CLINICAL RESEARCH

Aldosterone-to-renin ratio for diagnosing aldosterone-producing adenoma: A multicentre study

Le rapport aldostérone/rénine pour diagnostiquer un adénome de Conn : une étude multicentrique

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KEYWORDS
Aldosteronism; Renin; Aldosterone-producing adenoma; Hypertension; Hypokalaemia;

Summary
Background. — Biological diagnostic criteria for diagnosing aldosterone-producing adenoma (APA) are not well-established.
Aim. — The aim of the study was to establish the best biological predictors of APA.
Methods. — A prospective register was implemented in 17 secondary or tertiary hypertension centres. The inclusion criterion was one of the following: onset of hypertension before 40 years of age; history of hypokalaemia; drug-resistant hypertension (resistant to three drugs); or spironolactone efficiency on BP.

Abréviations: APA, aldosterone-producing adenoma; ARR, aldosterone-to-renin ratio; AVS, adrenal venous sampling; BP, blood pressure; CT, computed tomography; PA, primary aldosteronism; ROC, receiver operating characteristic.
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Background

Primary aldosteronism (PA) is one of the commonest forms of secondary hypertension [1] and diagnosis of PA has received much attention in recent years [2–6]. Once PA is confirmed, the subtype must be determined in order to guide treatment. Almost one-third of PA cases are due to aldosterone-producing adenoma (APA) and are surgically curable. Two-thirds of PA cases are related to idiopathic adrenal primary aldosteronism, frequently with nodular adrenal hyperplasia [7]. APA has been reported to carry a higher risk of left ventricular hypertrophy, stroke and chronic kidney disease [8–13]. A surgical alternative to long-term drug therapy would reduce costs and increase patients’ quality of life. PA was initially defined as hypertension associated with increased aldosterone and low renin concentrations. High concentrations of aldosterone lead to the sodium retention responsible for hypertension and renal potassium loss causing hypokalaemia. However, in half of PA cases, potassium is within the normal range and sometimes even blood pressure (BP) is normal. Thus, diagnosis of PA is not easy and screening for possible PA, which was initially restricted to hypertension with hypokalaemia, had to be extended to cases of resistant hypertension [14,15]. The diagnosis of PA remains biological, associating high concentrations of aldosterone with low concentrations of renin. Many authors have used the aldosterone-to-renin ratio (ARR) to define PA and several cut-off values have been proposed [14,16–22]. Furthermore, to avoid diagnosis of low-renin hypertension, minimum values for renin and/or aldosterone have been advocated for calculating the ARR. As noted by Kaplan [23], there are ‘considerable differences in the definition of an elevated ARR’. Two strategies have been used to define the ARR. The first, representing the upper values

Results. — Among the 338 collected cases, 192 patients had two aldosterone-to-renin ratio (ARR) determinations (after 1 hour supine and at least 1 hour upright) on the same occasion. Twenty-five patients (8.2%) had biological hyperaldosteronism and an adrenal adenoma identified by computed tomography. APA was histologically confirmed in all 12 patients who underwent surgery. Histologically proven APAs were used as the ‘gold standard’ in receiver operating characteristic (ROC) curve analysis. ARRs were computed with a minimum renin value set at 5 ng/L to avoid misclassification of so-called ‘low-renin hypertension’. To predict an APA, the ARR area under the ROC curve was 0.93. A supine APA cut-off value of 32 ng/ng provided the highest sum of sensitivity (92%) plus specificity (92%). On the basis of an ARR ≥ 32 ng/ng in the supine and/or upright position, sensitivity reached 100%.

Conclusion. — The proposed cut-off value of 32 ng/ng for ARR (minimum renin value set at 5 ng/L) in one of two determinations had 100% sensitivity and 72% specificity with 20% positive and 100% negative predictive values for diagnosing APA.

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obtained in unselected essential hypertensive subjects, led to a lower cut-off value and a consequently higher percentage of PA (up to 39% of hypertensives). The second is based on minimal ARR values in patients with an APA. A more suitable strategy used by Bernini et al. [24] to assess ARR cut-off values is based on receiver operating characteristic (ROC) analysis. In their single centre study, they proposed an aldosterone-to-plasma renin activity cut-off value and reported a high percentage of APA (8.4%) and IHAP (author to define at proof stage) (12.7%) in newly diagnosed hypertensives. In the PAPY study, Rossi et al. [25] reported that ARR based on measurement of active renin is a valuable alternative to that based on plasma renin activity. Using a similar strategy, we conducted a prospective multicentre study to determine an ARR threshold for detecting an APA.

Methods

A prospective register was implemented in 17 secondary or tertiary hypertensive centres. The inclusion criterion was one of the following: onset of hypertension before 40 years of age; history of hypokalaemia; drug-resistant hypertension (resistant to three drugs); or spironolactone efficiency on BP. The register opened in October 2006 and closed in June 2007 when 300 consecutive case reports were recorded as specified in the study protocol.

For each patient, the following data were collected: anthropomorphic variables and lifestyle (weight, height, smoking habits, alcohol consumption, liquorice consumption); hypertension history (duration, family history, efficiency of spironolactone if available and potassium supplementation; sitting BP [measured three times at 2-minute intervals after a 5-minute rest; the averages of the three BP measurements were calculated; validated automatic sphygmomanometers were used in all centres]); cardiovascular complications (myocardial infarction, arrhythmia and stroke); biological variables (kalaemia, creatininemia, plasma bicarbonate, 24-hour urinary excretion of creatinine, sodium, potassium, albumin and aldosterone); plasma aldosterone and active renin (both expressed in ng/L), determined after 1 hour in a supine position and after at least 1 hour in an upright position.

A minimum plasma renin value of 5 ng/L was set to calculate the ARR. Treatments that might interfere with the renin-angiotensin system were not allowed; however, oral contraceptive and hormonal replacement therapies were not stopped or modified. A wash-out period prior to ARR screening was 2 weeks for angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers and diuretics (except for spironolactone) and 4 weeks for spironolactone; however, in 22 patients with ischaemic myocardial disease, beta-blockers could not be interrupted. When necessary, authorized treatments were calcium channel blockers (non-dihydropriydine or long-acting dihydropriydine), central-acting agents (rilmenidine) and alpha-blockers (slow-release prazosin, urapidil). Sodium diet and potassium supplementation (if applicable) were not modified. Cases reports containing unauthorized drugs were discarded (n = 6).

Using complete case report forms, a blind outcome committee (J.-P.F. and J.-P.B.) established the diagnosis of PA. The ARR cut-off value of 23 ng/ng was used to suspect a PA, with the minimal renin concentration set at 5 ng/L. This cut-off value was chosen because it was the lowest reported in the literature [17]. However, in five case reports, the ARR was greater than 23 ng/ng and computed tomography (CT) scans were not available; the diagnosis could not be made and these cases were excluded for incomplete data. Actually, in the remaining patients, the minimum ARR value used by the investigators for high-resolution angiographic CT-scan was 14.5 ng/ng. Thus, a CT-scan was also performed in 99 patients (70%) in whom the ARR was lower than 23 ng/ng. Twenty-two other case reports were also excluded due to incomplete data.

Results

As shown on the flow-chart (Fig. 1), among the 338 collected cases, 305 met all the inclusion criteria, 192 patients had two ARR determinations (supine and upright) and 25 patients (8.2%) had biological PA and an adrenal adenoma. Among them, 12 patients chose adrenalectomy and 13 preferred pharmacological treatments. Five patients (two among those who chose adrenalectomy) who had biological PA and an adrenal adenoma were taking a beta-blocker. Among the 12 operated patients, only six had adrenal venous sampling (AVS); in each case, it confirmed the unilateral aldosterone secretion. No technical failure was reported by the investigators. In all those who underwent unilateral adrenalectomy, an APA was confirmed by histology. Patients treated by spironolactone achieved either normalization (n = 9) or significant reduction (n = 4) in BP (BP decrease > 20% or use of fewer antihypertensive drugs).

Thus, for our purpose, the 12 patients with histologically proven APAs were compared with the 167 remaining patients who did not have APAs. The mean characteristics of patients with histologically proven APAs and those without APAs are shown in Table 1. BP was slightly, but not significantly, higher in APA patients undergoing similar therapy at presentation. Four of the 12 (33%) APA patients had normal serum
Figure 1. Flow-chart. APA: aldosterone-producing adenoma; ARR: aldosterone-to-renin ratio.

Table 1 Mean characteristics of patients with and without aldosterone-producing adenoma.

<table>
<thead>
<tr>
<th></th>
<th>APA (n = 12)</th>
<th>Non-APA (n = 167)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45 ± 10.5</td>
<td>51 ± 13</td>
<td>0.14</td>
</tr>
<tr>
<td>Men/women (n/n)</td>
<td>5/7</td>
<td>73/94</td>
<td>0.87</td>
</tr>
<tr>
<td>Duration of hypertension (years)</td>
<td>7.2 ± 1.4</td>
<td>10.1 ± 9.1</td>
<td>0.30</td>
</tr>
<tr>
<td>Office SBP (mmHg)</td>
<td>162 ± 21</td>
<td>159 ± 13</td>
<td>0.68</td>
</tr>
<tr>
<td>Office DBP (mmHg)</td>
<td>97 ± 17</td>
<td>95 ± 13</td>
<td>0.56</td>
</tr>
<tr>
<td>Number of antihypertensive drugs</td>
<td>2.3 ± 1.1</td>
<td>2.2 ± 6.5</td>
<td>0.76</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7 ± 4.6</td>
<td>27.6 ± 5.2</td>
<td>0.46</td>
</tr>
<tr>
<td>Kalaemia (mmol/L)</td>
<td>3.4 ± 0.35</td>
<td>3.8 ± 1.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>eGFR (mL/minute)</td>
<td>102 ± 42</td>
<td>103 ± 26</td>
<td>0.87</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>27.7 ± 2.5</td>
<td>26.4 ± 3.9</td>
<td>0.18</td>
</tr>
<tr>
<td>Supine active renin (ng/L)</td>
<td>2.9 ± 2.5</td>
<td>5.4 ± 3.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Standing active renin (ng/L)</td>
<td>4.7 ± 6.7</td>
<td>10.6 ± 7.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Supine aldosterone (ng/L)</td>
<td>276 ± 115</td>
<td>88 ± 78</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Standing aldosterone (ng/L)</td>
<td>483 ± 318</td>
<td>200 ± 156</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Supine ARR (ng/ng)</td>
<td>52.5 ± 24.5</td>
<td>14.0 ± 10.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Standing ARR (ng/ng)</td>
<td>76.3 ± 42.7</td>
<td>25.0 ± 19.5</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation unless otherwise indicated. APA: aldosterone-producing adenoma; ARR: aldosterone-to-renin ratio (with a minimum renin value set at 5 ng/L); BMI: body mass index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure.

<sup>a</sup> P is the alpha risk of Student’s t test to compare mean values and of the Chi² test to compare percentages.
potassium concentrations ($\geq 3.5$ mmol/L). Bicarbonate and estimated renal function (Cockcroft and Gault formula) did not differ between groups. Supine and standing active renin concentrations were significantly lower and aldosterone concentrations were significantly higher in APA patients. Consequently, supine and standing ARRs were significantly higher in APA patients. The area under the ROC curve that plotted sensitivity versus (1—specificity) for supine active renin was 0.72 (Fig. 2), while that for supine aldosterone was 0.92 (Fig. 2). The highest area under the ROC curve was obtained with an ARR calculated with the minimal value of active renin set at 5 ng/L (data not shown for other renin threshold). The highest area under the ROC curves provided the highest Youden index and thus the best compromise between sensitivity and specificity. As shown in Fig. 2, the AUC for the ROC analysis was 0.93 for supine
ARR. The highest Youden coefficients with corresponding cut-off values for aldosterone, active renin and ARR are given in Table 2. The corresponding ARR cut-off value in the supine position was 32 ng/L. According to this cut-off value, 11 APAs were diagnosed. An ARR > 32 ng/L in the upright position revealed the remaining APA. The cut-off value of 32 ng/L for ARR in at least one of two determinations had 100% sensitivity, 72% specificity and 20% positive and 100% negative predictive values for diagnosing an APA. Absolute values of active renin and aldosterone did not provide any additional information. When applied to the 25 patients, in whom the CT-scan revealed an adrenal adenoma, the ARR cut-off value of 32 ng/L provided a specificity of 92% and a sensitivity of 72% (Fig. 2).

According to the CT-scan and the determined threshold of 32 ng/L, there were five cases of bilateral primary hyperplasia, four cases of unilateral primary hyperplasia (no adenoma) and 34 cases of idiopathic hyperaldosteronism.

Discussion

Primary aldosteronism is defined as inappropriately high and autonomous aldosterone secretion, which is not suppressible by sodium loading. Among PA, diagnosing an APA is of clinical interest as it is a curable cause of hypertension in about half of the patients [26], adrenalectomy has become simpler by coelioscopy and APAs are associated with high cardiovascular morbidity and renal impairment [27]. The ARR, which reflects aldosterone hypersecretion in relation with renin, is currently considered to be the most reliable tool for PA screening. The aim of our study was to propose a reliable ARR threshold that is usable as a first step in the diagnosis of an APA.

The ARR first proposed by Dunn and Espiner [16] in 1976 proved to be the best for differentiating PA from essential hypertension. There is marked heterogeneity in the conditions of ARR determination and therefore in proposed ARR cut-off values [17,18,22,23,28]. However, although performed in different conditions in a multicentre study (PAPY study), ARR determinations showed satisfactory within-patient reproducibility [29]. The population in which the ARR cut-off value is determined is of major importance. Our study population is in accordance with current recommendations [30]. In our study, the percentage of patients with an APA was very similar to that reported in the literature. As recommended by Plouin et al. [20], minimum values for renin and/or aldosterone must be determined to avoid classifying low-renin hypertension as PA. In our study, the highest area under the ROC curve for ARR was obtained with a minimal value of active renin set at 5 ng/L. To set a minimal renin concentration at 5 ng/L is equivalent to setting a minimal aldosterone concentration at 160 ng/L.

There are considerable differences in definitions of elevated ARR, with most ARR cut-off values set as the mean + 2 standard deviations of values obtained in patients with essential hypertension. A methodology well suited to defining an ARR cut-off is ROC analysis. In our study, diagnosis of APA was based on the association of biological PA, an adrenal adenoma on CT-scan and a histological proven adrenal adenoma in operated patients. The proportion of operated patients was similar to that usually reported [18]. In the 12 patients who underwent surgery, histological analysis always confirmed the APA. For the other cases, spironolactone was always efficient in normalizing (n = 9) or reducing (n = 4) BP.

In our study, an ARR determination was performed at rest and during an orthostatic stimulation test, as recommended when our study was implemented. In 2006, there was no clear consensus on the diagnostic usefulness of confirmatory tests (sodium loading test, fludrocortisone suppression test, captoril challenge test) [4]. Considering the further published recommendations [30], the lack of confirmatory tests should be taken into account when interpreting our study.

The population in which the ROC analysis is applied is also of major importance. To our knowledge, ROC curve analysis has been used mainly in case-control studies. Such a selection of subjects artificially increases the number of APAs in the tested population and the definition of controls is also somewhat arbitrary. Sensitivity and specificity could be artificially increased. Thus, a small bias in selecting the control population may result in great variations in cut-off values, which are based on sensitivity and specificity. Furthermore, positive and negative predictive values cannot be calculated accurately in case-control studies. To avoid such sampling bias, we implemented prospective registers in 17 secondary or tertiary hypertension centres. Adherence of centres to the register was voluntary; this can be regarded as a limitation of our study. On the other hand, the centres were scattered all over France and can be considered as representative. To avoid selection bias, investigators were instructed to register consecutive case reports from October 2006 to June 2007. We checked the possible centre effect by analysis of variance (Analysis of variance). The main characteristics of our included patients were similar to those usually reported in the literature [18].

Table 2. Diagnostic power of the different biological tests determined in supine position.

<table>
<thead>
<tr>
<th></th>
<th>Renin</th>
<th>Aldosterone</th>
<th>ARR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut-off values (ng/L)</td>
<td>4.0</td>
<td>167</td>
<td>32</td>
</tr>
<tr>
<td>AUC</td>
<td>0.72 (0.65–0.79)</td>
<td>0.92 (0.90–0.97)</td>
<td>0.93 (0.89–0.96)</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>83 (52–98)</td>
<td>92 (61–100)</td>
<td>92 (63–100)</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>54 (46–62)</td>
<td>87 (81–92)</td>
<td>92 (87–96)</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>11 (6–20)</td>
<td>33 (18–52)</td>
<td>46 (25–68)</td>
</tr>
<tr>
<td>PNV (%)</td>
<td>97 (92–100)</td>
<td>99 (96–100)</td>
<td>99 (97–100)</td>
</tr>
</tbody>
</table>

Data are mean (95% confidence interval) unless otherwise indicated. ARR: aldosterone-to-renin ratio (with a minimum renin value set at 5 ng/L); AUC: area under the receiver operating characteristic curve; PPV: positive predictive value; PNV: negative predictive value.
The main limitation in our ‘real-life multicentre study’ is that biological variables were determined in each centre and thus were not standardized. However, when a central laboratory is used, results are valid only if performed in that laboratory. Our approach was more pragmatic and thus the results, which may be considered as less accurate, can be applied to different centres.

AVS is the most accurate means of differentiating between uni- and bilateral forms of PA. However, it remains difficult and should be undertaken only by trained physicians. AVS can lead to false-negative results, especially for right APA [31]. Thus, AVS that was performed only in six operated patients over 12 was not taken into account when analysing our results. However, the percentage of PA patients systematically submitted to AVS ranged from 19% to 100% in referral centres for endocrine hypertension worldwide [32]. Even in specialized centres, success rates for AVS were poor [33]. However, AVS remains recommended before surgery [30]. In our study, the absence of AVS should not have interfered with our results as only patients with histologically documented adenoma were used as references in the ROC analysis. Usually, surgery efficiency is confirmed by BP and kalaemia determinations. A postsurgery measure of plasma aldosterone was not performed in our pragmatic clinical study.

One criterion for deciding the cut-off value of a test is to maximize the sum of sensitivity and specificity values (Youden coefficient). According to this strategy, the proposed ARR cut-off value is 32 ng/ng. However, a single determination of ARR did not provide sufficient sensitivity for clinical practice. A second value of ARR ≥ 32 ng/ng after at least 1-hour standing increased the sensitivity to 100%, together with a satisfactory specificity of 72%. However, CT-scans are not sensitive enough to reveal all the unilateral APAs. Thus, our ARR cut-off value can only be considered for screening for APAs that can be visualized by CT-scan. What to do if an ARR is > 32 ng/ng without any CT-scan adrenal abnormality remains to be explored. Our proposed ARR cut-off value of ≥ 32 ng/ng is not far from the one determined in the PAPY study (27.3 ng/mU corresponding to 38 ng/ng) [25].

Conclusion

In conclusion, our proposed pragmatic approach, based on an ARR cut-off value ≥ 32 ng/ng in one of two determinations, with a minimal active renin value set at 5 ng/L (or minimal aldosterone at 160 ng/L), diagnosed APA with very good sensitivity and satisfactory specificity. Although determined in a prospective multicentre study, this cut-off value must be confirmed.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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