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References

2. Coresh J, Astor BC, McCul[...]

Rapid Cortisol Assay during Adrenal Vein Sampling in Patients with Primary Aldosteronism, Giulio Mengozzi,1 Denis Rossato,2 Chiara Bertello,3 Corrado Garetti,4 Alberto Milan,5 Roberto Pagliu,1 Franco Veglio,3 and Paolo Mulatero3 (1 Clinical Chemistry Laboratory and Divisions of 2 Radiology, 3 Internal Medicine and Hypertension, and 4 Surgery, University of Torino, Torino, Italy; * address correspondence to this author at: Clinical Chemistry Laboratory, ASO S. Giovanni Battista, University of Torino, 10126 Torino, Italy; fax 39 011 676052, e-mail gmengozzi@molinette.piemonte.it)

Background: Adrenal vein sampling is considered the gold standard test to identify primary aldosteronism, the most frequent form of secondary hypertension. Technical difficulties with this procedure may be overcome by monitoring cortisol concentrations in the different sampling sites during catheterization.

Methods: We applied a rapid automated cortisol assay performed on a benchtop immunoassay analyzer near the operating suite during the catheterization procedures in 5 hypertensive patients. A mean of 7.8 samples (range, 5–13) were collected from the vena cava as well as from right and left adrenal veins.

Results: Cortisol concentrations measured by the rapid assay and by our routine method were comparable. Two of 5 patients were found to be affected by an aldosterone-producing adenoma and 3 of 5 by a bilateral adrenal hyperplasia. Cortisol determination during the adrenal vein sampling procedure allowed a successful cannulation in all patients, including a patient in whom it was necessary to cannulate 9 different candidate right adrenal veins before finding the correct one.

Conclusions: Intraoperative cortisol assays appeared safe, reproducible, simple to perform, rapid, and cost-effective. The approach represents a service-oriented model for the laboratory and can provide valuable and timely information for improving the success rate of adrenal vein catheterization.

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Initial studies on rapid hormone immunoassays have shown promising results in preoperative localization studies of endocrine hormone-secreting tumors and/or for assessing the effectiveness of surgical resection (1–8). Since the publication of the 1st report of a modified cortisol immunoassay as a rapid intraoperative adjunct for the localization of aldosteronomas during catheterization procedures (3), there have been no other published studies to further support the adoption of this test.

Primary aldosteronism (PA) is the most frequent form of secondary hypertension, accounting for up to 5% or 10% of all hypertensive patients (9). When a diagnosis of PA is made, then the subtype should be identified, because patients with an aldosterone-producing adenoma (APA) benefit from adrenalectomy, whereas patients with bilateral adrenal hyperplasia (BAH) should be treated with spironolactone (9). To this end, adrenal vein sam-
pling (AVS) is considered the gold standard test, although it may be technically difficult, especially in the catheterization of the right adrenal vein. Because of the difficulty of placing the catheter tip within the small adrenal veins, the sample is often obtained from near the orifice of the vein. When this happens, the concentration of aldosterone and other adrenal hormones in adrenal vein blood is diluted by other blood. The simultaneous measurement of cortisol concentrations in the blood taken from the adrenal vein allows a correction for this dilution and is a measure of the adequacy of the cannulation. Left adrenal vein catheterization is relatively easy to perform because the left adrenal vein feeds directly into the left renal vein. By contrast, the right adrenal vein usually feeds directly into the inferior vena cava (IVC) and is therefore difficult to locate and to distinguish from other adjacent small vessels. As many as one-third of adrenal vein samples from the right side turn out to be improperly collected. Monitoring of cortisol during the catheterization procedure allows any improperly collected adrenal samples to be immediately re-collected, thus reducing the frequency of repeat procedures and thereby reducing the cost as well as the discomfort to the patient and avoiding delay in diagnosis.

We report here a rapid cortisol assay performed near the operating room. Cortisol measurements were carried out on an AIA®-360 benchtop analyzer by ST AIA-PACK CORT (TOSOH Bioscience), a competitive enzyme immunoassay with individual test cups, each containing lyophilized and ready-to-use reagents. A specimen volume of 10 µL is required, and the incubation time is 10 min. The detection limit (limit of the blank) of the assay, calculated as 2 SD above the mean of 20 repeated measurements of the zero calibrator, is 2 µg/L, with a dynamic range up to 600 µg/L. No cross-reactivity with aldosterone up to 10 000 µg/L has been detected. Precision studies yielded within-run CVs of 3.1%, 2.6%, and 2.5% and between-run CVs of 3.9%, 4.3%, and 4.6% at cortisol concentrations of 53, 217, and 412 µg/L, respectively. No interferences were detected for hemoglobin, bilirubin, lipemia, or proteins.

The instrument requires minimal counter space and is easily transportable on a cart including a small centrifuge. Samples were collected in heparinized tubes. After 2 min centrifugation, plasma specimens were processed both undiluted using primary tubes and after 10-fold and 100-fold dilutions. Linearity tests showed a good correlation among serial dilution samples, allowing measurement of cortisol up to 60 000 µg/L. When all instrument settings are ready, samples can be processed on a random access basis. After 18 min to obtain the 1st result, subsequent results are available every 2 min. Results were correlated with those found with our routine method, ARCHITECT® Cortisol (Abbott Laboratories), using chemiluminescent microparticle immunoassay technology.

Patients were selected as previously described (10, 11) (Table 1). Patients with a confirmed PA further underwent a computed tomographic scan of the adrenals and AVS, performed through sequential catheterization during low-dose continuous adrenocorticotropic hormone infusion. Under these conditions, it is not necessary to

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex (M/F)</th>
<th>Aldo/PRA (ng·dL⁻¹/ ng·mL⁻¹·h⁻¹)</th>
<th>CT scan finding</th>
<th>Diagnosis after AVS</th>
<th>Number of sampling</th>
<th>Aldo (ng·dL⁻¹)</th>
<th>Cortisol (µg·dL⁻¹)</th>
<th>A:C</th>
<th>Lateralisation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) D.S.</td>
<td>44</td>
<td>M</td>
<td>228</td>
<td>Right nodule</td>
<td>Right APA</td>
<td>13</td>
<td>LA = 173.8</td>
<td>RA = 419.6</td>
<td>LA = 0.46</td>
<td>Dx/Sx = 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td>12 mm, left</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>nodule 4 mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) A.M.</td>
<td>42</td>
<td>F</td>
<td>600</td>
<td>Left adrenal</td>
<td>Left APA</td>
<td>8</td>
<td>IVC = 48.5</td>
<td>LA = 2349</td>
<td>LA = 15.9</td>
<td>Sx/Dx = 9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41.3</td>
<td>nodule 25 mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) R.M.</td>
<td>41</td>
<td>M</td>
<td>247</td>
<td>Normal adrenal</td>
<td>BAH</td>
<td>6</td>
<td>LA = 1168</td>
<td>LA = 598</td>
<td>LA = 1.95</td>
<td>Sx/Dx = 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.9</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal adrenal</td>
<td>BAH</td>
<td>7</td>
<td>LA = 7634</td>
<td>LA = 710</td>
<td>LA = 10.7</td>
<td>Sx/Dx = 11.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left APA</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4) P.G.</td>
<td>52</td>
<td>M</td>
<td>155</td>
<td>Left nodule</td>
<td>BAH</td>
<td>5</td>
<td>LA = 657</td>
<td>LA = 440</td>
<td>LA = 1.5</td>
<td>Sx/Dx = 1.3</td>
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<td></td>
<td></td>
<td></td>
<td>28</td>
<td>12 mm</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) S.M.</td>
<td>51</td>
<td>M</td>
<td>420</td>
<td>Left nodule</td>
<td>BAH</td>
<td>5</td>
<td>LA = 1237</td>
<td>LA = 1080</td>
<td>LA = 1.15</td>
<td>Sx/Dx = 1.3</td>
</tr>
</tbody>
</table>

* M, male; F, female; Aldo, plasma aldosterone; PRA, plasma renin activity; SLT, i.v. saline load test; CT, computed tomography; AVS, adrenal vein sampling; APA, aldosterone-producing adenoma; BAH, bilateral adrenal hyperplasia; pSLT, postsaline load test; A:C, aldosterone:cortisol ratio; lateralisation ratio, ratio between A:C in the higher side and A:C in the lower side; LA, left adrenal vein; RA, right adrenal vein; IVC, inferior vena cava.
dilute the samples for cortisol measurements, because results above the highest point of the calibration curve, even if the measurement is not precise, are sufficient to establish that the correct vein has been cannulated.

Adrenal vein cannulation was considered successful if the adrenal vein/IVC cortisol gradient was at least 2; lateralization was considered when the aldosterone/cortisol ratio (A:C) from 1 adrenal was at least 3 times the ratio from the other adrenal gland, together with an A:C in the contralateral lower than the A:C in the IVC.

Two of 5 patients were found to be affected by an APA and 3 of 5 by a BAH. Cortisol determination during the AVS procedure allowed a successful cannulation in all patients; a mean of 7.8 samples (range, 5–13) were collected from each patient. The cannulation of the right adrenal vein was identified as successful only after 8 unsuccessful attempts in 1 patient, at the 3rd attempt in 2 other patients, and at the 1st attempt in another 2 patients. The left adrenal vein was successfully cannulated at the 1st attempt in all cases.

The mean time to obtain a complete AVS result was 33.7 min (range, 24–52 min). The clinical interpretation in terms of the lateralization of the adrenal tumors was the same whether the rapid or routine cortisol assays were used in the calculation of the A:C ratio.

Cortisol concentrations measured by the rapid assay on plasma and by our routine method on serum were comparable ($r = 0.93, P < 0.0001$; Fig. 1A). Considering plasma values <1500 μg/L, we observed an even closer correlation between the 2 methods ($r = 0.98, P < 0.0001$; Fig. 1B). The Bland-Altman plot (12) showed that for cortisol <4000 μg/L the differences between the 2 meth-

![Fig. 1. Comparison of results of rapid and routine (Abbott Architect) assays for plasma and serum samples.](image)

(A), rapid cortisol assay on plasma samples and routine cortisol assay on sera. (B), same as (A) but restricted to cortisol values <1500 μg/L. (C), Bland-Altman plot for the differences between the rapid plasma cortisol and routine methods. (D), rapid and routine assays on serum.
ods were small (Fig. 1C). In the high part of the range a larger error was acceptable because the importance of the cortisol assay during AVS is to demonstrate the correct cannulation of the vein. We also found a very good correlation when we compared the serum (rather than plasma) cortisol measurements obtained with the TOSOH Bioscience analyzer and routine methods ($r = 0.98$, $P < 0.0001$; Fig. 1D).

In patients with PA, AVS is the key procedure to distinguish between surgically treatable forms and subtypes that should be treated medically. Unfortunately, the technical difficulty of performing AVS successfully has limited the widespread use of this test, and often the choice of therapeutic option is based only on computed tomographic scan findings, which were found to be unreliable (13–15). In fact, 3 of 5 patients of the present study would have been misdiagnosed if only computed tomographic findings had been followed.

We report here a quick and reliable cortisol assay that can be performed during the AVS, allowing the radiologist further attempts at cannulation until cortisol measurements demonstrate the success of the sampling. If the rapid cortisol assay had not been available in our study, at least 1 of 5 patients would have required a repetition of the AVS procedure owing to unsuccessful cannulation.

The adoption of this technically easy and user-friendly system in the immediate vicinity of the radiologist operating suite is a good example of the rational use of laboratory services in a patient-focused setting (16). This approach appeared safe and cost-effective, and it provided good diagnostic performance and technical requirements to meet clinical needs, with an excellent instrument stability and portability, no reagent wastage and no reagent preparation, long reagent stability, and long calibration stability. The analysis time of cortisol rapid assays is short enough to be used during the procedure.

Distributed laboratory testing is a cost-saving, service-oriented model for the laboratory. The advantages are faster turnaround time, ability to interact with the operative team, increased visibility for the laboratory, and more involvement in patient care for the technologists (17). The 20% reduction in AVS procedures will lead to $200,000 US dollar savings per 100 patients compared with an expense of approximately $55,000 US dollar for rapid cortisol testing. Moreover, the same instrument may be used to perform other tests over a wide-range panel menu, mainly in low-volume or satellite laboratories.

In summary, the rapid intraoperative cortisol assay has been shown to meet all of the criteria for an intraoperative test (18): it is safe, reproducible, simple to interpret, rapid, and cost-effective. The results compare favorably with our routine cortisol assay, and the approach can potentially provide valuable and timely information for improving the success rate of adrenal vein catheterization in the localization of adrenal tumors while the patient is still in the operating room. This feature is of particular importance in patients with PA, because of the high prevalence of this form of secondary hypertension, and is useful for the differentiation of the major PA subtypes.

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References