CLINICAL STUDY

A stress reaction affects assessment of selectivity of adrenal venous sampling and of lateralization of aldosterone excess in primary aldosteronism

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Abstract

Background: A stress reaction involving increased cortisol release, which has not been documented thus far, might affect the assessment of selectivity of catheterization during adrenal venous sampling (AVS).

Objective: To investigate whether an ACTH-driven cortisol release occurs during AVS and whether it influences the assessment of selectivity by the step-up of cortisol (plasma cortisol concentrations, PCC) between the adrenal vein blood (PCC_{SIDE}) and the inferior vena cava (PCC_{IVC}), e.g. the selectivity index (SI).

Design and methods: We determined the SI in samples obtained simultaneously at starting AVS (t-15) and again after 15 min (t0) in 34 consecutive patients with proven aldosterone-producing adenoma. We then calculated the SI with PCC_{SIDE} obtained at t-15 and at t0, and the PCC_{IVC} values obtained at the different time point, thus simulating sequential AVS.

Results: The PCC_{SIDE} and the SI fell significantly from t-15 to t0 on both the sides. When PCC_{SIDE} obtained at t-15 was combined with PCC_{IVC} at t0, the SI values were higher than those obtained with simultaneously drawn samples. This led to label as selective more AVS studies than with bilaterally simultaneous data, especially when using higher cutoffs for the SI.

Conclusions: A transient increase in cortisol release from both adrenal glands occurs in the majority of the patients who undergo AVS. This stress reaction can influence the assessment of both the selectivity of the catheterization during the sequential AVS technique and the lateralization of aldosterone excess.

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Introduction

Adrenal venous sampling (AVS) is the most accurate technique available for the identification of the surgically curable forms of primary aldosteronism (PA). Hence, the Endocrine Society practice guidelines endorse its systematic use in the PA patients who seek surgical cure (1, 2). However, notwithstanding recent improvements (3), unresolved issues concerning the performance and interpretation of AVS still remain (4, 5, 6, 7, 8). This prompted a concerted international effort – the Adrenal Vein Sampling International Study (AVIS; <http://clinicaltrials.gov/ct2/show/NCT01234220>) – that showed that the major referral centers worldwide are almost equally split between those that have adopted the bilaterally simultaneous catheterization (9) and those using the more traditional sequential technique. The latter implies first catheterization of the right side followed by that of the left side and then of the infrarenal inferior vena cava (IVC).

While both techniques can easily be performed by experienced radiologists, whether one outperforms the other remains contentious (10, 11).

It is conceivable that AVS, even despite being minimally invasive, can imply a stress reaction, entailing an ACTH-induced cortisol release. As the selectivity of catheterization is assessed by the step-up of cortisol between the adrenal veins and the IVC blood, usually referred to as the selectivity index (SI), waning of this cortisol discharge during AVS might affect the assessment of selectivity. This might occur when using sequential AVS because the time elapsing between blood sampling from the right and the left side could lower the cortisol step-up on the left side and thus the SI, more than that on the right side. As selective catheterization is easily accomplished on the left side, this issue could be regarded as trivial. Nonetheless, it might lower the rate of clinically usable AVS studies by causing exclusion of those studies that are not bilaterally selective.

To minimize the impact of both time-dependent stress-related differences and the pulsatile pattern of cortisol secretion on selectivity of catheterization and on lateralization of aldosterone excess, some centers perform AVS during 1–24 ACTH (cosyntropin) stimulation. By enhancing cortisol release, the latter facilitates the ascertainment of selectivity (12), but its usefulness is limited by its confounding effect on the assessment of lateralization of aldosterone excess (8). Hence, it is unlikely that cosyntropin stimulation can provide a final solution to the issue of time-dependent stress-related effects on AVS results (8). In spite of all these premises, a stress reaction during AVS has never been systematically investigated as yet (13, 14, 15). Likewise, whether it could differently affect assessment of selectivity when using the bilaterally simultaneous or the sequential catheterization technique remains unknown. We therefore sought to ascertain whether and how commonly a stress reaction occurs and whether it affects the step-up of cortisol between the adrenal vein blood and the IVC.

Patients and methods

Study design

We recruited consecutive PA patients among those referred to the Specialized Center for Hypertension, who were offered AVS according to current guidelines' recommendations (1). The patient's refusal to undergo adrenalectomy and/or AVS and/or contraindications to the general anesthesia required for laparoscopic adrenalectomy was the only exclusion criteria.

The protocol followed the principles of the Declaration of Helsinki and the institutional guidelines. An informed written consent was obtained from each participant. Before AVS, the patients were asked to eat a normal sodium diet (16), and were pharmacologically prepared as described (16). Hypokalemia was systematically corrected when present before AVS because it blunts aldosterone secretion (17).

Serum K^+ levels, plasma renin activity (PRA), plasma aldosterone concentration (PAC), and plasma cortisol concentration (PCC) levels were measured as reported (16). The cross-reactivity of the antibody against aldosterone or cortisol for the other adrenal steroids was <0.001% (16). Normal ranges and intra- and interassay coefficients of variation were 0.65–2.64 ng/ml per h and 8 and 10% for PRA; 1–15 ng/dl and <5.6% for PAC; and 7–25 µg/dl and 6 and 10% for PCC levels (16).

AVS procedure

AVS was performed between 0800 and 1200 h by the same experienced radiologist (D M) using a catheter shaped for each adrenal vein and the bilateral

simultaneous technique as reported (3). From both the sides and (within 1–2 min) the infrarenal IVC, blood samples were obtained by gravity for measurement of PAC and PCC. Blood was identically obtained again 15 min later (t0). During this interval, the catheter remained in the adrenal vein on the left side; on the right side to avoid the risk of thrombosis, it was withdrawn from the vein after obtaining the t-15 sample and thereafter repositioned into the vein to collect the blood sample at t0.

The SI was calculated as the ratio between the right or left adrenal vein PCC (PCC_{SIDE}) and the infrarenal IVC PCC (17). The cutoffs most widely used worldwide under unstimulated conditions were used to define the selectivity (13). These cutoffs range from 1.10 to 2.00 and were chosen because they were formally validated using receiver operator characteristic curve analysis and Youden index (18). Moreover, they were shown to allow clinical use of most of the AVS studies (17). The common egress in the IVC of the right adrenal with hepatic accessory veins that carry blood with much lower PCC also justifies use of these seemingly low values (3).

Recognizing that an unequivocal diagnosis of PA is possible only for aldosterone-producing adenoma (APA) with the 'four corner' criteria (16), only the APA cases were considered for this study. These criteria include i) biochemical diagnosis of PA, ii) unequivocal evidence of lateralized aldosterone secretion at bilaterally selective AVS, iii) evidence of adrenocortical nodule at histopathology, iv) cure or improvement of hypertension, and v) correction of the biochemical picture of PA at follow-up.

To investigate whether a stress reaction occurs during AVS, the initially obtained PCC values at t-15 were within-patient compared with those measured at t0 on each side and in the IVC. The SI calculated for each side using the samples obtained simultaneously at t-15 and at t0 was also compared between times. To simulate conditions occurring with the sequential AVS technique, the SI values were calculated using the PCC of the APA or the contralateral side at either t-15 or t0, and the PCC values obtained in IVC at the different time point.

The lateralization index was calculated as the ratio of PAC/PCC at the APA side to PAC/PCC at the contralateral side.

Statistical analysis

Results are expressed as mean and s.d., or median and interquartile range, as appropriate. PRA, PAC, PCC, and SI values were examined after log transformation because of their skewed distribution. A paired *t*-test was used to compare the log-transformed values obtained in each patient at t-15 and t0. The rate of selective AVS, both unilaterally and bilaterally, was compared with Fisher's exact test or χ^2 test, as

appropriate. Statistical significance was defined as $P < 0.05$ (two-sided). Statistical analysis was performed using SPSS 18.0 for Mac (SPSS, Inc., Bologna, Italy) and MedCalc (MedCalc Software, Mariakerke, Belgium).

Calculation of power

Based on a pilot study's findings, we had already calculated that a sample size of 34 conferred to the study a 92% power to detect a mean difference of 10 between the SI values measured at t-15 and t0, or between different sampling sites, assuming a common s.d. of 12 and using a two-sided paired t -test at $P < 0.05$ (nQuery Advisor (v. 7.0; Statistical Solutions, Saugus, MA, USA)).

Results

Patients and diagnosis

We initially recruited 56 consecutive PA patients (37 men and 19 women; age: 50 ± 12 years) for this study. All had high blood pressure, low PRA, and elevated PAC and therefore the aldosterone-to-renin ratio was high. Hypokalemia was found in 31% of the patients. Of these patients, 34 had an APA, and in all 34, the diagnosis was confirmed by the correction of PA (PAC decrease, PRA increase, and normalization of the aldosterone to renin ratio (ARR) and K^+ plasma levels) at post-adrenalectomy follow-up. The high blood pressure also fell significantly despite withdrawal, or tapering, of the antihypertensive therapy (Table 1). To avoid any diagnostic uncertainties, only the APA patients were considered for downstream analysis.

PCC and SI calculated with simultaneous AVS

The PCC fell from t-15 to t0 in both adrenal vein blood samples (APA side -63% , $P < 0.002$; contralateral side -45% , $P < 0.02$; Fig. 1 panel A), albeit with some variability among patients, while PCC in IVC fell (-8%)

Table 1 Clinical and biochemical features of the PA patients and effects of adrenalectomy. Mean value $\pm$ s.d., or median and interquartile range, as appropriate.

Variable	Adrenalectomy		<i>P</i>
	Before (<i>n</i> =34)	After (<i>n</i> =34)	
Systolic blood pressure (mmHg)	160 $\pm$ 15	128 $\pm$ 32	0.001
Diastolic blood pressure (mmHg)	97 $\pm$ 12	84 $\pm$ 9	0.001
Serum K^+ levels (mmol/l)	3.4 $\pm$ 0.4	4.2 $\pm$ 0.5	0.0001
Supine PRA (ng/ml per h)	0.37 (0.20–0.57)	0.96 (0.64–1.40)	0.0001
Supine aldosterone (ng/dl)	17.7 (15.0–26.7)	8.3 (5.6–13.5)	0.005
ARR (ng/dl):(ng/ml per h)	46.9 (33.2–77.6)	8.0 (4.8–12.9)	0.02

PRA, plasma renin activity; ARR, aldosterone to renin (PRA) ratio. To convert ng/dl aldosterone into pmol/l, multiply for 27.76.

Figure 1 Panel A shows PCC in the APA and contralateral vein blood and in the infrarenal IVC measured immediately after starting AVS (t-15) and again after 15 min (t0). A significant decrease was observed in each adrenal vein blood from t-15 to t0, with the resolution of the stress reaction. Panels B and C show no significant changes of PAC and PCC-corrected PAC between t-15 and t0 in the APA, the contralateral side, and the IVC blood. Panel D shows that the lateralization index significantly increased at t0, likely because of the different effects of the stress on PCC and PAC.

nonsignificantly (Fig. 1 panel A and Supplementary Figure 1, see section on supplementary data given at the end of this article). Thus, on both the sides, the SI was lower at t0 than at t-15 (APA side -52% , $P < 0.001$; contralateral side -40% , $P < 0.02$; Fig. 2 and Supplementary Figure 1). Most of the patients exhibited a fall of SI $> 30\%$ from both the APA (Supplementary Figure 2, see section on supplementary data given at the end of this article) and the contralateral sides (60 and 50% respectively) (Supplementary Figure 2). The fall of SI did not affect the proportion of AVS that was held to be selective, with cutoffs ranging from 1.10 to 2.00, when the AVS data obtained with the bilaterally simultaneous technique were used (Fig. 3). Age, gender, and BP did not predict the fall of SI on each side at regression analysis (Table 2).

The PAC showed a proportionally smaller decrease than PCC between t-15 and t0, which did not attain significance (Fig. 1 panel B), while PCC-corrected PAC tended to increase from both the APA and the contralateral from t-15 to t0 (Fig. 1 panel C). Because of the greater effect of the stress reaction on PCC than on PAC, the value of the lateralization index at the APA side increased in a highly significant fashion (Fig. 1 panel D).

PCC and SI calculated with sequential (nonsimultaneous) AVS data

Due to the higher PCC side values at t-15 (than at t0) and the lower values of PCC measured in IVC at t0

Figure 2 Panels A and B show box plots calculated using the samples obtained simultaneously at t-15 and t-0 (simultaneous AVS) and using the PCC of either adrenal vein (PCC_{SIDE}) at the numerator and the PCC_{IVC} at the denominator obtained at the different time points (non simultaneous) to simulate the conditions occurring with the sequential AVS technique. A decrease in SI values was observed on both the sides from t-15 to t-0 with bilaterally simultaneous AVS. The SI values calculated using adrenal vein PCC values obtained at t-15 at numerator and PCC values obtained at t-0 from the IVC at the denominator (PCC_{SIDE} t-15/PCC_{IVC} t-0) were significantly higher than those obtained with simultaneously drawn samples at t-0 (PCC_{SIDE} t-0/PCC_{IVC} t-0) but similar to those obtained at t-15. Conversely, the SI values obtained by combining the adrenal vein PCC values at t-0 with PCC_{IVC} values at t-15 (PCC_{SIDE} t-0/PCC_{IVC} t-15) were significantly lower than those obtained with simultaneously drawn samples at t-15 (PCC_{SIDE} t-15/PCC_{IVC} t-15) but similar to those simultaneously drawn at t-0.

(Fig. 1 panel A), when the adrenal vein PCC values at t-15 were combined with those obtained from the IVC at t-0, the SI values were higher than those obtained with simultaneously drawn samples (Fig. 2). For the same

reasons, the SI value calculated with PCC measured in the APA or the contralateral side at t-0 and PCC_{IVC} at t-15 fell markedly (Fig. 2).

These divergent effects of the timing of blood sampling changed the proportion of AVS studies defined as 'selective', both unilaterally (Supplementary Figure 3, see section on supplementary data given at the end of this article) and bilaterally (Fig. 3). Overall, the rate of selective AVS on the APA and the contralateral side (Supplementary Figure 3), or bilaterally (Fig. 3), was lower with the sequential than with the simultaneous AVS technique. The lowest rate of AVS studies judged to be bilaterally selective occurred when an SI cutoff of 2.00 was used with samples obtained at t-0 from either adrenal vein and at t-15 from IVC.

Discussion

The sequential and the bilaterally simultaneous AVS techniques are equally popular at major referral centers (13), but whether one technique is superior to the other is contentious. With a within-patient comparison of both techniques, we herein provide novel information on their performance for assessing the selectivity of catheterization. The effect of AVS-related stress on assessment of selectivity using either technique could also be established.

AVS-related stress reaction

We hypothesized that being brought to the hemodynamic laboratory to undergo AVS could be stressful for the patient (19) and might trigger a cortisol release that is variable, as suggested by the ample variability in adrenal vein catecholamine levels during AVS (20). Such a stress reaction has neither been systematically investigated nor been characterized thus far.

We found a fall of PCC in the adrenal vein blood over the 15 min from starting AVS, thus providing compelling evidence for such a stress reaction (Fig. 1 panel A). The prominent fall of PCC in adrenal veins, that is just downstream of the site of cortisol production, was paralleled by a fall of PCC in the IVC blood, which did not achieve statistical significance only because of the dilution of cortisol in the systemic vascular bed. This smaller fall minimized the difference between PCC levels measured at t-15 and t-0 and thus lowered (to 41%) the power of this study to show a significant difference. Hence, the lack of statistical significance at the IVC site most likely reflects a type 2 statistical error and therefore studies with a larger sample size are ongoing to test this hypothesis.

Judging from the fall of PCC in the adrenal veins, the stress reaction involved more than half of the patients who underwent AVS (Fig. 1 panel A and Supplementary Figure 1) and was short-lasting as it waned over 15 min. Moreover, it was markedly variable among

Figure 3 The technique of AVS (bilaterally simultaneous vs nonsimultaneous AVS) had no effect on the percentage of bilaterally selective AVS when using the more liberal SI cutoff values of 1.10 and 1.50 (left and mid panels). However, when using the more stringent SI cutoff value of 2.00, the proportion of bilaterally selective AVS studies fell significantly, particularly with sequential AVS data (right panel). When not reported in the graph, the comparison between the percentages performed using Fisher's exact test yielded no significant differences.

patients, which explains the large variability of the SI fall. Because of this variability with use of either liberal or more stringent cutoffs, the rate of selective AVS did not differ significantly between t-15, e.g. at peak of the stress, and at t0, e.g. after its resolution (Fig. 3 and Supplementary Figure 3). These results should call attention to the importance of stress during AVS, which is apparently largely neglected: of all major international centers worldwide that participated to the AVIS study, only one reported adopting precautions to minimize it (13).

The stress reaction seems to affect the release of aldosterone to a lesser extent than that of cortisol in that it did not significantly affect either PAC or PCC-corrected PAC. This smaller effect of the stress reaction on PAC than on PCC might be clinically important for the ascertainment of lateralization of aldosterone excess, which is the ultimate goal of AVS: the value of the lateralization index correctly pointing to the side with an APA increased significantly from t-15 to t0 (Fig. 1 panel D). Therefore, given the unpredictability of the stress reaction in individual patients, actions to minimize stress for the patients are needed in order to minimize its effect on AVS results.

Impact of sequential or simultaneous AVS on SI value

The comparison between the SI values obtained with bilaterally simultaneous AVS and under sequential AVS conditions showed that the two techniques provide different values (Fig. 2). These differences can largely be attributed to the aforementioned stress reaction because they became evident when the PCC measured at t-15 (at either the APA or the contralateral side) was replaced with PCC at t0 (Fig. 2). They account for the different rates of selective AVS detected under either condition. Hence, within the timeframe of a sequential AVS, use of samples taken at different times affects the assessment of selectivity. Overall, these results support the contention that the time elapsing between samples taken with the sequential technique should be considered when assessing the cannulation success rate by the PCC-based SI (21).

Conceivable factors affecting AVS selectivity include episodic cortisol fluctuations related to the circadian rhythm and stress-related bursts of ACTH and cortisol secretion. To minimize the impact of these factors, ACTH infusion was introduced by Weinberger *et al.* (22) and widely used thereafter (21, 23, 24), thus being held as an essential part of the sequential AVS (11, 19, 21, 25), or even of bilaterally simultaneous AVS (5, 26, 27). In our hands, cosyntropin stimulation confounded the assessment of lateralization and led to misclassification of the APA side (8, 17). While facilitating the ascertainment of selectivity, it did not eliminate the problems inherent to the stress reaction, particularly when using the sequential AVS technique. Moreover, given the high levels of endogenous ACTH occurring when starting AVS (12), cosyntropin likely acts in the context of an already maximally stimulated ACTH–cortisol axis and might therefore compete with endogenous ACTH. This can contribute to the unpredictable effects on aldosterone secretion (8).

Limitations and strengths of the study

Despite conclusively evidencing a stress reaction, this study falls short in identifying its predictors. Interestingly, however, the patients who exhibited a fall of PCC and SI, both bilaterally and from the APA and the contralateral sides, tended to have higher PCC and PAC at t-15, suggesting that they were more prone to raise their ACTH secretion in response to a stressful situation.

Table 2 PCC and PAC concentrations and SI values in the adrenal veins and the infrarenal IVC. Mean $\pm$ S.E.M.

Variable	APA side (time)		Contralateral side (time)		IVC (time)	
	t-15	t0	t-15	t0	t-15	t0
PCC (μ g/dl)	266.3 $\pm$ 47.8	119.7 $\pm$ 156.4	319.4 $\pm$ 55.1	144.6 $\pm$ 39.1	18.0 $\pm$ 12.1	16.5 $\pm$ 9.9
PAC (ng/dl)	2900.6 $\pm$ 804.2	1336.2 $\pm$ 473.7	382.8 $\pm$ 161.0	245.2 $\pm$ 110.4	30.3 $\pm$ 4.6	24.4 $\pm$ 3.0
SI	14.2 $\pm$ 2.1	6.8 $\pm$ 1.7	15.6 $\pm$ 2.8	9.9 $\pm$ 2.5	—	—

Considering that the cross-reactivity of the antibody used for the RIA measurement of PCC with aldosterone is <0.001%, and that the concentrations of the cortisol in the plasma are 10^2 – 10^3 higher than those of aldosterone, a cross-reaction bias is likely to be negligible.

A further potential limitation should, however, be noted: PCC was measured only during a 15 min period. By the end of this interval, the stress reaction could have resolved, yet it is to be proven whether the differences between the bilaterally simultaneous and the sequential technique are minimized when samples are obtained within the shorter times that can be achieved by experienced radiologists with the sequential technique. Collection of more blood samples would be needed to clarify this issue, but given that the catheters had to be removed soon after each blood collection from the right adrenal vein because of the risk of thrombosis, this was unfeasible. Moreover, repeated cannulation of the veins would have rendered the procedure not only more technically demanding but also more risky for the patient.

It could also be argued that repositioning of the catheter in the right adrenal vein might have introduced a bias because there is no certainty that the catheter was replaced at an identical site. However, the results obtained on the left side, where the catheter remained in place, were practically identical to those on the right side, indicating that this bias did not significantly affect our findings and conclusions. Moreover, findings were similar also when results were analyzed according to APA and contralateral sides, a result that could be anticipated as APAs were equally distributed between sides. Therefore, we can confidently exclude that the repositioning of the catheter on the right side was the only cause of the fall in PCC between t-15 and t0.

Finally, the cutoff value for the SI of 1.10 could be regarded as too low given the variation coefficient of the cortisol assay, but some considerations support its use. First, the occurrence of hepatic accessory veins that share a common egress with the right adrenal can cause a dilution of PCC in the right adrenal vein blood. Secondly, the practice of making a side hole close to the catheter's tip leads to dilution of adrenal vein from IVC blood. Finally, it has to be underlined that in this series of 34 consecutive unequivocally diagnosed APA cases, 14% had a SI <1.20, but notwithstanding, this could be correctly identified as having lateralized aldosterone secretion.

Conclusions

An increased cortisol release from the adrenal gland with and without the APA occurred in more than half of the patients who underwent AVS. This stress reaction waned over 15 min from the start of the procedure but

affects the assessment of selectivity of the catheterization when using the sequential catheterization technique. Hence, the SI values obtained with the sequential or the bilateral simultaneous AVS techniques differ substantively, as did the proportion of AVS studies that were held to be bilaterally selective when stringent cutoffs for SI were adopted. Further research should aim at determining the time course of this stress reaction and establishing protocols to minimize it. Moreover, efforts should be devoted to identify the effect of this surge and its changes over time on the identification of lateralization of aldosterone excess.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-11-0972>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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