Aldosterone

Impact of Different Diagnostic Criteria During Adrenal Vein Sampling on Reproducibility of Subtype Diagnosis in Patients With Primary Aldosteronism

Paolo Mulatero, Chiara Bertello, Norlela Sukor, Richard Gordon, Denis Rossato, Nicholas Daunt, David Leggett, Giulio Mengozzi, Franco Veglio, Michael Stowasser

Abstract—In patients with primary aldosteronism, adrenal vein sampling (AVS) is considered the only reliable technique to distinguish between unilateral and bilateral autonomous production of aldosterone, but agreement is lacking on the best criteria indicating successful cannulation and lateralization. The objective of this study was to assess the impact of differing criteria for the successful cannulation and lateralization on the reproducibility of subtype diagnosis. Sixty-two patients with confirmed primary aldosteronism underwent AVS on 2 separate occasions, because the first was unsatisfactory. We compared the different diagnoses of primary aldosteronism subtype reached using AVS data assessed by permissive (type 1), intermediate (type 2), and strict (type 3) criteria. Although 91.1% of all of the (both first and second) AVSs were “successful” by type 1 criteria (50.8% by type 2 and 33.9% by type 3), in only 35.3% of patients was the diagnosis concordant between the first and second AVS. Type 1 criteria also led to a higher rate of diagnosis of unilateral primary aldosteronism (67.3% of successful procedures) than type 2 (36.5%) or type 3 (26.2%). There was considerable disparity in the diagnosis reached using the 3 different criteria, with concordance in only 32.2%. Using either type 1 or 2 criteria, the minimal adrenal/peripheral vein cortisol ratio necessary to obtain the same diagnosis in the first and second AVS procedures was ≥2.75. In conclusion, permissive criteria for successful cannulation and lateralization on AVS achieve poor diagnostic reproducibility and should be avoided. (Hypertension. 2010;55:667-673.)

Key Words: endocrine hypertension • primary aldosteronism • aldosterone • aldosterone-producing adenoma • bilateral adrenal hyperplasia

Primary aldosteronism (PA) is currently believed to be the most frequent form of secondary endocrine hypertension, accounting for 5% to 10% of all hypertensive patients.1,2 The rate of diagnosis of PA increased markedly after use of the aldosterone/renin ratio (ARR) as a screening test became widespread.3 The diagnosis of PA should not be missed, because it has been demonstrated recently that patients with PA exhibit a higher rate of cardiovascular complications, target organ damage, and metabolic syndrome compared with matched essential hypertensives4–6 and that this increased rate is reversed with specific surgical or medical treatment.7 A definitive diagnosis is made after a positive confirmatory test (usually a suppression test) that is performed in patients with positive ARRs.8 After confirming the diagnosis of PA, it is fundamental to distinguish between unilateral and bilateral subtypes, because individuals with unilateral forms (mainly aldosterone-producing adenomas [APAs]) can be cured or at least experience significant amelioration of the disease by unilateral laparoscopic adrenalectomy,1,3,9,10 whereas patients with bilateral adrenal hyperplasia (BAH) are usually treated and benefit from targeted pharmacotherapy with aldosterone antagonists.1,11 Computed tomography scanning lacks sensitivity and specificity10,12–14 and should, therefore, be followed by adrenal venous sampling (AVS), which is the only reliable means of differentiating unilateral from bilateral PA and lateralizing APAs preoperatively.

During AVS, both plasma (or serum) aldosterone concentration (PAC) and plasma cortisol concentration are determined in blood collected from the adrenal veins and in simultaneously collected blood from a peripheral vein (PV) or the inferior vena cava (IVC). Comparison of adrenal venous and PV (or IVC) cortisol permits an assessment of the “adequacy” or “success” of cannulation of the adrenal veins. However, agreement is lacking on which criteria should be...
used for defining successful cannulation, with some centers using more permissive criteria (C<sub>adrenal vein/CIVC</sub>&gt;1.1)\textsuperscript{15} and others using more restrictive criteria (C<sub>adrenal vein/CIVC</sub>&gt;3.0 without adrenocorticotropic hormone [ACTH] stimulation\textsuperscript{10} or C<sub>adrenal vein/CIVC</sub>&gt;4.0 under low-dose continuous ACTH stimulation\textsuperscript{16}). Cortisol levels are also used in the calculation of aldosterone/cortisol ratios (A/Cs), which serve to “correct” adrenal venous aldosterone levels for differing degrees of dilution of adrenal versus nonadrenal venous blood. However, criteria for defining lateralization of aldosterone secretion are again not uniform, with some centers using more permissive criteria ([A/C]<sub>adrenal vein/contralateral adrenal vein</sub>&gt;2.0),\textsuperscript{15} and others using more restrictive criteria ([A/C]<sub>adrenal vein/contralateral adrenal vein</sub>&gt;2.0 and [A/C]<sub>contralateral adrenal vein/CIVC</sub>&lt;1.0) without ACTH stimulation\textsuperscript{10} or ([A/C]<sub>adrenal vein/contralateral adrenal vein</sub>&gt;4.0 under low-dose continuous ACTH stimulation\textsuperscript{16}).

In the current study, we sought to assess the impact of differing criteria for defining cannulation success and for defining lateralization on the rates of subtype (unilateral versus bilateral PA) diagnosis and the reproducibility of subtype diagnosis in patients who had undergone 2 separate AVS procedures, in each case because the cannulation during the first attempt was deemed unsuccessful according to locally used criteria.

**Methods**

The study was carried out in 2 referral centers: the Endocrine Hypertension Research Centre, Greenslopes and Princess Alexandra Hospitals, Brisbane, and the Division of Internal Medicine and Hypertension Unit, University of Torino. Patients were enrolled after receiving written informed consent and approval of the study protocol by the local ethics committees.

**Brisbane**

We selected all of the patients with PA who underwent 2 AVS studies in the period from 2000 to 2006, because the first one did not meet our criteria for successful cannulation. This was composed of 45 (8.5%) of 531 patients. Patients were studied as described previously.\textsuperscript{17,18} Briefly, all of the hypertensive patients with an ARR &gt;70 (plasma aldosterone expressed in picomoles per liter and plasma active renin activity [PRA] in nanograms per milliliter per hour) on ≥2 occasions were subjected to a fludrocortisone suppression test to definitively confirm or exclude PA. Blood was collected in the sitting position between 8:00 AM and 11:00 AM after ≥2 hours of upright posture for the measurement of ARR after hypokalemia had been corrected with potassium supplements and while patients were being encouraged to maintain a liberal dietary salt intake. Before testing, diuretics (including spironolactone and amiloride) were always ceased for ≥4 weeks, and, wherever possible, β-blockers, dihydropyridine-type calcium channel antagonists, angiotensin-converting enzyme inhibitors, and angiotensin II receptor antagonists were ceased for ≥2 weeks and withheld throughout the subsequent diagnostic steps. To maintain hypertension control, treatment was instituted where necessary with verapamil slow release, with or without added hydralazine and/or prazosin.\textsuperscript{18} The diagnosis of primary aldosteronism was based on fludrocortisone suppression test criteria.\textsuperscript{17} When PAC, measured at 10:00 AM in seated patients after ≥2 hours upright, failed to suppress to &lt;1.66 pmol/L (6 ng/dL) at the conclusion of 4 days of administration of a high-sodium diet, slow-release sodium chloride (Slow Na; 30 mmol 3 times daily with meals), and fludrocortisone acetate (0.1 mg every 6 hours), provided that, on day 4: (1) upright renin was suppressed to &lt;6.4 mU/L (for plasma active renin concentration) or 1 ng/mL per hour (for PRA); (2) plasma potassium was within the normal range; and (3) plasma cortisol was lower at 10:00 AM than at 8:00 AM, excluding an acute increase in adrenocorticotrophic hormone, which could prevent suppression of aldosterone. In all of the patients with primary aldosteronism confirmed by fludrocortisone suppression test, peripheral blood DNA was tested\textsuperscript{20} for the hybrid CYP11B1/CYP11B2 gene responsible for glucocorticoid-remediable primary aldosteronism and adrenal computed tomography performed with fine (2.5 to 3.0 mm) sections, before and after intravenous contrast. All who tested negative for the hybrid gene, regardless of computed tomography findings, underwent AVS\textsuperscript{17,20} to differentiate unilateral from bilateral adrenal aldosterone overproduction. “Gradients” of ≥3.0 between adrenal and peripheral venous cortisol concentrations were taken to indicate adequate sampling from adrenal veins. If the A/C ratio on 1 side was ≥2 times the simultaneous peripheral venous ratio and on the other side was the same as or less than the peripheral (“contralateral suppression”), the study was considered to demonstrate lateralization of aldosterone production. Patients demonstrating adrenal venous A/C ratios higher than peripheral on both sides were considered to have bilateral autonomous aldosterone production. For patients in whom the repeat (second) AVS procedure was successful by cannulation criteria but in whom adrenal A/C was less than peripheral venous ratio and on both sides between 1.1 and 2.0 times the peripheral in the other side, those AVS results were considered “inconclusive.” All of the AVS studies were performed between 8:00 AM and 11:00 AM to minimize the chance that “poor” adrenal/peripheral cortisol gradients could be attributed to low cortisol secretory rates from the adrenals, as might be expected in the afternoon.

Antihypertensive therapy was unaltered in the period between the 2 AVS studies. Hormonal assays were performed as described previously.\textsuperscript{17} Diagnosis of APA was confirmed after surgery, pathology, blood pressure outcome, and normal suppressibility of aldosterone after postoperative fludrocortisone suppression test.\textsuperscript{13} PAC was measured by solid-phase radioimmunoassay technique using Coat-A-Count assay (Diagnostic Product Corporation). The interassay coefficients of variation at 98, 631, and 1458 pmol/L were 15%, 6%, and 6%, respectively. The intra-assay coefficient of variation was 2.3% to 5.4% for values in the range of 180 to 2256 pmol/L. Plasma cortisol concentration was determined by chemiluminescent immunoassay on the DxI 800 Immunoassay System (Beckman Coulter). The interassay coefficient of variation at 140, 562, and 958 nmol/L is 6.5%, 5.0%, and 4.9%, respectively.

**Torino**

We selected all of the PA patients who underwent 2 AVSs in the period from 2002 to 2007 in our unit at the University of Torino. This was composed of 17 (18%) of 93 patients. PA patients were selected as described previously.\textsuperscript{14} Briefly, patients were screened using the ARR: the cutoff level for a positive ARR was 1100 (aldosterone in picomoles per liter and PRA in nanograms per milliliter per hour) together with a PAC of &gt;416 pmol/L. Blood samples were obtained in the sitting position between 8:00 and 10:00 AM. All of the antihypertensive drugs were stopped ≥3 weeks before the aldosterone and PRA measurements (≥6 weeks before for diuretics and ≥8 weeks before for spironolactone). Patients who, for clinical reasons, could not be left untreated were allowed to take an α-blocker (doxazosin) and/or a calcium channel blocker (verapamil oramlodipine) and maintained the same therapy during and for the period between the screening and the final diagnosis. The confirmatory test was an intravenous saline load (2 L of 0.9% NaCl infused over 4 hours) that was considered positive if posttest PAC levels were &gt;139 pmol/L.\textsuperscript{21}

Computed tomography scanning with fine cuts (2.5 mm) of the adrenal with contrast was performed in all of the PA patients. Adrenal vein cannulation, performed in all of the patients with a positive saline load test (unless found to have glucocorticoid-remediable aldosteronism by genetic testing),\textsuperscript{22} was considered successful if the adrenal vein/IVC cortisol gradient was ≥2. The study was considered to show lateralization when the A/C ratio from 1 adrenal gland was ≥4 times the ratio from the other adrenal gland.
Table 1. Clinical and Biochemical Parameters of Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Torino (n=17)</th>
<th>Brisbane (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SE, y)</td>
<td>53±2</td>
<td>53±2</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>13/4</td>
<td>14/31</td>
</tr>
<tr>
<td>SBP (mean±SE, mm Hg)</td>
<td>176±8</td>
<td>158±2</td>
</tr>
<tr>
<td>DBP (mean±SE, mm Hg)</td>
<td>104±5</td>
<td>91±1</td>
</tr>
<tr>
<td>Plasma potassium (mean±SE, mEq/L)</td>
<td>3.7±0.14</td>
<td>3.8±0.1</td>
</tr>
<tr>
<td>PAC (median, range, pmol/L)</td>
<td>757 (521 to 1559)</td>
<td>520 (264 to 1950)</td>
</tr>
<tr>
<td>PRA (median, range, ng/mL·l/h·1)</td>
<td>0.15 (0.1 to 0.8)</td>
<td>0.2 (0.1 to 0.4) [n=9]</td>
</tr>
<tr>
<td>Direct active renin (median, range, µL)</td>
<td>…</td>
<td>3.7 (2.0 to 10.0) [n=36]</td>
</tr>
<tr>
<td>PAC/PRA ratio (median, range)</td>
<td>5204 (1110–12760)</td>
<td>1940 (1207 to 11 150) [n=9]</td>
</tr>
<tr>
<td>PAC/direct active renin ratio (median, range)</td>
<td>…</td>
<td>175.5 (65 to 520) [n=36]</td>
</tr>
</tbody>
</table>

… indicates no data.

or if it was ≥3 times the contralateral together with an A/C in the contralateral vein lower than that in the PV.

All of the AVS studies were performed between 8:00 AM and 11:00 AM to minimize the chance that poor adrenal/peripheral cortisol gradients could be because of low cortisol secretory rates from the adrenals, as might be expected in the afternoon. Antihypertensive therapy was unaltered in the period between the 2 AVS studies. Diagnosis of APA was confirmed after surgery, pathology, blood pressure outcome, and normal suppressibility of aldosterone after postoperative intravenous saline loading.14 Hormonal assays were performed as described previously.14 PAC was assessed by solid-phase radioimmunoassay ALDOCTK-2 (DiaSorin, Saluggia). Within-run precision tests yielded coefficient of variations of 5.3%, 3.8%, and 2.7% on samples with mean aldosterone values of 8.6, 24.9, and 49.6 ng/dL, respectively. Between-run coefficients of variations were 13.7%, 10.4%, and 8.3% at concentrations of 9.1, 23.0, and 60.7 ng/dL, respectively. Plasma cortisol concentrations were determined using chemiluminescent microparticle immunoassay technology automated on an ARCHITECT analyzer (Abbott Laboratories). Precision studies yielded coefficient of variations were 13.7%, 10.4%, and 8.3% at cortisol concentrations of 8.6, 24.9, and 49.6 ng/dL, respectively. Between-run coefficients of variations were 13.7%, 10.4%, and 8.3% at cortisol concentrations of 8.6, 24.9, and 49.6 ng/dL, respectively.

### Results

The study involved 62 patients (45 assessed at the Brisbane center and 17 at the Torino center) who underwent 2 AVS procedures (a total of 124 procedures). Clinical and biochemical parameters are summarized in Table 1. During the period of the study, the success rate of cannulation was 83% in Torino and 81% in Brisbane, using the criteria specific to the corresponding institution. We analyzed the AVS data according to a relatively permissive set of criteria currently in use elsewhere23 (type 1), a relatively strict set of criteria (type 3, used in the Brisbane center), and an intermediate set of criteria (type 2, used in the Torino center; Table 2).

### Cannulation Success Rates

Of the first AVS studies (all deemed unsatisfactory in either Brisbane or Torino), 83.4% would have been considered successful using the type 1 criteria. As expected, 98.4% of the second, repeat AVS studies met the type 1 criteria (Table 3). In contrast, using the type 3 criteria, cannulation would have been judged successful in 0% with first AVS and 67.7% with second AVS (33.9% overall), and using the type 2 criteria, 19.4% would have been judged successful with the first and 82.3% with the second (50.8% overall).

### Rates of Subtype Diagnosis

Application of type 1 criteria led to a high rate of diagnosis of unilateral PA, with 67.3% of those deemed successful AVSs by type 1 criteria lateralizing (61.3% of the total 124). Using type 2 criteria, 36.5% of successful AVS procedures lateralized (18.5% of the total), and, using type 3 criteria, only 26.1% of the successful AVS procedures lateralized (8.9% of the total).

With marked differences among the 3 criteria sets in terms of diagnostic conclusion reached for each AVS study, only 32.2% of them were concordant (Table 4). However, in >75% of the AVS studies, the same conclusion was reached using criteria type 2 or type 3. Results using criteria type 1 were concordant with results using criteria type 2 in only 46.7% and concordant with criteria type 3 in only 33.0%.

### Table 2. Criteria for Successful Cannulation and Lateralization After AVS

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Criterion Type 1</th>
<th>Criterion Type 2</th>
<th>Criterion Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannulation criteria</td>
<td>C&lt;sub&gt;adrenal vein&lt;/sub&gt;/C&lt;sub&gt;PV&lt;/sub&gt;≥1.1</td>
<td>C&lt;sub&gt;adrenal vein&lt;/sub&gt;/C&lt;sub&gt;peripheral vein&lt;/sub&gt;≥2</td>
<td>C&lt;sub&gt;adrenal vein&lt;/sub&gt;/C&lt;sub&gt;PV&lt;/sub&gt;≥3</td>
</tr>
<tr>
<td>Laterization criteria</td>
<td>(A/C)&lt;sub&gt;adrenal vein&lt;/sub&gt;/ (A/C)&lt;sub&gt;contralateral adrenal vein&lt;/sub&gt;≥2</td>
<td>(A/C)&lt;sub&gt;adrenal vein&lt;/sub&gt;/ (A/C)&lt;sub&gt;contralateral adrenal vein&lt;/sub&gt;≥4 or (A/C)&lt;sub&gt;adrenal vein&lt;/sub&gt;/ (A/C)&lt;sub&gt;contralateral adrenal vein&lt;/sub&gt;≥3 + (A/C)&lt;sub&gt;adrenal vein&lt;/sub&gt;/ (A/C)&lt;sub&gt;contralateral adrenal vein&lt;/sub&gt; (A/C)&lt;sub&gt;PV&lt;/sub&gt;&lt;1</td>
<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

### Table 3. Success and Final Diagnosis After AVS With the 3 Different Criteria

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Type 1, n (%)</th>
<th>Type 2, n (%)</th>
<th>Type 3, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successful AVS</td>
<td>113 (91.1)</td>
<td>63 (50.8)</td>
<td>42 (33.9)</td>
</tr>
<tr>
<td>APA right</td>
<td>39 (31.5)</td>
<td>16 (12.9)</td>
<td>9 (7.3)</td>
</tr>
<tr>
<td>APA left</td>
<td>37 (29.8)</td>
<td>7 (5.6)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>BAH</td>
<td>37 (29.8)</td>
<td>40 (32.3)</td>
<td>31 (25.0)</td>
</tr>
<tr>
<td>Unsuccessful AVS</td>
<td>11 (8.9)</td>
<td>61 (49.2)</td>
<td>82 (66.1)</td>
</tr>
</tbody>
</table>

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Importantly, in 21 of the 25 studies in which conclusions reached using criteria 2 and 3 were discordant, this was because of a lack of any satisfactory diagnosis being reached because of failed cannulation when the stricter type 3 criteria were used. This left only 4 studies where diagnoses were reached by both sets of criteria 2 and 3 that were truly discordant.

Because results for the first performed AVS for each patient showed failed cannulation (and, hence, lacked a definitive subtype diagnosis) in all 62 of the cases using type 3 criteria and in 50 patients using type 2 criteria, we analyzed rates of subtype diagnosis for the second AVS alone (Table 4). In 30 (48.4%) of the 62 patients, the conclusion reached was the same with all 3 of the criteria. In 19 (30.6%), the conclusion was similar according to criteria types 1 and 2 but differed from that of criteria type 3. In 9 (14.5%), it was similar for criteria types 1 and 3 but differed from that of criteria type 2. In 1 (1.6%), it was similar between criteria types 1 and 3 but differed from type 2, and in 3 (4.8%) patients the diagnosis was different among all 3 of the criteria sets.

Reproducibility of Subtype Diagnosis

We first analyzed all of the AVS studies using the most permissive criteria (type 1) both for success and lateralization. With these criteria, cannulation of one or both sides was unsuccessful during =1 AVS procedure in 11 of the 62 patients, leaving 51 patients in whom AVS was successful on both occasions that it was performed and in whom a comparison of diagnoses reached between the 2 studies was therefore possible. Only in 18 (35.3%) of the 51 patients was the diagnosis concordant between the first and the second AVS using type 1 criteria, leaving 33 (64.7%) in which the diagnoses differed (Table 5). Of these, in 7 patients (13.7%) who were deemed unilateral after the first AVS, the diagnosis changed to unilateral in the contralateral gland after the second AVS. In 19 (37.2%), a diagnosis of unilateral made after the first AVS changed to bilateral after the second. In the remaining 7 (13.7%), a diagnosis of bilateral after the first AVS became unilateral after the second (Table 6). The minimal adrenal/PV cortisol ratio that was necessary to obtain the same diagnosis in the 2 AVS procedures using the type 1 lateralization criteria was =2.75.

We also analyzed reproducibility using the intermediate criteria (type 2). Because criteria for cannulation success were stricter than for type 1, they were met for both the first and second AVS studies in only 8 patients (Table 5). Of these, the diagnosis was concordant between the 2 studies in 4 (50%), whereas, in the other 4, a diagnosis of unilateral made after the first AVS became bilateral after the second (Table 6). Interestingly, even in this analysis, the minimum cutoff adrenal/PV cortisol ratio required to obtain the same diagnosis between each pair of studies was again 2.75.

Discussion

Adrenal vein sampling is the only reliable approach to distinguish between unilateral, and therefore surgically correctable, forms of PA and bilateral forms, which are usually treated with mineralocorticoid receptor antagonists. However, different, necessarily arbitrary cutoffs are used in different centers, both to define successful cannulation of the adrenal veins and to determine lateralization of aldosterone secretion. The current study has demonstrated that the use of differing AVS cutoffs or criteria can have a profound impact on the diagnostic conclusions reached.

With regard to the effect on cannulation success rates, raising the cutoff adrenal/PV cortisol ratio from 1.1 to 3.0 dropped the proportion of studies regarded as being success-
ful from 91% to 34%, a difference of great practical significance. It must be emphasized, however, that patients were chosen for this study on the basis of having had 2 AVS procedures (to allow for an assessment of diagnostic reproducibility) because of a failed study on the first attempt, which could have exaggerated the differences in success rates observed among the 3 criteria sets. Taking the second AVS study into consideration alone, for example, the differences were much less marked but still considerable (98% versus 68%). It appears clear from this study that the use of permissive cannulation success criteria can lead to significant numbers of patients being given a definitive subtype diagnosis when stricter criteria would have led to the study results being rejected (and the study repeated) or interpreted with great caution.

The impact of differing AVS criteria on subtype diagnosis was considerable. The more permissive criteria led to a much higher rate of diagnosis of unilateral PA, ~6 times (61% versus 9%) that of the least permissive criteria for all of the studies combined and more than double (67% versus 26%) when only those studies considered successful were considered. Concordance of diagnosis between criteria sets was better between type 2 and 3 criteria than between type 1 and either of the other 2 types.

Because this study did not include an analysis of outcomes of surgery as a gold standard, it is not possible from these data alone to make firm conclusions about the relative accuracy of subtype diagnosis reached by each criteria set. As an alternative approach, we examined the reproducibility of diagnostic conclusions reached by comparing results obtained between 2 separate studies on individual patients. Using the most permissive criteria, which resulted in studies rejected in Brisbane or Torino being included, diagnostic reproducibility was poor, with a similar diagnosis being obtained for first and second studies in only 18 (35%) of 51 patients. All of the combinations of discordance were observed, but of particular concern was the finding that, in 7 patients, lateralization actually changed from one side to the other. A somewhat higher rate of reproducibility (50%) was seen with the application of type 2 criteria, but because only 8 patients satisfied those criteria in terms of successful cannulation for both first and second AVS procedures and were, therefore, the only subjects eligible for this analysis, it is difficult to draw firm conclusions in this respect. Similarly, we could not assess reproducibility of type 3 criteria, because the nature of selection of patients for this study meant that none had successful AVS procedures during the first attempt. Importantly, however, whether type 1 or type 2 lateralization criteria were applied, reproducibility was found to be 100% only when studies in which adrenal/PV cortisol ratios of ≥2.75 were considered. This would argue for the adoption of the stricter type 3 criteria (which uses a cutoff of 3) for defining cannulation success to minimize diagnostic inaccuracy.

In agreement with our results are the reported lower cure rates (30%) among operated patients diagnosed as having APA using permissive criteria,24 which is approximately half the rate reported by the Torino and Brisbane groups.3 In this context it should be considered that cure reported by Sukor et al25 in 15% of patients with BAH who underwent adrenalectomy was mainly restricted to patients who only had milder forms of PA and milder hypertension.25

AVS is a difficult procedure, especially for the cannulation of the right adrenal vein, which is small and usually empties directly into the IVC (unlike the left, which usually empties into the left renal vein, making it easier to cannulate). The success rate depends on the experience and dedication of the radiologist. It has been argued that, because using higher cortisol cutoff ratios will lower the percentage of usable AVS studies, a more permissive ratio should be recommended.23 However, as the current study demonstrates, this would likely be at the cost of risking incorrect subtype diagnoses. In 1 report, in which 4 radiologists performed AVS in 60 patients, the success rate of bilateral adrenal vein cannulation was only 44%.26 By contrast, with experience and focusing the expertise to 1 or 2 radiologists at each center, the AVS success rate can rise to 96%16 or at least to >80%.10,14,20 Recently, the introduction of a method for real-time rapid cortisol assay27 during AVS, which provides the radiologist with an assessment as to the success or otherwise of attempted cannulation at the time of the procedure, offers the radiologist a new tool to improve the performance of AVS.

In a recent article, the use of AVS as the gold standard has been questioned, mainly because of the lack of standardization among different groups with regard to the criteria used to define cannulation success and to define lateralization.28 We agree entirely that this reduces the usefulness of AVS as a diagnostic tool. Our contention is that criteria that incorporate less stringent requirements to define cannulation success and lateralization will result in reduced diagnostic accuracy. This may help to explain the lower sensitivity and specificity rates reported in some studies.

Coexisting autonomous aldosterone and cortisol production has been rarely reported among patients with APA,29 and this would be expected to result in absence of an adrenal/peripheral venous cortisol gradient on the side contralateral to the lesion. Although this may have accounted for the absence of a cortisol gradient in some of our patients, we would expect the number to be very small. Furthermore, this would not account for the change in diagnosis between the first and second AVSs, because both studies should have been affected similarly.

We believe that the difference between the diagnosis obtained with the first and the second AVS is not attributed to the fact that both centers use a sequential cannulation technique and not a simultaneous cannulation of the adrenal veins; in fact, because of the level of experience of our radiologists, there was usually a ~10- to 15-minute gap between sampling of the 2 adrenal veins. Importantly, in no patient were there significant alterations in peripheral aldosterone or cortisol levels (of which ≥5 were available for analysis for each procedure) during either of the 2 AVS studies, which would argue against there being significant ACTH- or angiotensin II–induced fluctuations in adrenal steroid output during those periods.

In our study, AVS was performed without the use of ACTH stimulation. Others have used continuous cosyntropin infusion (50 μg/h started 30 minutes before sampling and...
continued throughout the procedure) during AVS\textsuperscript{16,30} to minimize stress-induced fluctuations in aldosterone secretion during nonsimultaneous AVS, to maximize the gradient in cortisol from adrenal vein to IVC, and to maximize the secretion of aldosterone from an aldosterone-producing adenoma. However, it has been suggested that, in some cases, this could result in stimulation of the gland contralateral to an APA, blunting the difference between the sides and leading to a mistaken diagnosis of bilateral autonomous aldosterone production. This is perhaps most likely to happen when renin suppression has not been of long duration, and aldosterone production by the contralateral gland is not thoroughly suppressed. The use of a high-dose ACTH bolus (250 μg) during simultaneous adrenal vein catheterization has been reported recently not to result in a significant improvement in diagnosis accuracy.\textsuperscript{24} However, we propose that our results are also applicable to AVS performed during ACTH infusion, although an adrenal/peripheral cortisol gradient would be expected to be obtained more easily with this protocol. In a recent study published by Ceral et al\textsuperscript{31} in which AVS was performed during ACTH infusion (160 μg/h), reproducibility in lateralization diagnosis (determined by comparing results among multiple samples collected during the same AVS procedure and using those with cortisol gradients of ≥10 as the “s” samples) was found to be critically dependent on adrenal/peripheral cortisol gradients. Interestingly, the optimum cutoff again appeared to be at gradients of ≥3.

The results of the present study are of importance for the interpretation of a technique that is considered to be the gold standard for the diagnosis of PA subtypes. Indeed, as a result of our findings, the Torino center has adopted the policy to increase the adrenal vein/IVC cortisol cutoff to 3 for defining adrenal venous cannulation success.

**Disclosures**

None.

**References**


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