Detection of Primary Aldosteronism by the 6-Hour Integrated Aldosterone/Renin Ratio

Zvi Zadik, Philip A. Levin, Bruce P. Hamilton, and A. Avinoam Kowarski

SUMMARY An outpatient diagnostic procedure measuring the 6-hour integrated plasma concentration of aldosterone and plasma renin activity was used to detect primary aldosteronism in 12 patients with low renin hypertension, including six with mild hypertension and normal urinary excretion and spot plasma levels of aldosterone. The ratio of integrated plasma concentration of aldosterone to plasma renin activity in the 12 patients (mean, 339; range, 116–700; p < 0.0001) did not overlap with that measured in 105 normotensive controls (mean, 27.8; range, 5–97) or in 87 subjects with essential hypertension (mean, 29.2; range, 4–67). Eight patients had surgically proven adenomas (3 of which measured <5 mm) with normalization of blood pressure following adrenalectomy. The four remaining patients had bilateral hyperplasia. The 6-hour integrated plasma concentration of aldosterone to plasma renin activity ratio was found to be a useful new outpatient diagnostic tool for evaluation of primary hyperaldosteronism. (Hypertension 8: 285–289, 1986)

KEY WORDS • hyperaldosteronism • hypertension • aldosterone/renin ratio

Although primary hyperaldosteronism is an infrequent cause of hypertension, early diagnosis and detection is important since it can be treated satisfactorily by surgical excision of a unilateral adenoma or by administration of spironolactone. Unfortunately, the disorder is often overlooked, especially in its early stages, because of the insidious nature of the symptoms. A normal serum potassium concentration is not always an adequate criterion for excluding primary aldosteronism, since potassium levels above 3.6 mEq/L were found in 22% of surgically proven aldosterone (ALDO)-secreting adrenal adenomas. As recently reviewed, some studies have reported normal plasma renin activity (PRA) and a normal ALDO urinary excretion rate in numerous patients with primary aldosteronism.

Since the biological activity of ALDO is directly related to its plasma concentration, measuring the plasma level of ALDO and PRA should be helpful in the diagnostic workup of primary aldosteronism. Unfortunately, these determinations in single blood samples are of little practical use because of the wide range found in normal subjects, which overlap with the concentrations in patients suffering from distinct endocrine disease. The variability of the concentrations of ALDO and PRA in plasma throughout the day is attributed to numerous factors, such as time of day and body position. Consequently, marked increases or decreases in the plasma levels of ALDO and PRA may remain unrecognized when only single, discrete samples are assayed.

We have developed a method for measuring the integrated mean 6-hour concentration of blood components by using a small, portable pump that draws blood at a constant rate through a nonthrombogenic intravenous catheter. The integrated concentration (IC) of various hormones was obtained in this manner and caused minimal interference with normal activity. Simultaneous determination of the IC of ALDO and IC of PRA rather than measurement of the concentration in a single blood specimen drawn at a single point in

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time decreases the variability caused by fluctuation, thus improving the diagnostic usefulness of the determination.\textsuperscript{9, 12, 13} We have previously reported the integrated concentrations of ALDO, PRA, cortisol, epinephrine, and norepinephrine in normal and hypertensive patients as well as the ratio between IC of ALDO and IC of PRA in normal subjects and in patients with mild essential hypertension.\textsuperscript{9-11}

With the use of this method in the present study, we detected primary hyperaldosteronism in 12 patients, including six with normal spot plasma ALDO levels and urinary ALDO excretion.

**Subjects and Methods**

The study group comprised 105 normotensive control subjects, 87 control subjects with mild essential hypertension who had no detectable or known endocrine abnormalities by standard endocrine evaluation and workup (including renin stimulation and 24-hour urine ALDO determination), and 12 patients with surgically proven ALDO-secreting adrenal adenoma or bilateral hyperplasia. These 12 patients had been selected for evaluation because of initial hypertension and hypokalemia or hypertension with normal baseline potassium levels that decreased markedly with diuretic therapy. Their initial evaluation included renin stimulation testing and 24-hour urine ALDO excretion or ALDO suppression testing (or both), which in some but not all patients suggested the diagnosis of primary hyperaldosteronism. Diagnosis of bilateral hyperplasia in four patients was based on a clinical evaluation that included failure of adrenal computed tomographic scan to demonstrate an adenoma in all patients and an increase in plasma ALDO level on upright posture or a bilateral increase in plasma ALDO level on adrenal vein catheterization.

Subjects with essential hypertension had been treated with diuretics, \(\beta\)-blockers, clonidine, or prazosin singularly or in combination. They had no evidence of congestive heart failure or marked renal disease at time of study. All participants were volunteers who signed a consent form approved by the University of Maryland and Johns Hopkins Committees on Human Investigation. The integrated hormone study was performed in addition to the traditional diagnostic workup establishing the diagnosis of the 12 patients with ALDO-secreting adenoma or bilateral hyperplasia. In four of the patients with ALDO-secreting adenoma (Patients 3–6), the diagnosis was supported by a high rate of ALDO excretion. In the remaining four patients with ALDO-secreting adenoma, the diagnosis of primary hyperaldosteronism was held in doubt because of a normal urinary excretion of ALDO and a borderline high IC of ALDO.

Patients and control subjects were admitted for 24 hours to the Clinical Research Unit (Johns Hopkins) or Endocrine Diagnostic Unit (University of Maryland). Activity and food intake were not restricted. Diet included ad libitum salt intake for the week before admission; all evaluated subjects had sodium intake exceeding 100 mEq/24 hours on the day of study. Hypertensive subjects had not received any medication, including diuretics, for at least 1 week before the study began. Potassium-sparing antihypertensive therapy had been discontinued at least 3 weeks before the study began. No oral potassium supplements had been given for 5 days before the study began.

Blood was collected at a constant rate for 6 hours beginning at 0900, as previously described.\textsuperscript{9-11} A portable peristaltic pump and nonthrombogenic intravenous catheter were used. In Patient 2 the blood was collected by intermittent sampling. The blood was delivered into ethylenediaminetetraacetic acid–containing Vacutainers that were replaced at 30-minute intervals. The plasma was separated and frozen at the end of each 30-minute collection period, the samples were pooled, and a single sample for assay of IC of ALDO and PRA was obtained. As previously reported, ALDO and PRA remain stable in the blood withdrawn during the continuous withdrawal period.\textsuperscript{11} All urine excreted during a 24-hour period was collected at the Clinical Research Center so that full collections could be obtained. This was confirmed by measurement of urine creatinine, or collection was repeated. The 24-hour excretion of creatinine, ALDO, sodium, and potassium were measured. At the end of the 24-hour urine collection period, the subjects were given furosemide (1 mg/kg i.v., up to 80 mg) and remained upright for 4 hours. Blood was taken for measurement of PRA at the beginning while the subject was supine and at the end of this 4-hour period while the subject was standing.

The PRA and plasma and urinary ALDO levels were determined in our laboratories by previously described methods.\textsuperscript{14-16} Data are presented as means ± SD. Statistical analyses were performed using Student’s unpaired \(t\) test to compare the different study groups.

**Results**

Table 1 summarizes the results of our endocrine evaluation of the 12 patients, in eight of whom the diagnosis of primary aldosteronism was confirmed by operation. In six patients the urinary ALDO excretion rates and the discrete plasma ALDO levels were within the normal range.

The IC of ALDO, IC of PRA, and the IC ALDO/IC PRA ratio in the 12 patients, 105 normotensive control subjects, and 87 essential hypertensive controls are depicted in Figure 1. The IC of ALDO was distinctly above the normal range in seven patients, while it was only slightly elevated or within the normal range in the remaining five patients.

The PRA did not respond to furosemide stimulation in most patients with primary aldosteronism and remained equally low in many of the subjects with essential hypertension. Of 43 hypertensive control subjects tested, 26 responded to furosemide stimulation with increased PRA above 1.0 ng of angiotensin I per milliliter per hour while 17 did not respond to furosemide stimulation. Table 2 summarizes and compares the endocrine workup results in the three groups. No significant difference was noted between the values in the
TABLE 1. Clinical and Diagnostic Data for 12 Patients with Primary Hyperaldosteronism

<table>
<thead>
<tr>
<th>Patient no., sex, age (yr)</th>
<th>BP (mm Hg)</th>
<th>Na⁺ (mEq/L)</th>
<th>K⁺ (mEq/L)</th>
<th>PRA* (ng ANG I/ml/hr)</th>
<th>ALDO (µg 24 hr)</th>
<th>CT scan</th>
<th>IC ALDO (ng/dl)</th>
<th>IC PRA (ng ANG I/ml/hr)</th>
<th>IC ALDO/IC PRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal adenoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, M, 30</td>
<td>180/135</td>
<td>140</td>
<td>2.4</td>
<td>0.1</td>
<td>8.0</td>
<td>177</td>
<td>—</td>
<td>23.4</td>
<td>0.1</td>
</tr>
<tr>
<td>2, M, 66</td>
<td>185/100</td>
<td>136</td>
<td>3.7</td>
<td>0.1</td>
<td>8.0</td>
<td>138</td>
<td>—</td>
<td>23.0</td>
<td>0.1</td>
</tr>
<tr>
<td>3, F, 36</td>
<td>150/105</td>
<td>141</td>
<td>3.1</td>
<td>0.1</td>
<td>31.8</td>
<td>160</td>
<td>+</td>
<td>50.8</td>
<td>0.1</td>
</tr>
<tr>
<td>4, F, 33</td>
<td>140/110</td>
<td>138</td>
<td>4.4</td>
<td>0.1</td>
<td>101</td>
<td>112</td>
<td>+</td>
<td>72.4</td>
<td>0.2</td>
</tr>
<tr>
<td>5, F, 44</td>
<td>190/120</td>
<td>139</td>
<td>2.8</td>
<td>—</td>
<td>53.0</td>
<td>216</td>
<td>+</td>
<td>41.5</td>
<td>0.2</td>
</tr>
<tr>
<td>6, F, 49</td>
<td>190/140</td>
<td>144</td>
<td>3.7</td>
<td>0.2</td>
<td>59.0</td>
<td>305</td>
<td>+</td>
<td>49.0</td>
<td>0.07</td>
</tr>
<tr>
<td>7, M, 51</td>
<td>184/110</td>
<td>143</td>
<td>2.8</td>
<td>0.22</td>
<td>5.0</td>
<td>—</td>
<td>+</td>
<td>58.4</td>
<td>0.2</td>
</tr>
<tr>
<td>8, M, 46</td>
<td>150/104</td>
<td>143</td>
<td>3.9</td>
<td>0.1</td>
<td>12.3</td>
<td>128</td>
<td>—</td>
<td>23.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Bilateral hyperplasia</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>9,‡ M, 51</td>
<td>195/110</td>
<td>145</td>
<td>3.5</td>
<td>0.1</td>
<td>6</td>
<td>223</td>
<td>—</td>
<td>23.0</td>
<td>0.2</td>
</tr>
<tr>
<td>10, F, 46</td>
<td>140/100</td>
<td>143</td>
<td>2.9</td>
<td>0.1</td>
<td>5.5</td>
<td>150</td>
<td>—</td>
<td>12.4</td>
<td>0.1</td>
</tr>
<tr>
<td>11,‡ F, 50</td>
<td>160/100</td>
<td>140</td>
<td>2.8</td>
<td>1.2</td>
<td>—</td>
<td>166</td>
<td>+</td>
<td>31.5</td>
<td>0.07</td>
</tr>
<tr>
<td>12, M, 64</td>
<td>160/100</td>
<td>145</td>
<td>3.3</td>
<td>0.16</td>
<td>35</td>
<td>316</td>
<td>—</td>
<td>31.4</td>
<td>0.05</td>
</tr>
</tbody>
</table>

BP = blood pressure; PRA = plasma renin activity; ALDO = aldosterone; CT = computed tomographic; IC = integrated concentration; ANG I = angiotensin I; N = normal.

*Measured after furosemide (1 mg/kg i.v., up to 80 mg) was administered.
‡Adrenal vein aldosterone levels bilaterally increased.

87 hypertensive controls and those in the 105 normotensive control subjects. The IC ALDO/IC PRA ratio in all 12 patients (mean, 339.4), an indicator of independent primary secretion of ALDO,⁹,¹⁰ was more than 8 SD above the mean that was found in the 105 normal subjects (mean, 27.8; p < 0.001) and in the 87 essential hypertensive controls (mean, 29.2; p < 0.001).

Discussion

As has been recently emphasized the clinical spectrum of hyperaldosteronism is wider than previously appreciated.¹⁷,¹⁸ Primary hyperaldosteronism, especially in patients with very small adenomas or mild bilateral hyperplasia,¹⁹ may present a difficult diagnostic problem. Traditional diagnostic studies for primary hyperaldosteronism under varying conditions of testing are prone to false-negative results.¹⁷,¹⁸ Bravo et al.¹⁷ evaluated the limitations of several standard screening tests for hyperaldosteronism: the PRA stimulation test, the ALDO suppression test, and the presence of hypokalemia, all of which failed to detect more than 20% of patients with hyperaldosteronism. Bravo et al.¹⁷ found that measurement of 24-hour urinary ALDO excretion was most useful, but as several studies have documented, there are patients with mild primary hyperaldosteronism who may not have an excessive urinary excretion of ALDO.⁴⁻⁶ Furthermore, individual variation in metabolic clearance rate of ALDO or its conversion to 18-glucuronide,¹² 20, 21 as well as collection inaccuracies of a 24-hour sample, leads to further limitations of the diagnostic value of the urinary excretion test.

We have found that single basal plasma samples of both ALDO and renin show much greater variance than do the 6-hour IC of ALDO and IC of PRA determinations performed on the same patients. In a group

FIGURE 1. The 6-hour integrated concentration (IC) of plasma aldosterone, IC of plasma renin activity, and the ratio of IC of aldosterone to IC of plasma renin activity in 12 patients with primary aldosteronism (1° ALDO) is compared with corresponding parameters in 87 age-matched adults with hypertension (black dots). Shaded area depicts the range found in 105 normotensive age-matched adults.
of 78 hypertensive and control subjects, the variance in a single plasma sample of ALDO was 2.7 times as great as that found with integrated sampling \((p < 0.001)\) in the same patient and renin variance was 3.2 times that found with IC sampling \((p < 0.01)\). Furthermore, recent studies by Bravo et al.\(^7\) in 70 patients with primary hyperaldosteronism support the conclusion that the variance of IC of ALDO and IC of PRA is significantly lower than the variances seen in ALDO and renin levels in single, discrete blood samples, regardless of whether they are compared in normotensive subjects or subjects with essential hypertension or hyperaldosteronism. Bravo et al.\(^7\) found that single ALDO samples had a variance of 81\% in 70 patients with primary hyperaldosteronism, while the present study found 48\% variance for IC of ALDO in our patients with hyperaldosteronism. Furthermore, single renin samples had a variance of 140\% in patients with primary hyperaldosteronism,\(^17\) while the present study found a 60\% variance for IC of PRA in patients with hyperaldosteronism.

When Hiramatsu et al.\(^22\) used discrete plasma ALDO/PRA ratios in screening for hyperaldosteronism, the results were effective but did not give the same degree of separation of normal subjects from those with primary aldosteronism as did the IC ALDO/IC PRA ratios used in our study. Use of discrete plasma ALDO to PRA ratio was associated with overlap of three of nine patients with hyperaldosteronism within the upper normal range.\(^22\) In contrast, the smaller variance in IC ALDO/IC PRA ratios in the present study allowed for complete separation of subjects with primary hyperaldosteronism from the control subjects. The 6-hour IC of ALDO also shows significantly less variability when compared with that seen for 24-hour urinary ALDO excretion. In a study of 91 patients, the urinary ALDO excretion had 2.5 times the variance of the IC of ALDO \((p < 0.01)\).\(^23\)

We have not directly compared the saline suppression test with the IC ALDO/IC PRA ratios in all of our patients. A recent study has shown that the saline suppression test has a marked incidence of falsely normal suppression in some patients with hyperaldosteronism, especially in those with bilateral hyperplasia.\(^24\) In one of our patients with a normal saline suppression test result, the IC ALDO/IC PRA ratio was elevated and a small aldosteronoma was demonstrated. Three others had abnormal saline suppression test results and elevated IC ALDO/IC PRA ratios.

The discriminatory power of the IC method in patients with mild primary aldosteronism was demonstrated in Patients 2, 7, 8, 9, and 10. In these patients, 24-hour urinary ALDO excretion was within the normal range despite elevated IC of ALDO and IC ALDO/IC PRA ratios. In the four patients with normal urinary ALDO levels who were operated on, return of the blood pressure to normal range and the suppressed renin activity to normal levels subsequent to the removal of an adrenal adenoma is an indication of the primary nature of their hyperaldosteronism. The adenomas were small in three of these four patients, but the return of the IC of PRA and the blood pressure to normal is strong evidence that even those small adenomas were the source of a PRA-independent secretion of ALDO. Blood pressure in the two patients with hyperplasia and normal 24-hour urinary ALDO excretion returned to normal when aldactone therapy was instituted.

We have previously reported on the 24-hour IC ALDO/IC PRA ratio in a small number of hypertensive patients and normotensive control subjects.\(^11\) Since a 24-hour collection of blood cannot be used in an outpatient endocrine clinic, we have shortened the procedure to 6 hours. As noted by Carey,\(^25\) a recent study reported that, unlike the PRA or ALDO concentration, the ALDO/PRA ratio was much less influenced by variations in sodium intake or diuretic therapy.\(^22\) That study found single plasma ALDO and renin concentrations increased by a similar degree after diuretic administration so that the ALDO/PRA ratio in their patients remained unchanged in diagnostic category.\(^22\) In our study, one patient (Patient 11) demonstrated a reactive spot PRA following furosemide administration but still had a low 6-hour IC of PRA and an elevated IC ALDO/IC PRA ratio.

Hypokalemia may have played a role in four of our five patients with primary aldosteronism who had normal urinary excretion of ALDO. Hypokalemia will

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**Table 2. Comparison of 12 Patients with Primary Hyperaldosteronism, 105 Normotensive Subjects, and 87 Subjects with Essential Hypertension**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Primary hyperaldosteronism</th>
<th>Normotension</th>
<th>Essential hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC ALDO (ng/dl)</td>
<td>36.4 ± 17.8 (12.4–72.4)</td>
<td>11.5 ± 5.2 (4.8–25)</td>
<td>9.9 ± 4.5 (3.1–27)</td>
</tr>
<tr>
<td>IC PRA (ng ANG I/ml/hr)</td>
<td>0.1 ± 0.06 (0.05–0.2)</td>
<td>0.6 ± 0.5 (0.1–2.1)</td>
<td>0.4 ± 0.3 (0.1–1.5)</td>
</tr>
<tr>
<td>IC ALDO/IC PRA</td>
<td>339.4 ± 191.6 (116–700)*</td>
<td>27.8 ± 21.8 (2.6–97)*</td>
<td>29.2 ± 19.5 (9.2–90)*</td>
</tr>
<tr>
<td>Post-furosemide PRA (ng ANG I/ml/hr)</td>
<td>0.2 ± 0.3 (0.1–1.2)</td>
<td>5.0 ± 3.2 (1.6–12.5)</td>
<td>4.5 ± 1.5 (0.1–34)</td>
</tr>
<tr>
<td>Urinary ALDO excretion (µg/24 hr)</td>
<td>29.7 ± 31.2 (5–101)</td>
<td>7.6 ± 7.2 (0.6–27)</td>
<td>5.4 ± 5.9 (0.6–29)</td>
</tr>
</tbody>
</table>

Values are means ± SD; range is given in parentheses.

See Table 1 for key to abbreviations.*\(p < 0.001\), compared with values in control groups.
reduce plasma ALDO concentration and urinary ALDO excretion rates in patients with primary aldosteronism. Low ALDO parameters may rise to appropriate diagnostic levels following correction of hypokalemia with potassium supplements; however, it would be advantageous for a diagnostic method of primary hyperaldosteronism to be less influenced by the presence of hypokalemia. In the seven patients with potassium levels below 3.5 mEq/L, a diagnosis of primary aldosteronism was made on the basis of the IC ALDO/IC PRA ratio despite the presence of hypokalemia and, often, normal urinary ALDO excretion.

Taken together, these findings suggest that stringent preparation protocols may be less necessary when the IC ALDO/IC PRA ratio is used to diagnose hyperaldosteronism. This advantage increases the ease of using this method in clinical endocrine unit referral settings. The shortened 6-hour procedure has proved to be practical and convenient to patients and is now used in the diagnosis of primary aldosteronism by three major referral hospitals in Baltimore.

The present study also examined the 6-hour IC ALDO/IC PRA ratio in 87 patients with essential hypertension. None of these patients had ratios exceeding 100, which was the maximal ratio found in the presently reported enlarged group of 105 normotensive control subjects. Our results suggest that the 6-hour ratio of IC of ALDO to IC of PRA in essential hypertension is below 100 even in patients with low and unresponsive PRA. The ratio method distinguished patients with mild primary aldosteronism from patients with low renin essential hypertension.

Our results indicate that the 6-hour IC ALDO/IC PRA ratio is an effective outpatient method for the diagnosis of primary aldosteronism. It may be particularly useful when the results of the 24-hour urinary ALDO test, ALDO suppression test, or renin stimulation study are equivocal.

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