CONFIRMATORY TESTING IN NORMOKALAEMIC PRIMARY ALDOSTERONISM: THE VALUE OF THE SALINE INFUSION TEST AND URINARY ALDOSTERONE METABOLITES

Caroline Schirpenbach¹,³, Lysann Seiler¹, Christiane Maser-Gluth⁴, Frank Rüdiger², Christian Nickel², Felix Beuschlein¹ and Martin Reincke³

¹Medical Department II and ²Medical Department IV, Albert-Ludwigs-University, Freiburg, Germany, ³Medizinische Klinik – Innenstadt Klinikum der Ludwig-Maximilians-University, München Ziemssenstr. 1, D-80336 Munich, Germany and ⁴Institute of Pharmacology, Ruprecht-Karls-University, Heidelberg, Germany

(Correspondence should be addressed to M Reincke; Email: martin.reincke@med.uni-muenchen.de)

Abstract

Objective: Primary aldosteronism has recently been recognized as the most frequent cause of secondary hypertension. Since most patients are normokalaemic, differentiation to essential hypertension is challenging. As differentiation by baseline aldosterone/renin ratio may be insufficient, diagnosis should be confirmed by additional tests. However, as most confirmatory tests have been evaluated in hypokalaemic primary aldosteronism only, we reassessed the value of the saline infusion test and 24 h urinary aldosterone metabolites as confirmatory tests for both normo- and hypokalaemic primary aldosteronism under current antihypertensive medication.

Patients and methods: 25 patients with primary aldosteronism (11 hypokalaemic, 14 normokalaemic), 29 patients with essential hypertension and 47 normotensive subjects were studied. The hypertensives received their usual medication with the exception of spironolactone. All subjects underwent a standard saline infusion test (determination of plasma aldosterone before and after 2.0 liters of isotonic saline for 4 hours i.v.) and collected a 24 h urine sample for examination of urinary tetrahydroaldosterone and aldosterone-18-glucuronide.

Results: In hypokalaemic primary aldosteronism the saline infusion test showed a reasonable sensitivity (91%) and specificity (90%). However, the test failed to differentiate sufficiently between essential hypertension and normokalaemic primary aldosteronism (sensitivity 57%, specificity 90%). Similarly, urinary tetrahydroaldosterone had higher sensitivity in hypokalaemic than in normokalaemic primary aldosteronism (sensitivity 64% vs 36%, specificity 100%), whereas for aldosterone-18-glucuronide, no differences in hypo- and normokalaemic primary aldosteronism were found (sensitivity 45% and 43%, specificity 100%).

Conclusions: These data show that the saline infusion test as an established test in classical hypokalaemic primary aldosteronism is not a reliable test in the normokalaemic variant of the disease. Due to its low accuracy, determination of urinary aldosterone metabolites did not prove useful in confirming either normo- or hypokalaemic patients. We conclude from our data that these tests should not be used as confirmatory testing in the normokalaemic variant of primary aldosteronism.

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Introduction

Primary aldosteronism has recently been recognized as the most frequent cause of secondary hypertension, occurring with a prevalence of 5–18% within the hypertensive population (1–4). Being a potentially curable disease, correct diagnosis is crucial for specific treatment, such as mineralocorticoid antagonist therapy and adrenalectomy (3). Only 10–30% of patients present with the classical symptoms characterized by hypertension and hypokalaemia, whereas the large majority presents with normal serum potassium, thus showing a milder variant of this disorder (4–6).

The aldosterone/renin ratio is currently the most recommended screening test for primary aldosteronism (3, 6–10). Application of the ratio even under antihypertensive medication is increasingly advocated (8, 11, 12). A positive test result requires confirmation by functional testing (13), as the specificity of the aldosterone/renin ratio is low (14, 15). Various confirmatory tests have been proposed, but an ideal test which is simultaneously simple to perform, sensitive and specific is currently lacking. A common approach to confirming the diagnosis of primary aldosteronism is to demonstrate the insufficient suppression of aldosterone after oral sodium loading, acute saline infusion, and
administration of captopril or fludrocortisone (3, 6, 13, 16). The fludrocortisone suppression test is regarded by some investigators as the definitive confirmatory test for primary aldosteronism (3, 17), but the need for hospital admission due to an associated risk of severe hypokalaemia requiring 6 hourly potassium controls limits its usefulness in practice. The saline infusion test, however, can be performed on an outpatient basis. Based on the principle of a decrease in aldosterone in response to saline-induced renin suppression, the saline infusion test enables a distinction to be made between those patients with essential hypertension and patients with primary aldosteronism who fail to suppress aldosterone sufficiently (18–21). However, all previous studies on the saline infusion test were performed in patients with hypokalaemic primary aldosteronism, whereas the only study investigating both normo- and hypokalaemic patients, based on a previously published aldosterone cut-off value, resulted in the ascription of low sensitivity to the saline infusion test (3).

Another approach in diagnosing primary aldosteronism is to measure aldosterone metabolites in a 24 h urine sample. This is thought to have the advantage of being independent of circadian variations in plasma levels (22, 23). Aldosterone is excreted mainly as tetrahydroaldosterone after metabolism in the liver, and as aldosterone-18-glucuronide produced mainly in the kidney (24).

Appropriate validation of the saline infusion test and urinary aldosterone metabolites as confirmatory tests for normokalaemic primary aldosteronism has not been demonstrated so far. This study, therefore, was designed to reassess the value of the acute saline infusion test and of urinary aldosterone metabolites in patients with confirmed primary aldosteronism receiving their usual hypertensive medication by comparing them with results obtained in normotensive subjects and patients with essential hypertension.

Subjects and methods

Subjects

Twenty-five patients with confirmed primary aldosteronism (mean age ± S.D. 54.8 ± 10.3 years; 10 females) and 29 patients with essential hypertension (51.1 ± 12.2 years; 16 females) referred to our institution over a 15 month period and 47 normotensive subjects (24.1 ± 10.3 years; 16 females) referred to our institution over a 15 month period and 47 normotensive subjects (24.1 ± 10.3 years; 16 females) were enrolled in a prospective controlled trial. Twelve of the twenty-five patients with primary aldosteronism were diagnosed with bilateral adrenal hyperplasia and four had an aldosterone-producing adenoma. In the remainder, subtype evaluation had not been completed at the end of this study.

With the exception of spironolactone, which was withdrawn at least 6 weeks prior to testing, patients took their regular antihypertensive medication while being studied. Sodium intake was unrestricted. Table 1 shows the clinical data of the patients.

The study protocol was approved by the ethics committee of the University of Freiburg, according to the requirements of the Helsinki Declaration, and informed written consent was obtained from all subjects.

Diagnostic criteria

All 47 normotensive subjects had a blood pressure below 140/90 mmHg, no history of hypertension or renal disease, and did not take contraceptives.

Criteria for essential hypertension were blood pressure above 140/90 mmHg, exclusion of secondary forms of hypertension, normal aldosterone/renin ratios (cut-off values according to the mean ± 2 S.D. of normotensive subjects: aldosterone/renin concentration < 21 pg/ml:µU/ml, aldosterone/renin activity < 670 pg/ml:ng/ml/h), normal serum potassium and normal urinary aldosterone excretion (< 15 µg/24 h (41.6 nmol/day)).

The diagnosis of primary aldosteronism was based on biochemical criteria including repeatedly elevated aldosterone/renin ratios (aldosterone/renin concentration > 21 pg/ml:µU/ml), elevated urinary aldosterone excretion (> 15 µg/day), a prior pathological saline infusion test (serum aldosterone at 240 min > 80 pg/ml), adrenal venous sampling (n = 15) and surgery, as described elsewhere (6, 15). Diagnosis of the underlying subtype required unequivocal results of posture testing and computed tomography/magnetic resonance imaging (CT/MRI) or a gradient of the aldosterone/cortisol ratio during adrenal venous sampling. The patients were divided according to serum potassium status: patients with previous documented hypokalaemia (< 3.4 mmol/l) were assigned to the hypokalaemic group (n = 11), and potassium supplementation was started which normalized potassium in all but three subjects. The remainder with normal potassium were assigned to the normokalaemic group (n = 14).

Saline infusion test and 24 h urine excretion

All subjects underwent the acute saline infusion test, which was performed by administration of 2000 ml of 0.9% saline i.v. over 4 h, beginning between 8000 and 9030 h. Subjects remained recumbent during infusion. Before infusion and after 4 h, blood was drawn from a forearm vein for the measurement of plasma aldosterone, plasma renin concentration and plasma renin activity. Blood for measurement of renin activity was kept in crushed ice until centrifuged.

All subjects collected a 24 h urine sample, which was kept refrigerated until analysis. The urine was collected either the day before or at least 2 days after saline...
infusion testing. All plasma and urine samples were frozen at −20°C until measured.

**Assays**

Plasma aldosterone as well as urinary aldosterone metabolites were determined in the Steroid Laboratory of the University of Heidelberg by radioimmunoassay after extraction and chromatography, as described previously (25, 26). Plasma renin concentration was measured on a fully automated immuno-chemiluminescence analyzer (DirectRenin, Nichols Institute Diagnostics, USA). Measurement of plasma renin activity was performed using an angiotensin-I-radioimmunoassay (RENCTK, DiaSorin, Italy). The intra- and inter-assay coefficients of variations were <10% and <15%, respectively.

**Statistical analysis**

Results are expressed as mean ± S.D. or S.E.M., as appropriate. Data were compared through use of the unpaired Wilcoxon test, with P < 0.05 being considered statistically significant. Sensitivity and specificity were calculated using receiver-operating characteristic (ROC) curves. Statistical analysis of the data was performed using Microcal Origin 6.0 (Microcal Origin, Northampton, MA, USA) and SAS 6.12 (SAS, North Carolina, MA, USA).

**Results**

**Saline infusion test**

Figure 1 shows the baseline hormone characteristics of the patients. Whereas baseline aldosterone concentrations were similar between normal subjects and patients with essential hypertension, baseline renin levels were higher in patients with essential hypertension.

During saline infusion a decrease of mean plasma renin concentration and renin activity was observed in normal controls and patients with essential hypertension (Fig. 1 and Table 2), with the latter still having higher renin levels than the controls. In both groups, the decrease in renin was paralleled by a decrease in mean plasma aldosterone concentrations. In comparison, mean renin levels remained suppressed during saline infusion in primary aldosteronism, whereas aldosterone concentrations decreased after 4 h. In primary aldosteronism, mean aldosterone concentration after saline infusion was significantly higher (P < 0.0001) than in the other groups (mean ± S.D.: primary aldosteronism 14.2 ± 15.0 ng/dl (394 ± 416 pmol/l), essential hypertension 2.1 ± 1.9 ng/dl (58 ± 53 pmol/l), normal controls 2.3 ± 1.8 ng/dl (64 ± 50 pmol/l)) (Fig. 2). Calculation of ROC curves
revealed a sensitivity and specificity of 68% and 90%, respectively, for differentiation between primary aldosteronism and essential hypertension (cut-off value 5.1 ng/dl (141 pmol/l)) (Table 3). However, the response of aldosterone differed highly between normo- and hypokalaemic primary aldosteronism (Fig. 2). Thus, when comparing essential hypertension and hypokalaemic primary aldosteronism, aldosterone after saline infusion had an acceptable specificity (90%) and sensitivity (91%), whereas the latter was considerably lower in normokalaemic patients (57% sensitivity, 90% specificity). Analysis of renin and aldosterone levels in the two subgroups of primary aldosteronism showed that aldosterone excess seems to be less severe in normokalaemic patients compared to hypokalaemic patients (Table 2). For example, although there were no marked differences between normo- and hypokalaemic patients, plasma renin activity tended to be higher in normokalaemic patients before and after saline infusion than in hypokalaemic patients. Figure 3 shows individual aldosterone curves of patients with essential hypertension and primary aldosteronism, demonstrating that, in general, aldosterone secretion does not appropriately respond to saline infusion in the majority of patients with primary aldosteronism.
Blood pressure medication did not have a significant influence on aldosterone concentrations after saline infusion in essential hypertension: aldosterone concentration in patients receiving 12.5 mg hydrochlorothiazide \((n = 8)\) was \(2.7 \pm 2.6\) ng/dl, in patients with angiotensin converting enzyme (ACE)-inhibitors \((n = 13)\) \(1.6 \pm 1.0\) ng/dl, in patients with ATII type I receptor blockers \((n = 7)\) \(2.7 \pm 2.8\) ng/dl, and in patients with beta-blockers \((n = 11)\) \(1.7 \pm 1.0\) ng/dl. Some variability was seen in primary aldosteronism, but it was probably due to sample size and intra-group fluctuations: aldosterone concentration in patients receiving 12.5 mg hydrochlorothiazide \((n = 10)\) was \(6.4 \pm 3.5\) ng/dl, in patients with ACE-inhibitors \((n = 10)\) \(19.3 \pm 21.1\) ng/dl, in patients with ATII type I receptor blockers \((n = 7)\) \(13.9 \pm 21.8\) ng/dl, and in patients with beta-blockers \((n = 13)\) \(11.3 \pm 8.3\) ng/dl.

**Aldosterone metabolites**

Mean urinary excretion of tetrahydroaldosterone and aldosterone-18-glucuronide was significantly higher in primary aldosteronism compared to essential hypertension and normal subjects (Table 4). However, substantial overlap was observed between patients with primary aldosteronism and the two other groups (Fig. 4A and B). Since the distribution of the single values suggested these parameters to be suitable for a specific rather than a sensitive test, cut-off values were determined considering a maximum specificity. Thus, cut-off values of 126 μg tetrahydroaldosterone/g creatinine and 19.7 μg aldosterone-18-glucuronide/g creatinine with a specificity of 100% revealed a sensitivity of 48% and 44%, respectively, when separating primary aldosteronism from essential hypertension, with a diagnostic accuracy of 76% for tetrahydroaldosterone and 74% for aldosterone-18-glucuronide. Whereas sensitivity of tetrahydroaldosterone was found to be better in hypokalaemic than in normokalaemic patients (64% vs 36%, respectively), we did not see a difference between hypokalaemic and normokalaemic patients with respect to aldosterone-18-glucuronide (45% vs 43%, respectively).

**Table 3** ROC of plasma aldosterone concentration after saline infusion in patients with normokalaemic and hypokalaemic primary aldosteronism.

<table>
<thead>
<tr>
<th>Cut-off aldosterone after saline infusion (ng/dl)</th>
<th>Specificity (%)</th>
<th>Sensitivity for normo-kalaemic patients (%)</th>
<th>Specificity for hypo-kalaemic patients (%)</th>
<th>Sensitivity for hypo-kalaemic patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.65</td>
<td>100</td>
<td>52</td>
<td>29</td>
<td>82</td>
</tr>
<tr>
<td>5.8</td>
<td>97</td>
<td>68</td>
<td>57</td>
<td>82</td>
</tr>
<tr>
<td>5.35</td>
<td>93</td>
<td>68</td>
<td>57</td>
<td>82</td>
</tr>
<tr>
<td>5.1</td>
<td>90</td>
<td>72</td>
<td>57</td>
<td>91</td>
</tr>
<tr>
<td>4.7</td>
<td>86</td>
<td>76</td>
<td>64</td>
<td>91</td>
</tr>
<tr>
<td>4.5</td>
<td>86</td>
<td>80</td>
<td>71</td>
<td>91</td>
</tr>
</tbody>
</table>

**Discussion**

Primary aldosteronism is meanwhile considered the most common cause of secondary hypertension. However, debate concerning the rationale for screening of primary aldosteronism and concerning the best screening test is continuing (23, 27, 28, 30). Opponents of widespread screening within the hypertensive population argue that the benefit for patients with an elevated aldosterone/renin ratio with respect to outcome measures such as mortality and morbidity has not been shown in randomized controlled trials (31). In addition, screening with the aldosterone/renin ratio has been criticized for a lack of standardization and low
specificity (10, 28). Since differentiation to low renin states such as low renin hypertension and essential hypertension treated with beta-blockers is difficult (32), the putative diagnosis of primary aldosteronism based on a raised aldosterone/renin ratio requires confirmatory testing in order to establish the autonomous character of aldosterone secretion in Conn Syndrome (3, 13).

We reassessed the saline infusion test as a confirmatory test for primary aldosteronism. All patients received their usual hypertensive medication with the exception of spironolactone. The rationale for this approach was threefold: (i) in previous studies we and other groups demonstrated that hypertensive medication such as beta-blockers mainly affects renin concentrations but has little, if any, effect on aldosterone levels (12, 32); (ii) in the context of an acute volume expansion which is known to strongly suppress aldosterone secretion (33), continuation of antihypertensive medication is even less likely to alter the results of the saline infusion test; and (iii) taking patients with primary aldosteronism off their usual medication is time- and cost intensive, and may harm the patient.

In our study, both patients with essential hypertension and normal controls showed a similar decrease of aldosterone following saline infusion. In addition, there were no significant differences in aldosterone concentrations between patients receiving diuretics, ACE inhibitors, or beta-blockers. Therefore, the normal range in both groups seems to be identical, despite the fact that essential hypertensives used their usual medication. We believe that this is indirect evidence for our hypothesis that hypertensive medication does not influence the aldosterone response to volume loading. However, only a prospective study evaluating the aldosterone response under different blood pressure regimens would provide conclusive evidence for our hypothesis.

In patients with primary aldosteronism, aldosterone also declined during sodium loading but remained significantly higher than in essential hypertension and in normal controls. Thus, aldosterone after saline infusion allowed differentiation between hypokalaemic primary aldosteronism and essential hypertension, with a sensitivity of 91% and a specificity of 90% (cut-off value 5.1 ng/dl (141 pmol/l)). However, the test proved to be inappropriate for differentiating between normokalaemic primary aldosteronism and patients with essential hypertension (57% sensitivity, 90% specificity) or normal subjects. The most likely explanation for this phenomenon is that aldosterone excess is less severe and less autonomous in the case of normokalaemic

Table 4 Results of urinary measurements in normotensive subjects, patients with essential hypertension and patients with primary aldosteronism (mean ± s.d.).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Tetrahydroaldosterone (µg/g creatinine)</th>
<th>Aldosterone-18-glucuronide (µg/g creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>47</td>
<td>47.3 ± 20.0</td>
<td>8.3 ± 4.7</td>
</tr>
<tr>
<td>Essential hypertension</td>
<td>29</td>
<td>57.1 ± 23.6</td>
<td>8.7 ± 3.7</td>
</tr>
<tr>
<td>Primary aldosteronism</td>
<td>25</td>
<td>119.0 ± 49.7*†</td>
<td>18.4 ± 8.4*†</td>
</tr>
<tr>
<td>Hypokalaemic</td>
<td>11</td>
<td>138.1 ± 51.8</td>
<td>20.1 ± 8.1</td>
</tr>
<tr>
<td>Normokalaemic</td>
<td>14</td>
<td>103.9 ± 44.1</td>
<td>17.1 ± 8.1</td>
</tr>
</tbody>
</table>

*Significant difference (P < 0.001) between patients with primary aldosteronism and normal subjects.
†Significant difference (P < 0.001) between primary aldosteronism and essential hypertension.
primary aldosteronism than in the hypokalaemic variant of the disease. This concept is supported by biochemical data in our study showing in normokalaemic primary aldosteronism slightly higher renin levels and lower aldosterone levels both before and after saline infusion. Our data suggest that it is less sensible to use the saline infusion test as a confirmatory test in that the poor detection of normokalaemic primary aldosteronism may lead to inadequate classification of these patients.

Previous studies have employed mainly the saline infusion test to separate Conn’s adenoma from essential hypertension (18, 19, 21) and were performed in hypokalaemic patients. Another study in hypokalaemic aldosteronism investigated the value of the test in differentiating both adenoma and hyperplasia from essential hypertension (34), showing a sensitivity of 96% and a specificity of 84%, which is comparable to our findings in hypokalaemic patients. In contrast, the low sensitivity of this test was recently demonstrated by Stowasser et al. (3), who reported a sensitivity of 18–30% in a group of mainly normokalaemic patients with primary aldosteronism. Although these results are partly in line with our data, the extremely low sensitivity in the Stowasser study may also be explained by the use of a previously reported cut-off value of 8 ng/dl for aldosterone. The ROC analysis performed with our data suggested a lower cut-off value of 5.1 ng/dl using a highly specific aldosterone assay and thus confirms the results of Kem et al. (21) and Holland (24) who have both suggested a cut-off value of 5 ng/dl. In another study we demonstrated that aldosterone cut-offs after saline infusion varied up to 100%, depending on the assay used (C Shirpenbach et al., unpublished observations). Thus, this reinforces the need to define appropriate cut-off values for each aldosterone assay used.

As another confirmatory test measurement of urinary aldosterone metabolites has been used in order to demonstrate aldosterone excess in primary aldosteronism. Less than 5% of secreted aldosterone is excreted as free aldosterone into urine, whereas approximately 10% is excreted as aldosterone-18-glucuronide (24). Tetrahydroaldosterone accounts for a higher proportion of aldosterone excretion than aldosterone glucuronide, but nonetheless accounts for only a third of total aldosterone excretion. Some
aldosterone metabolites are unknown or not determined in clinical practice (36). This is probably the reason why urinary aldosterone determination does not have a better diagnostic value than plasma determinations. Our findings revealed a high overlap for tetrahydroaldosterone and aldosterone-18-glucuronide when comparing primary aldosteronism with essential hypertension. Employing appropriate cut-off values, urinary metabolites proved to have a high specificity for primary aldosteronism, but — owing to high overlap in the lower range of values — a rather low sensitivity. The sensitivity of tetrahydroaldosterone and aldosterone-18-glucuronide did not exceed 48% and 44%, respectively, for a specificity of 100%. These findings are more or less in line with previous retrospectively collected data by our own group which likewise showed a low sensitivity (28%) for tetrahydroaldosterone but a higher one for aldosterone-18-glucuronide (74%) at a specificity of 100% (32). Also, Ulick et al. did not find a significant difference between essential hypertension and primary aldosteronism when measuring urinary tetrahydroaldosterone (35).

Contrary to these findings, Abdelhamid et al. reported a sensitivity and specificity of 95% and 96% for tetrahydroaldosterone and 91% and 71%, respectively, for aldosterone-18-glucuronide (22) in a large study population of 1865 patients using the same assay as in our study. Evaluation of the data differs in that Abdelhamid’s group calculated the metabolite concentration as µg/day whereas we expressed the excretion per gram of creatinine in order to correct collection errors. However, this does not seem to be a sufficient explanation for these discrepancies. It is more likely that differences in the design of the studies and diagnostic criteria for primary aldosteronism varied between studies. As a major difference in the study design, Abdelhamid et al. withdrew antihypertensive medication in their patients 2 weeks prior to testing, whereas in our study antihypertensive medication was continued. Thus, in our experience, both tetrahydroaldosterone and aldosterone-18-glucuronide seem to be of limited value for confirming primary aldosteronism under conditions as used in our study.

In summary, both aldosterone concentrations after saline infusion and measurement of urinary aldosterone metabolites did not prove to be useful as a single confirmatory test for normokalaemic primary aldosteronism owing to a high rate of false negatives. Therefore, these tests cannot be recommended as confirmatory tests in patients with suspected normokalaemic primary aldosteronism.

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References
8 Lim PO & MacDonald TM. Primary aldosteronism, diagnosed by the aldosterone to renin ratio, is a common cause of hypertension. Clinical Endocrinology 2003 59 427–430.
10 Tiu SC, Choi CH, Shek CC, Ng YW, Chan FK, Ng CM & Kong AP. The use of aldosterone-renin ratio as a diagnostic test for primary hyperaldosteronism and its test characteristics under different conditions of blood sampling. Journal of Clinical Endocrinology and Metabolism 2005 90 72–78.
31 Kaplan NM. Caution about the overdiagnosis of primary aldosteronism. *Clinical Endocrinology and Metabolism* 1977 45 141–146.

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