BAH after ACTH infusion. In both cases the patients were operated confirming the basal diagnosis.

Twenty-five (69%) AVS were both successful in basal and post-ACTH conditions using intermediate criteria (Table 4). Eighteen out of twenty-five (72%) 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 8 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 BAH became APA and 1 APA became BAH after ACTH. In the 4 patients having SI between 1.1 and 2 in basal conditions, none had the diagnosis confirmed after ACTH (2 BAH became APA and 2 APA became BAH).

Interestingly, in 24/26 (92%) cases in which post-ACTH was successful with both criteria the diagnosis reached was the same. In the two cases with different diagnosis, this was due to a LI between 3 and 4 and not to the different SI.

Effect of i.v. ACTH bolus on final diagnosis

Eighteen out of forty (45%) AVS were successful under both basal and post-ACTH conditions using restrictive criteria (Table 5). Fourteen out of eighteen (78%) had the same diagnosis before and after ACTH. In all four cases the difference was due to a diagnosis of APA becoming a diagnosis of BAH after ACTH infusion. In two cases patients were adrenalectomized confirming the diagnosis of BAH.

Thirty-two out of forty (80%) AVS were successful under both basal and post-ACTH conditions using intermediate criteria. Twenty-six out of thirty-two (81%) had the same diagnosis before and after ACTH. Using basal permissive criteria and post-ACTH intermediate criteria all AVS were successful. However, only 26/40 (65%) displayed the same diagnosis before and after ACTH. In one case the diagnosis of BAH became diagnosis of APA after ACTH, whereas in all the other 13 cases the change in the diagnosis was from an APA to a BAH.

Interestingly, in 25/26 (96%) 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 due to a LI between 3 and 4 and not to 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 3/7 of these BAH cases the diagnosis was confirmed by histology with immunohistochemical staining of steroidogenic enzyme and post-surgical clinical evaluation (17).

Contralateral 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 (supplemental Table S2, please see http://hyper.ahajournals.org). 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 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 microAPAs and UAH and lack of specificity for non-secreting adrenal nodules (3). For this reason recent Endocrine Society (1) and Japan Endocrine Society (20) 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 ^{11}C-metomidate PET-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 since 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 stimulation during the procedure. ACTH infusion could theoretically be of help in reducing fluctuation of aldosterone and cortisol production during non-simultaneous sampling but also non-synchronous fluctuation of these hormones during simultaneous AVS, and to maximize aldosterone production from an APA (9). 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 clinicans: centers with low success rates under basal conditions should consider performing AVS after ACTH stimulation since a successful adrenal cannulation is more easily obtained with this protocol. Overall, the success rate at cannulation after ACTH was 87% compared to 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 misleading subtype diagnosis (10). Surprisingly, we did not observe an increase in LI after ACTH stimulation and therefore, our findings are in disagreement with the hypothesis that cosynthropin infusion maximizes the secretion from an APA. Theoretically, this may have been the case for angiotensin-II unresponsive APA whereas for angiotensin-II responsive APA, a phenotype comprising 30-50% of adenomas (22), cosynthropin may cause a reduction of LI by stimulating the gland contralateral to the APA. However, it has been shown that angiotensin-II responsive APA also display a response to ACTH infusion (23), and the results of the present study further rule out the possibility of significant false negative lateralisation findings after ACTH stimulation.

Some discrepancy between the final diagnosis obtained before and after ACTH was shown. In 2 cases the diagnosis was different after ACTH infusion compared to basal conditions in Italian patients; in both cases, the final diagnosis was in agreement with the basal rather than the stimulated results. In one case (from APA to BAH diagnosis after ACTH) contralateral retroinhibition was absent in basal conditions and in the other (from BAH to APA) both LI values were around the cut-off of 4. By contrast, bolus ACTH resulted in 4 changes of diagnosis: in two cases the correct diagnosis was confirmed to be that obtained post-cosyntropin. It should be noted that in all these last cases the diagnosis of APA made in basal conditions was due to LI> 4 but without contralateral retroinhibition on the contralateral adrenal (i.e. $\text{aldosterone/cortisol}_{\text{adrenal vein non dominant}}$/ $\text{aldosterone/cortisol}_{\text{peripheral vein}}$ was > 1). This finding could be compatible with the presence of bilateral hyperplasia with one side producing slightly more than the contralateral side. In agreement with this hypothesis, the contralateral inhibition associated to LI>4 may be considered necessary to suggest adrenalectomy (24). It should be noted that a previous study showed that 93.4% of APA and 100% of UAH display contralateral aldosterone/cortisol ratios <1 (6).

Another important finding is that the higher concordance between diagnosis before and after ACTH was achieved when strict cannulation and lateralisation criteria were used. This is in agreement with a previous study on patients who underwent two samplings, showing that only the use of strict criteria resulted in concordance of the diagnosis between first and second AVS, whereas using more permissive criteria for cannulation could be detrimental for the patients because of errors in the final subtype diagnosis and even wrong determination of the side of the APA (11). When more permissive criteria were used a concordance in the diagnosis between basal and stimulated conditions dropped by 13-26% depending on the protocol for ACTH infusion. Therefore, conservative SI should be used to considered cannulated successfully an adrenal vein.

A potential limitation of the present study is that one ACTH protocol was used in Japanese patients and another in Caucasians. Therefore, direct comparison of findings using between the two protocols should be done cautiously.

We would like to underline the extreme difficulty of finding the ideal cut-off that allows discrimination between unilateral and bilateral PA. In fact, this cut-off value could only be obtained by removing every single dominant adrenal in PA patients, regardless of the level of SI and LI and re-evaluate the post-surgery outcomes. Such a study, which could be ethically challenged, would also be hampered by the fact that a consistent number of BAH patients also display blood pressure reduction and sometimes a cure of hypertension and hypokalemia after unilateral adrenalectomy as shown recently by Sukor et al. (24).

Perspectives

Endocrine Society Guidelines (1) 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 standardisation for AVS protocols created confusion for clinicians both in term of performance and interpretation of the AVS results. The present study demonstrated that cosynthropin 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 lateralisation indexes are of primary importance to ensure diagnostic reproducibility. Future Guidelines should consider establishing widely accepted protocols for AVS performance and interpretation in order to more easily compare diagnostic results and to allow the diffusion of this technique to a larger number of centers.

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Figure Legends.

Figure 1.

Serum cortisol and aldosterone levels under basal conditions and after cosyntropin infusion and i.v. bolus. *$p<0.001$ compared to basal conditions.

Figure 2.

Effect of ACTH stimulation on SI (a) and LI (b) indexes. Selectivity index (SI) is defined as $\text{cortisol}_{\text{adrenal vein}}/\text{cortisol}_{\text{peripheral vein}}$ and lateralisation index (LI) as $\text{aldosterone/cortisol}_{\text{adrenal vein}}/\text{aldosterone/cortisol}_{\text{contralateral adrenal vein}}$. *$p<0.001$ compared to basal conditions; $^{\S}p<0.05$ compared to basal conditions.

Table 1. Clinical and biochemical characteristics of PA patients

Parameters	Torino (n=16)	Ancona (n=20)	Sendai (n=40)	p
Age (years)	50±12	47±11	49±13	n.s.
Sex (M/F)	9/7	9/11	25/15	n.s.
SBP (mmHg)	170±16	158±17	148±16	<0.001
DBP (mmHg)	104±8	97±13	90±12	0.001
drug number (n)	2.8±0.8	2.3±0.9	1.9±1.7	0.03
sK$^+$ (mEq L^{-1})	3.4±0.7	3.4±0.9	3.7±0.7	n.s.
sAldosterone (ng dL^{-1})	41.8 (33.3-44.9)	29.8 (20.3-50.4)	26 (16.5-40)	0.04
PRA (ng mL^{-1} h^{-1})	0.3 (0.2-0.5)	0.2 (0.2-0.4)	0.3 (0.2-0.6)	n.s.
CT (uni nod/bil nod/no)	7/3/6	15/2/3	22/4/14	n.s.
Nodule diameter (mm)	16.2±9.6	12.8±5.4	15.2±5.5	n.s.

SBP and DBP levels were measured under standard therapy, before changing the type of drug and placing the patients under therapy not interfering with hormonal measurements.

Table 2. Effect of continuous i.v. ACTH infusion on success rate at adrenal veins cannulation.

(Data are expressed as total number and percentage and after subdivision into Torino and Ancona units).

basal AVS	**strict** (SI>3)	**intermediate** (SI>2)	**permissive** (SI>1.1)	**unsuccessful** (SI<3/2/1.1)
LAV cannulated (n,%)	28 (78%)	31 (86%)	34 (94%)	8/5/2 (22/14/6%)
Torino/Ancona	15/13 (94/65%)	15/16 (94/80%)	16/18 (100/90%)	
RAV cannulated (n,%)	19 (53%)	26 (72%)	29 (81%)	17/10/7 (47/28/19%)
Torino/Ancona	13/6 (81/30%)	13/13 (81/65%)	14/15 (88/75%)	
both AV (n,%)	19 (53%)	25 (69%)	29 (81%)	17/11/7 (47/31/19%)
Torino/Ancona	13/6 (81/30%)	13/12 (81/60%)	14/15 (88/75%)	
post-ACTH infusion (n,%)	**strict** (SI>4)	**intermediate** (SI>2)		**unsuccessful** (SI<4/2)
LAV cannulated (n,%)	31 (86%)	33 (92%)		5/3 (14/8%)
Torino/Ancona	16/15(100/75%)	16/17 (100/85%)		
RAV cannulated (n,%)	26 (72%)	33 (92%)		10/3 (28/8%)
Torino/Ancona	15/11 (94/55%)	16/17 (100/85%)		
both AV (n,%)	26 (72%)	33 (92%)		10/3 (28/8%)
Torino/Ancona	15/11 (94/55%)	16/17 (100/85%)		

Table 3. Effect of ACTH i.v. bolus infusion on success rate at adrenal veins cannulation

basal AVS	**strict** (SI>3)	**intermediate** (SI>2)	**permissive** (SI>1.1)	**unsuccessful** (SI<3/2/1.1)
LAV cannulated (n,%)	21(53%)	33(83%)	(100%)	19/ 7/ 0 (47/ 17/ 0%)
RAV cannulated (n,%)	24(60%)	38(95%)	(100%)	16/ 2/ 0 (40/ 5/ 0%)
both AV (n,%)	18(45%)	33(83%)	(100%)	22/ 7/ 0 (55/ 17/ 0%)
post-ACTH i.v. bolus	**strict** (SI>4)	**intermediate** (SI>2)		**unsuccessful** (SI<2/4)
LAV cannulated (n,%)	40 (100%)	40 (100%)		0/ 0 (0/ 0%)
RAV cannulated (n,%)	40 (100%)	40 (100%)		0/ 0 (0/ 0%)
both AV (n,%)	40 (100%)	40 (100%)		0/ 0 (0/ 0%)

Table 4. Effect of continuous ACTH infusion on final diagnosis

Parameters	**Basal Strict Cr. + post-ACTH Strict Cr.**	**Basal Intermediate Cr. + post-ACTH Intermediate Cr.**	**Basal Permissive Cr. + post-ACTH Intermediate Cr.**
Successful Cannulation	17/36 (47%)	25/36 (69%)	29/36 (81%)
Diagnosis Concordance	15/17 (88%)	18/25 (72%)	18/29 (62%)
Diagnosis Changes	1 APA → BAH 1 BAH → APA	3 APA → BAH 4 BAH → APA	5 APA → BAH 6 BAH → APA

Table 5. Effect of ACTH i.v. bolus infusion on final diagnosis

Parameters	**Basal Strict Cr. + post-ACTH Strict Cr.**	**Basal Intermediate Cr. + post-ACTH Intermediate Cr.**	**Basal Permissive Cr. + post-ACTH Intermediate Cr.**
Successful Cannulation	18/40 (45%)	32/40 (80%)	40/40 (100%)
Diagnosis Concordance	14/18/ (78%)	26/32 (81%)	26/40/ (65%)
Diagnosis Changes	4 APA → BAH	5 APA → BAH 1 BAH → APA	13 APA → BAH 1 BAH → APA

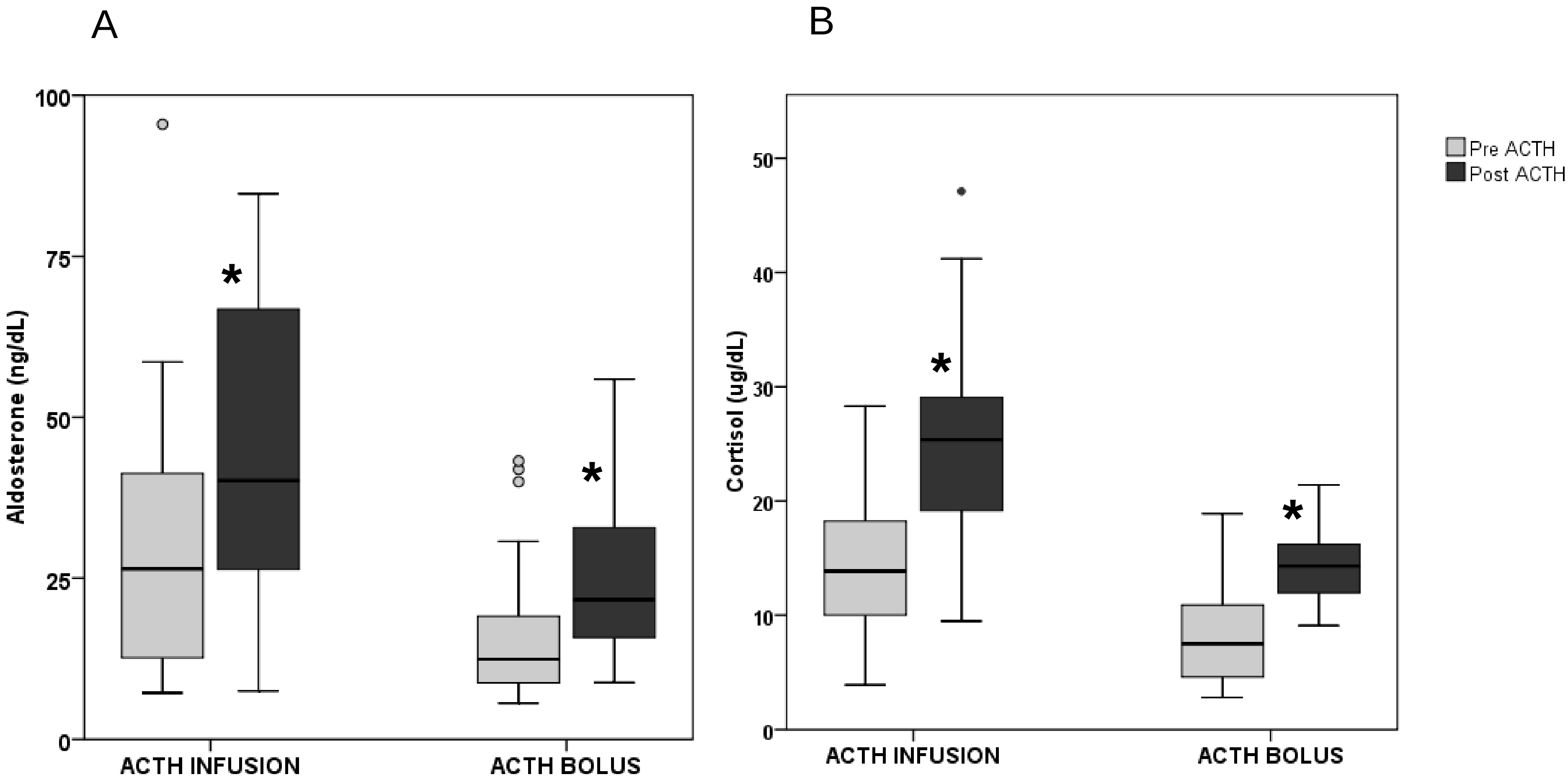
A
B
100
75
50
25
0
Aldosterone (ng/dL)
ACTH INFUSION
ACTH BOLUS
*
*
50
40
30
20
10
0
Cortisol (ug/dL)
ACTH INFUSION
ACTH BOLUS
*
*
Pre ACTH
Post ACTH

Figure 1

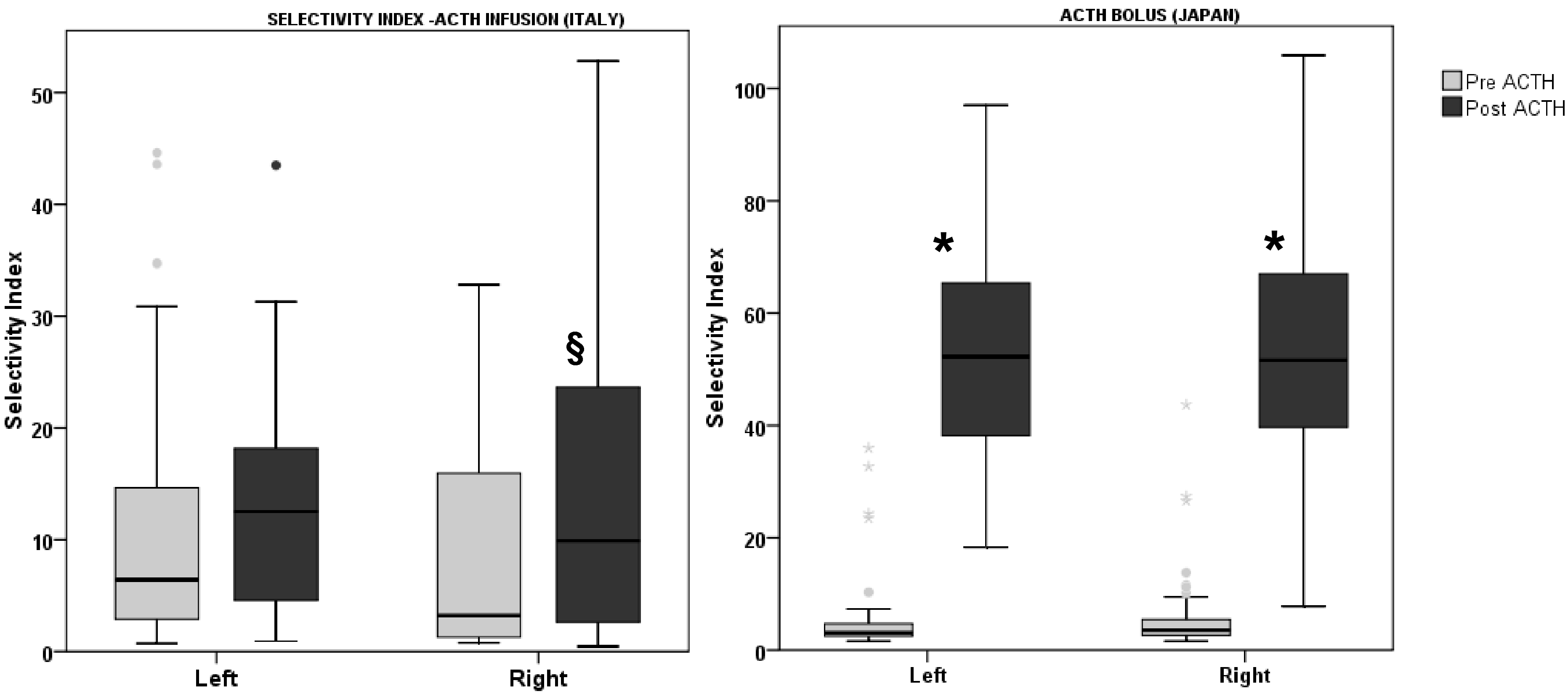
SELECTIVITY INDEX -ACTH INFUSION (ITALY)
Selectivity Index
0
10
20
30
40
50
§
Left
Right
ACTH BOLUS (JAPAN)
Selectivity Index
0
20
40
60
80
100
*
*
Left
Right
Pre ACTH
Post ACTH

Figure 2A

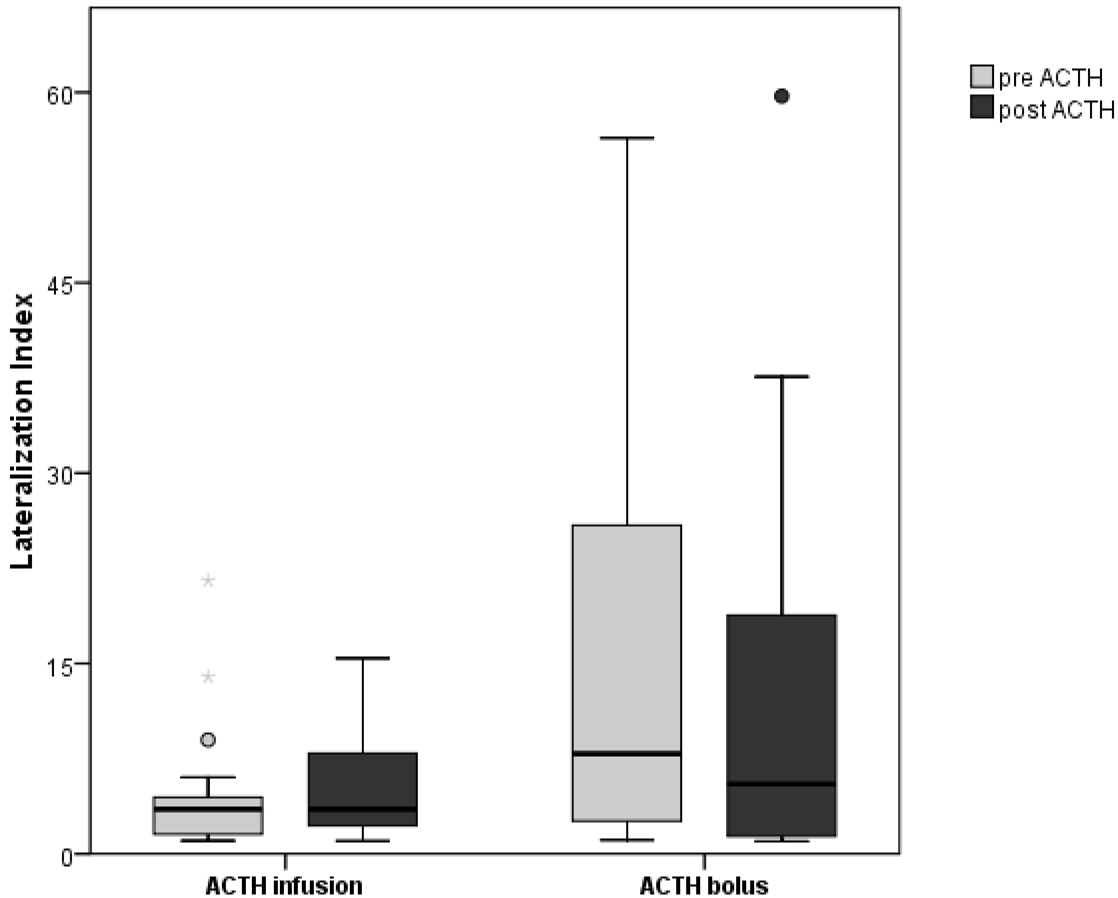
pre ACTH
post ACTH
Lateralization Index
60
45
30
15
0
ACTH infusion
ACTH bolus

Figure 2B