Intraprocedural Cortisol Measurement Increases Adrenal Vein Sampling Success Rate in Primary Aldosteronism

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BACKGROUND
Adrenal venous sampling (AVS) is the gold standard for the identification of unilateral primary aldosteronism (PA), but is technically difficult. The aim of our study was to assess whether intraprocedural cortisol measurement (IPCM) increases AVS success rate.

METHODS
Twenty-five consecutive PA patients underwent cosyntropin-stimulated AVS. Cortisol was measured immediately in a first set of samples drawn from adrenal veins and inferior vena cava. The selectivity criterion was an adrenal vein-to-inferior vena cava cortisol ratio ≥5. If bilateral selectivity was not achieved in a first set of samples, a second set was obtained during the same radiological session. PA was judged as unilateral if the gradient of cortisol-corrected aldosterone between dominant and nondominant side was >3.5. Twenty-five consecutive PA patients who had previously been submitted to AVS without IPCM served as historical controls. Lateralizing patients who underwent unilateral adrenalectomy were followed for 2 years after surgery.

RESULTS
Bilateral selectivity using IPCM was achieved in 19/25 patients in the first set of samples, and in an additional four cases in the second set (92% vs. 76%; \( P = 0.06 \)). The final rate of bilateral selectivity was higher than that obtained in the historical series (23/25 vs. 16/25, \( P = 0.04 \)), whereas bilateral selectivity in the first set of samples was not different from that achieved in the historical series. Nineteen lateralizing patients (13 of the present series, six of the historical series) were submitted to adrenalectomy, resulting in reversal of PA.

CONCLUSIONS
IPCM increases the success rate of AVS.

Keywords: adrenal hyperplasia; adrenal venous sampling; aldosterone; aldosterone-producing adenoma; blood pressure; cortisol; hypertension; primary aldosteronism

American Journal of Hypertension, advance online publication 18 August 2011; doi:10.1038/ajh.2011.148
**METHODS**

**Patients.** Clinical and laboratory data were collected prospectively in 25 consecutive patients with biochemically confirmed PA willing to undergo surgical treatment. They were submitted to AVS between April 2007 and December 2009; plasma cortisol was measured in the first set of blood samples from adrenal veins immediately after sampling. Twenty-five consecutive patients with PA who had been submitted to AVS between January 2005 and December 2006 served as HIS controls.

**Diagnosis of PA.** Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers were withdrawn 3 weeks before testing, and β-blockers, α₂-selective adrenergic agonists, diuretics, and mineralocorticoid receptor antagonists were withdrawn 6 weeks before testing. If necessary, the above mentioned drugs were replaced by α₁-blockers and/or long-acting calcium channel antagonists. Hypokalemia, if present, was treated with KCl supplements orally until the attainment of a plasma K⁺ concentration ≥3.3 mmol/l. Blood samples for the measurement of aldosterone/renin ratio were collected mid-morning after the patients had been ambulatory for 2 h and seated for 30 min. An intravenous saline load (2 l 0.9% NaCl over 4 h from 8 AM to noon with the patient supine) was performed on a separate occasion as a confirmatory test. The diagnosis of PA was based on the following criteria: plasma aldosterone (ng/dl)-to-plasma–renin activity (ng/ml/h) ratio ≥30 and plasma aldosterone ≥7.5 ng/dl.

All PA patients underwent a high-resolution computed tomography (CT) scan of the adrenals. In all PA patients, a long-PCR test for glucocorticoid-remediable aldosteronism was performed.

**Protocol for AVS.** All patients were hospitalized in the morning of the day of AVS, and discharged the day after.

Sequential AVS was performed during continuous cosyntropin infusion (50 μg/h started 30 min before sampling and continued throughout the procedure).

Each sampling session typically included two patients. In each patient, a first set of samples (right and left adrenal vein and then external iliac vein) were drawn, and cortisol was measured in all blood specimens. While the cortisol assay was being performed, the catheter was removed, the catheter sheath was left in place, and the patient was moved to the contiguous recovery room; the other patient was then submitted to a first set of samples. If the first set of venous samples of a patient turned out to be nonselective, then that patient was returned to the radiology suite to undergo resampling. After obtaining a second set of samples in a given patient, the procedure was stopped independent of the results of the second set, to reduce the risk of major complications. The procedure duration (median and interquartile range) for each patient was 80 min (75–90 min) for the first set of samples, plus 60 more minutes (56.2–67.5 min) for resampling in each patient whose first set of samples turned out to be nonselective. Thus, the time interval (median and interquartile range) during which the radiology suite was occupied during each AVS session varied from 200 min (190–220 min) when the first set of samples turned out to be selective in both patients to 280 min (262.5–315 min) when both patients underwent resampling.

For each set of samples, a variable number of blood specimens were drawn from putative RAVs when the radiologist had doubts on the correct catheter tip location based on the venographic images. The same radiologist (G.G.) performed all of the procedures, alone in the HIS series, and under the supervision of a more skilled radiologist (D.M.) in the IPCM series.

Each adrenal venous sample was considered selective if the adrenal vein-to-external iliac vein cortisol ratio (selectivity index, SI) was ≥5.

Among bilaterally selective AVSs, unilateral PA was diagnosed whether the ratio of cortisol-corrected aldosterone concentration between the dominant and nondominant side (lateralization index, LI) was >3.5. Indeed, while a LI >4 is observed in most patients with unilateral PA, a LI between 3 and 4 may be consistent with either unilateral or bilateral PA.²⁵ Therefore, we considered a cutoff of 3.5 a reasonable compromise.

Informed consent was obtained from all patients. The protocol was approved by the Provincial Ethical Committee of Reggio Emilia.

**Hormonal measurements.** Plasma–renin activity was measured by radioimmunoassay for generated angiotensin I with a commercial kit (Radim Diagnostics, Pomezia, Italy), after a 3-h incubation at pH 5.5 at 37 °C. The intra- and interassay coefficients of variation were ≤6 and 9%, respectively. The lower limit of detection was 0.1 ng/ml/h.

Samples drawn during AVS were collected in K2-EDTA tubes and centrifuged. Plasma samples were frozen at −20 °C until assayed for aldosterone concentration, whereas plasma concentrations of cortisol were measured immediately using the LIAISON kit (DiaSorin, Stillwater, MN), which exploits a competitive luminometric assay based on the solid-phase antigen linked technique.

Samples with high cortisol concentrations were tested as such and after serial dilution. The intra- and interassay coefficients of variation were 5.1% (20 assays) and 6.9% (59 assays), respectively, for samples with cortisol concentrations ranging between 2.4 and 4.8 μg/dl. The analytical and functional sensitivities of the plasma cortisol concentration method were 0.2 and 0.5 μg/dl, respectively.

Aldosterone was measured using a solid-phase¹²⁵I RIA kit (Immunotech-Beckman Coulter, Marseille, France). High-concentration samples were serially diluted. The intra- and interassay coefficients of variation were 9.5 and 9.9%, respectively. The analytical sensitivity of the plasma aldosterone concentration method was 0.6 ng/dl.

**Exclusion of glucocorticoid-remediable aldosteronism.** The CYP11B1/CYP11B2 chimeric gene was excluded by a long-PCR test.⁶

**Statistics.** Anthropometric, clinical, and laboratory data collected in the patients of the series with IPCM and the HIS
series were compared by the Student’s *t* test for independent samples or the Mann–Whitney test, as appropriate. The numbers of RAV blood samples drawn within the first session in each of the two series of patients were compared by the Mann–Whitney test; the numbers of RAV blood samples drawn during each of the two sessions within the IPCM series were compared by the Wilcoxon signed-rank test. Categorical variables and frequencies in the two series were compared by χ² or Fisher’s exact test, as appropriate.

**RESULTS**

Patients’ characteristics are reported in Table 1. Apart from slightly lower diastolic blood pressure values, there were no significant differences between the IPCM and the HIS series of patients. One patient of the IPCM series showed both an aldosterone/renin ratio (26.9 ng/dl/ng/ml/h) and a postsaline plasma aldosterone level (6.9 ng/dl) slightly below the pre-specified cutoff values. One patient of the HIS series showed a postsaline aldosterone level (7.2 ng/dl) barely below the pre-specified cutoff value. Nevertheless, both patients underwent AVS because of a clear-cut PA syndrome. Indeed, at presentation both patients had stage 2 hypertension, severe and spontaneous hypokalemia (2.1 mEq/l and 2.4 mEq/l, respectively), inappropriate urine K⁺ excretion (≥40 mmol/24 h), and metabolic alkalosis (plasma (HCO⁻₃) 38 mEq/l and 35 mEq/l, respectively). Despite oral KCl supplements (56 mmol/day), plasma (K⁺) before testing was still low (3.1 mEq/l and 3.0 mEq/l, respectively). Despite oral KCl supplements (56 mmol/day), plasma (K⁺) before testing was still low (3.1 mEq/l and 3.0 mEq/l, respectively), which may have dampened the aldosterone hypersecretion.

In the IPCM series, the length of time (median and interquartile range) for cortisol results was 40 min (38–45 minutes), which included 3 min for sample transport to the laboratory, 8 min for centrifugation, 5–7 min for manual dilution, and 20–22 min for assay.

One patient of the HIS series and one of the IPCM had self-limited groin hematoma; one patient of the IPCM had transient hypotension. No other complications were observed.

All samples drawn from the left adrenal vein were selective based on a SI >5, whereas selectivity was not always achieved in the samples from the RAV. The number of samples drawn from the putative RAVs in the first set in the patients of the IPCM series was not significantly different from that obtained in the single set in the patients of the HIS series (2 (1–4) vs. 2 (1–3), median (range); *P* = 0.29). Also, there was no significant difference between the number of samples drawn from the putative RAVs in the first and second set in the IPCM series (2 (1–4) vs. 1 (1–2), median (range) *P* = 0.56).

Bilateral selectivity was found in 19/25 (76%) in the first set of samples in the IPCM series. Among the six patients with unsuccessful AVS in the first set, the second set of samples turned out to be bilaterally selective in four. Eventually, bilateral selectivity was achieved in 23/25 (92%) vs. 19/25 (76%) in the first set of AVS (one-sided *P* = 0.06). The overall rate of bilateral selectivity obtained with IPCM was significantly higher than that obtained in the HIS series (23/25 vs. 16/25, *P* = 0.04), whereas the rate of bilateral selectivity in the first set of samples of IPCM series was not different from the overall rate of bilateral selectivity of the HIS series (19/25 vs. 16/25, *P* = 0.54).

Among the 23 patients with bilaterally successful AVS in the IPCM series, median SI was 27.0 (range 5.0–97.7) in the right adrenal samples and 26.3 (range 6.1–66.8) in the left adrenal samples. Among the 16 patients with bilaterally successful AVS in the HIS series, median SI was 26.5 (range 4.7–61.0) in the right adrenal samples (*P* = 0.753 vs. IPCM series) and 25.6 (8.3–43.7) in the left adrenal samples (*P* = 0.387 vs. IPCM series).

Lateralization of aldosterone secretion was found in 14 of the 23 patients of the IPCM series with bilaterally selective AVS, with a median cortisol-corrected aldosterone LI of 10.1 (range 3.78–54.3), and in eight of the 16 patients of the HIS series with bilaterally selective AVS, with a median cortisol-corrected aldosterone LI of 17.4 (range 9.7–33.1; *P* = 0.08 vs. the IPCM series). Lateralizing patients of both series also showed suppression of aldosterone secretion on the contralateral side (Figure 1).

Thirteen of the 14 lateralizing patients of the IPCM series and six of the eight lateralizing patients of the HIS series were submitted to unilateral adrenalectomy, with histological findings of an adrenal adenoma in 18 patients and of adrenal hyperplasia in one patient. One of the 14 lateralizing patients of the IPCM series and two of the eight lateralizing patients of the HIS series declined surgery. The median diameter of adeno-mas was 13 mm (range 6–23 mm). Complete clinical and biochemical follow up at 2 years was available in 18 (94.7 %) out of 19 adrenalectomized patients of the two series combined. In all cases, surgery was followed by reversal of PA (Table 2), and cure (*N* = 7; 38.9%) or improvement (*N* = 11; 61.1%) of hypertension (Figures 2 and 3). In one patient, postoperative plasma–renin activity was still suppressed (0.4 ng/ml/h),
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though associated with a normal aldosterone/renin ratio (7.25). In another patient both plasma aldosterone (45 ng/dl) and aldosterone/renin ratio (37.5) turned out to be still high. In this case, an intravenous saline test excluded the persistence of PA based on a plasma aldosterone concentration of 3.1 ng/dl at the end of the infusion. The duration of hypertension was not significantly related to the reduction in either systolic ($r = -0.31, P = 0.18$) or diastolic ($r = 0.24, P = 0.29$) blood pressure values after adrenalectomy. Age had a significant direct relationship with the reduction in diastolic ($r = 0.47, P = 0.03$) but not systolic ($r = 0.17, P = 0.46$) blood pressure.

The patients in whom high blood pressure persisted after adrenalectomy were older and had a longer duration of hypertension compared with those who were completely cured after surgery, although these differences did not reach statistical significance (age, 50.6 ± 3.4 vs. 42.9 ± 2.7 years, $P = 0.15$; duration of hypertension, 10.5 ± 2.3 vs. 5.3 ± 1.4 years, $P = 0.15$).

### Table 2 | Changes in biochemical data in adrenalectomized patients (n = 19) at follow up

<table>
<thead>
<tr>
<th></th>
<th>Prior to surgery</th>
<th>Follow up (2 years after adrenalectomy)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum K$^+$ (mEq/l)</td>
<td>3.4 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PRA (ng/ml/h)</td>
<td>0.35 (0.10–1.00)</td>
<td>2.7 (0.40–6.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma aldosterone (ng/dl)</td>
<td>35.3 (9.0–73.5)</td>
<td>7.5 (2.0–45.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALDO/PRA (ng/dl/ng/ml/h)</td>
<td>96.7 (36.6–192.9)</td>
<td>2.7 (1.1–37.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
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Values are mean ± s.e.m. or median (range). In patients requiring postoperative pharmacological treatment, drugs interfering with the renin–angiotensin–aldosterone system were replaced by α1-blockers and/or long-acting calcium channel antagonists before testing.

ALDO/PRA, plasma aldosterone-to-plasma–renin activity ratio; PRA, plasma–renin activity.
Among the 39 bilaterally selective AVSs, concordance between AVS and CT scan was observed in 26 (66.7%) cases. In seven (17.9%) patients, the CT scan showed a unilateral adrenal nodule, while the AVS indicated bilateral aldosterone hypersecretion. In four (10.3%) patients, the CT scan showed normal adrenals, while the AVS demonstrated unilateral aldosterone hypersecretion, with confirmation of an aldosterone-producing adenoma at both surgery and follow up.

**DISCUSSION**

The results of our study show that IPCM improves the AVS success rate; in fact, four of 23 bilaterally selective AVSs would have been unsuccessful if IPCM had not been performed.

The differentiation between unilateral and bilateral PA should be based on the results of AVS. However, the technical difficulty in identifying the RAV may result in failed cannulation in a variable proportion of AVSs. Usually, as the radiologist’s experience increases over time, AVS success rate also improves, reaching 95% in some referral centers. This improvement involves a training phase, with a suboptimal success rate of AVS before the achievement of a plateau phase of high performance; the duration of the training phase will depend on both the intrinsic radiologist’s skill and the number of AVSs performed by the same radiologist per year. The availability of IPCM may increase the success rate of AVS, as the awareness of a previously failed attempt provides the interventional radiologist with a feedback that may be particularly relevant in the linear phase of the learning curve. On the other hand, this feedback is conceivably less important in centers with a high procedure volume, where the interventional radiologist(s) has (have) already reached high technical skills.

While the low number of AVSs performed in the time interval between the beginning of the HIS series and the completion of the IPCM series does not warrant sufficient statistical power to assess the additional contribution of a training effect, some considerations may suggest a trend towards an improvement in the radiologist’s expertise. In fact, in all of the last 9 patients of the IPCM series, compared to only 10 out of the previous 16 patients of the same series ($P = 0.06$), bilateral selectivity was achieved on the first set of samples; this suggests that resampling guided by the knowledge of a previously failed attempt can ameliorate the radiologist’s ability to interpret the venographic images correctly in the subsequent AVS procedures, resulting in a steeper radiologist’s learning curve as a result. However, while the overall rate of bilateral selectivity obtained with IPCM (92%) was significantly higher than that obtained in the HIS series (64%), the rate of bilateral selectivity in the first set of samples of IPCM series was not different from the rate of bilateral selectivity achieved in the HIS series. This suggests that the increase in AVS success rate was mainly due to the feedback provided by the IPCM during the same session.

Our results show that even a routine cortisol assay, provided that the time of the preanalytical phase is minimized and the assay is immediately performed, may improve AVS success rate in centers where a more rapid modified immunoenzymatic assay is not available.

We followed the AVS protocol suggested by Young et al. The outcomes of our lateralizing patients submitted to surgery are completely consistent with the results reported by Young et al. as all of the lateralizing patients eventually turned out to harbor a unilateral adrenal lesion; however, since nonlateralizing patients were not submitted to surgery, the design of our study did not allow the assessment of either the sensitivity or the true specificity of stimulated AVS.

The diagnostic accuracy of stimulated AVS has recently been challenged and contrasted with that achieved with unstimulated AVS. In particular, infusion of cosyntropin might result in either missed lateralization or lateralization on the wrong side in 33% of the patients. However, in that study, at variance with the method used by Young et al. and ourselves, continuous infusion was preceded by a bolus of cosyntropin. Indeed, the kinetics of cortisol and aldosterone following a cosyntropin bolus are not necessarily concordant, and could possibly confound the interpretation of the aldosterone–cortisol ratios. Furthermore, other studies have shown that unstimulated LI is less accurate than adrenocorticotropic hormone-stimulated LI in identifying unilateral PA. In addition, in the case of unstimulated AVS, a very low SI cutoff value has been suggested as appropriate, corresponding to an adrenal vein cortisol concentration just 10% higher than that in inferior vena cava and falling within the coefficient of variation of most cortisol assays. Thus, a low SI cutoff value is expected to inflate the rate of selectivity of adrenal venous samples and may eventually increase the false-positive rate in detecting unilateral PA; indeed, comparisons of multiple adrenal venous samples collected from the same PA patients have shown that a high reproducibility in lateralization diagnosis is achieved only among samples with a SI ≥3 for unstimulated AVS. Similarly, in stimulated AVS, only adrenal samples with a SI of at least five have turned out to be concordant in lateralizing diagnosis.

The prevalence of unilateral subtypes of PA (49%) among the 39 bilaterally selective AVSs in our study is high compared to previously published studies. Moreover, the prevalence of hypokalemia (70%) among our PA patients is a figure much higher that that found in other studies. Actually, many patients submitted to AVS in our center had been referred from their general practitioners due to suspicion of secondary hypertension based on the finding of hypokalemia, which is more frequently associated with unilateral than with bilateral PA. Therefore, a possible selection bias among the patients undergoing AVS in our study should be acknowledged.

In the present study, the practical importance of achieving an elevated rate of successful AVSs is also stressed by the observation that CT imaging missed 10% of unilateral PA cases, and misclassified as unilateral 18% of the cases with successful AVS. In addition, the clinical impact of successful AVS is confirmed by the results of clinical and biochemical follow up of our PA patients submitted to unilateral adrenalectomy, in whom we observed both complete reversal of the biochemical syndrome and clear-cut improvement of hypertension control. Parenthetically, we cannot exclude that the benefits of adrenalectomy on blood pressure may have been
diluted by the coexistence of essential hypertension in a fraction of the patients.

Finally, we propose a few considerations concerning the additional costs associated with IPCM, as compared with a sampling procedure exclusively guided by the venographic images. In our clinical setting, the radiology suite was primarily dedicated to a single session of AVS (two patients for each session). No lag time was allowed during which the radiology suite was left unoccupied; thus, if bilateral selectivity was reached with the first set of samples in both patients, after ~200 min the radiology suite was potentially available for other procedures. However, if resampling was necessary after ~200 min the radiology suite was left unoccupied; thus, if bilateral selectivity was achieved with the first sample set in both patients.

The global costs for a single session of AVS with IPCM ranged from 2,150 euros (3,032 USD) in the event of bilateral selectivity achieved with the first sample set in both patients (640 euros (902 USD) for staff time plus 738 euros (1,041 USD) for the use of the radiologic facility, 290 euros (409 USD) for analytical processing of the samples, and 241 euros (340 USD) for 1-day hospital stay for each patient) to 2,994 euros (4,222 USD) (580 euros (818 USD) for analytical processing of the samples, and 241 euros (340 USD) for 1-day hospital stay for each patient) in the event of bilateral selectivity achieved with the first sample set in both patients. Compared to the procedure without IPCM, AVS incorporating IPCM would increase the global costs of a single sampling session (two patients) by 128 euros (180 USD) in the event of bilateral selectivity achieved with the first sample set in both patients. As a rough estimate, given the low procedure volume in our center (i.e., not more than 14 patients/year corresponding to seven AVS sessions/year), the increase in total costs for IPCM compared to the usual AVS procedure in the case of bilateral selectivity achieved with the first sample set in both patients would sum up to 896 euros (1,263 USD) per year. On the other hand, taking into account that failure to achieve bilateral selectivity in the setting of a procedure without IPCM would require a repeated patient hospitalization to perform a second sampling session, AVS with IPCM would decrease the costs by 1,050 euros (1,480 USD) every two AVSs with nonselective first sample set.

In conclusion, IPCM may be an effective tool to improve AVS success rate, particularly in the temporal interval which precedes the peak of performance in the radiologist’s learning curve.