The aldosterone/renin ratio as a diagnostic tool for the diagnosis of primary hypoaldosteronism in newborns and infants

Ruecker, Beate; Lang-Muritano, Mariarosaria; Spanaus, Katharina; Welzel, Maik; l’Allemand, Dagmar; Phan-Hug, Franziska; Katschnig, Claudia; Konrad, Daniel; Holterhus, Paul-Martin; Schoenle, Eugen J

Abstract: BACKGROUND/AIMS: Primary hypoaldosteronism is a rare inborn disorder with life-threatening symptoms in newborns and infants due to an aldosterone synthase defect. Diagnosis is often difficult as the plasma aldosterone concentration (PAC) can remain within the normal range and thus lead to misinterpretation and delayed initiation of life-saving therapy. We aimed to test the eligibility of the PAC/plasma renin concentration (PRC) ratio as a tool for the diagnosis of primary hypoaldosteronism in newborns and infants. Methods: Data of 9 patients aged 15 days to 12 months at the time of diagnosis were collected. The diagnosis of primary hypoaldosteronism was based on clinical and laboratory findings over a period of 12 years in 3 different centers in Switzerland. To enable a valid comparison, the values of PAC and PRC were correlated to reference methods. RESULTS: In 6 patients, the PAC/PRC ratio could be determined and showed constantly decreased values <1 (pmol/l)/(mU/l). In 2 patients, renin was noted as plasma renin activity (PRA). PAC/PRA ratios were also clearly decreased. The diagnosis was subsequently genetically confirmed in 8 patients. CONCLUSION: A PAC/PRC ratio <1 pmol/mU and a PAC/PRA ratio <28 (pmol/l)/(ng/ml × h) are reliable tools to identify primary hypoaldosteronism in newborns and infants and help to diagnose this life-threatening disease faster. © 2015 S. Karger AG, Basel.

DOI: https://doi.org/10.1159/000381852

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-111380
Published Version

Originally published at:
DOI: https://doi.org/10.1159/000381852
The Aldosterone/Renin Ratio as a Diagnostic Tool for the Diagnosis of Primary Hypoaldosteronism in Newborns and Infants

Beate Ruecker\textsuperscript{a, b}, Mariarosaria Lang-Muritano\textsuperscript{a, b}, Katharina Spanaus\textsuperscript{c}, Maik Welzel\textsuperscript{f}, Dagmar l’Allemand\textsuperscript{d}, Franziska Phan-Hug\textsuperscript{e}, Claudia Katschnig\textsuperscript{a, b}, Daniel Konrad\textsuperscript{a, b}, Paul-Martin Holterhus\textsuperscript{f}, Eugen J. Schoenle\textsuperscript{a, b}

\textsuperscript{a}Department of Endocrinology/Diabetology and \textsuperscript{b}Children’s Research Centre, University Children’s Hospital Zurich, and \textsuperscript{c}Department of Clinical Chemistry, University Hospital Zurich, Zurich, \textsuperscript{d}Department of Endocrinology, Children’s Hospital of Eastern Switzerland, St. Gallen, and \textsuperscript{e}Department of Endocrinology, University Hospital Lausanne, Lausanne, Switzerland; \textsuperscript{f}Division of Paediatric Endocrinology and Diabetes, University Hospital of Schleswig Holstein, Kiel, Germany

Abstract

Background/Aims: Primary hypoaldosteronism is a rare inborn disorder with life-threatening symptoms in newborns and infants due to an aldosterone synthase defect. Diagnosis is often difficult as the plasma aldosterone concentration (PAC) can remain within the normal range and thus lead to misinterpretation and delayed initiation of life-saving therapy. We aimed to test the eligibility of the PAC/plasma renin concentration (PRC) ratio as a tool for the diagnosis of primary hypoaldosteronism in newborns and infants. Methods: Data of 9 patients aged 15 days to 12 months at the time of diagnosis were collected. The diagnosis of primary hypoaldosteronism was based on clinical and laboratory findings over a period of 12 years in 3 different centers in Switzerland. To enable a valid comparison, the values of PAC and PRC were correlated to reference methods. Results: In 6 patients, the PAC/PRC ratio could be determined and showed constantly decreased values <1 (pmol/l)/(mU/l). In 2 patients, renin was noted as plasma renin activity (PRA). PAC/PRA ratios were also clearly decreased. The diagnosis was subsequently genetically confirmed in 8 patients. Conclusion: A PAC/PRC ratio <1 pmol/mU and a PAC/PRA ratio <28 (pmol/l)/(ng/ml × h) are reliable tools to identify primary hypoaldosteronism in newborns and infants and help to diagnose this life-threatening disease faster.

Introduction

Primary hypoaldosteronism is a rare inborn disorder due to an aldosterone synthesis defect. It is transmitted as an autosomal recessive trait. Usually, aldosterone synthase is affected, which is a terminal enzyme in the aldosterone biosynthetic pathway that mediates the two last steps in the production of aldosterone [1]. There are two types of aldosterone synthase defects (ASD), both

Key Words

Aldosterone/renin ratio · Plasma aldosterone concentration · Plasma renin concentration · Diagnostic tool · Diagnosis of primary hypoaldosteronism · Aldosterone synthase deficiency · CYP11B2
located in the CYP11B2 gene on chromosome 8q24.3. Type I (ASD I) is characterized by impaired hydroxylation of corticosterone and low concentrations of the metabolites aldosterone and 18-hydroxycorticosterone. Type II (ASD II) is characterized by impaired conversion of the 18-hydroxyl group to aldehyde and elevated 18-hydroxycorticosterone levels in combination with hypoaldosteronism [2, 3]. The clinical features, including salt wasting, hypovolemia, and failure to thrive, are identical in both types [1, 3, 4]. Especially in newborns and infants, primary hypoaldosteronism is clinically relevant and requires adequate therapy as the sodium/potassium metabolism is mostly regulated by aldosterone at that age. With complete kidney maturation, aldosterone-independent reabsorption rises and correspondingly renin and aldosterone levels fall. This may explain the fact that symptoms in patients suffering from hypoaldosteronism generally become attenuated towards the first years of life [1, 3].

Untreated primary hypoaldosteronism leads to a life-threatening electrolyte imbalance with hyponatremia and hyperkalemia in newborns and infants. Typically the cortisol concentration is normal and the plasma renin concentration (PRC) is high, with a proportional low serum plasma aldosterone concentration (PAC) [1, 3]. As PAC values can remain in the normal range, interpretation might be difficult and can lead to misdiagnosis and delayed initiation of life-saving treatment with fludrocortisone and salt.

The aldosterone/renin ratio is an accepted tool to diagnose hyperaldosteronism in adults [5–9]. The aim of our study was to proof the validity of this ratio as a diagnostic tool for the diagnosis of primary hypoaldosteronism in newborns and infants.

Materials and Methods

Patients
In order to include as many patients with diagnosed primary hypoaldosteronism as possible, we asked all 6 departments of pediatric endocrinology in Switzerland to submit data of patients with hypoaldosteronism. Thereby, we were able to collect data of 9 patients who were diagnosed with hypoaldosteronism and were being followed by 3 different departments of pediatric endocrinology in Switzerland (6/9 from the University Children’s Hospital Zurich, 2/9 from the Children’s Hospital of Eastern Switzerland, and 1/9 from the University Hospital Lausanne). The patients were diagnosed based on clinical and laboratory results at an age of 15 days to 12 months during the period of 2001–2013. Interestingly, all patients had a migration background with origins in the Balkan region (patients 1, 2, 3, 4, 5, 6, 7, and 9) and Tunisia (patient 8).

Molecular Genetic Analysis
In order to confirm the diagnosis genetically, all patients were screened for mutations in the CYP11B2 gene after obtaining informed consent.

DNA extraction was performed using an SOP DNA Kit (Applied Biosystems/Thermo Fisher Scientific, Waltham, Mass., USA)/QIAamp DNA Mini Kit (Qiagen, Venlo, The Netherlands). PCR amplification of the CYP11B2 gene and Sanger sequencing of all 9 coding exons and the exon/intron boundaries followed an established protocol [10]. Primers are available upon request. Mutations were designated according to the recommendations of the Human Genome Variation Society (HGVS) based on the reference sequence NM_000498.3 [11].

Analytical Methods
As patients who were diagnosed with primary hypoaldosteronism at 3 different centers in Switzerland from 2001 to 2013 were included, the analytic methods differed depending on the time and place where they were performed. In 7 patients, the PAC was measured by radioimmunoassay (RIA). In patients 2, 3, and 4, and a Coat-A-Count aldosterone RIA (Siemens, Eschborn, Germany) was used. In patients 6 and 7, PAC were determined using an aldosterone direct RIA (DRG Diagnostics GmbH, Marburg, Germany). The DSL active aldosterone RIA (Diagnostic Systems Laboratories, Inc./Beckman Coulter, Brea, Calif., USA) was used in patient 1. In patient 8, the ALDO-CTK-2 RIA (DiaSorin, Saluggia, Italy) was used. In patient 5, the PAC was determined via chemiluminescence immunoassay (CLIA; DiaSorin) on a Liaison immunoanalyzer. To compare the aldosterone levels of the patients, the PAC were traced back to an automated chemiluminescence assay (Nichols Advantage; Nichols Institute Diagnostics, San Clemente, Calif., USA) using equations from method comparison studies provided by DiaSorin, the laboratory of Dr. Limbach (Heidelberg, Germany), the Nichols Institute, Beckman Coulter, and K. Spanaus. The DRG and DSL RIA are the same assay and were being distributed by Beckman Coulter at the time of reporting. It was further possible to correlate Siemens Coat-A-Count aldosterone with DSL active aldosterone (information by Beckman Coulter) and ALDO-CTK-2 (information by DiaSorin). Liaison by DiaSorin was correlated with DRG (K. Spanaus, method evaluation on the Liaison immunoanalyzer in comparison to the DRG RIA). Accordingly, we converted the values using given factors in reference to the DRG RIA and the DSL assay. In a second step, we correlated the data to an automated chemiluminescence assay (Nichols Advantage) based on the correlation study by Schirpenbach et al. [12].

In patient 9, a noncommercial RIA was used based on the method published in 1984 by Nussberger et al. [13]. As no correlation studies for this test were available, we were not able to correlate these values exactly to the other methods.

In order to standardize the PAC values, which were documentented in nanograms per liter, picomoles per liter, or picograms per milliliter, they were converted to picomoles per liter.

The PRC was measured by RIA in 4 patients (1, 2, 6, and 7). In patient 1 as well as in patient 7, a CIS Bio RIA (Schering AG, Berlin, Germany) was used. In patient 2, the PRC was determined using a Bio-Rad third-generation RIA (Bio-Rad Laboratories, Inc., Hercules, Calif., USA). In patient 6, a Nichols RIA (Nichols Institute Diagnostics) was utilized.

In 3 patients (3, 4 and 5), the PRC was measured via chemiluminescence assay. In patients 3 and 5, the DiaSorin test on a Liaison Immunoanalyser (DiaSorin, Saluggia, Italy) was used. In patient 7, the PRC was determined by nephelometry (Cis Bio RIA; Cis Bio, France). In patient 9, a non-commercial RIA was used based on the method published in 1984 by Nussberger et al. [13]. As no correlation studies for this test were available, we were not able to correlate these values exactly to the other methods.
Aldosterone/Renin Ratio and Primary Hypoaldosteronism

Horm Res Paediatr 2015;84:43–48
DOI: 10.1159/000381852

son immunoanalyzer was used. In patient 4, the PRC was determined via chemiluminescence assay on a Nichols Advantage immunoanalyzer (Nichols Institute Diagnostics).

According to existent method comparison studies, it was possible to trace back the PRC of the patients to the CLIA on the Nichols Advantage analyzer. Liaison, Bio-Rad, and Nichols RIA assays could be directly correlated to the Nichols Advantage by given equations provided by DiaSorin, the laboratory of Dr. Limbach (Heidelberg, Germany), and the Nichols Institute. The values measured by the Cis Bio RIA first needed to be correlated to the DiaSorin CLIA and in a second step to the Nichols Advantage (information by DiaSorin) as there were no direct correlation studies available.

In patients 8 and 9, renin was measured as plasma renin activity (PRA). In patient 8, an RIA named RENCTK by DiaSorin was used. For determination of the PRA in patient 9, a radioimmunological microassay was used by Nussberger et al. [14], which is based on the method by Poulsen and Jorgensen [15].

The PRC values were noted in picomoles per liter, units per liter, or nanograms per milliliter and were converted to milliunits per liter to enable comparison. The PAC/PRC ratio (pmol/mU) was calculated for each patient (table 1).

The equations used to trace back the values to the Nichols Advantage are listed in online suppl. tables 1 and 2 (for all online suppl. material, see www.karger.com/doi/10.1159/000381852).

### Results

All 9 patients showed typical laboratory features with hyponatremia (mean serum sodium level 123.6 mmol/l, range 117–132 mmol/l) at the time of diagnosis. Seven patients showed hyperkalemia (mean serum potassium level 6.1 mmol/l, range 4.6–8.4 mmol/l). Patient 9 showed a potassium concentration within the upper range (4.6 mmol/l), and in patient 8 the potassium level was not documented (table 1).

Aldosterone and renin values were measured in all 9 patients at the time of diagnosis. The PAC was measured via 3 different assays in patients 1–6. The values were correlated to Nichols Advantage in order to allow comparison of the data. In all 6 patients, the PAC was low but within the normal range (mean 744.5 pmol/l, range 126–1,906 pmol/l) (table 1). In these 6 patients, renin was measured as the PRC via 5 different methods. As described above (Materials and Methods), the values were correlated to Nichols Advantage. The corrected PRC values were strongly elevated (mean PRC 304,895 mU/l, range

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at the time of diagnosis</th>
<th>Sodium(^a), mmol/l</th>
<th>Potassium(^b), mmol/l</th>
<th>Corrected PAC(^c), pmol/l</th>
<th>Corrected PRC(^d), mU/l</th>
<th>PAC/PRC ratio(^e), pmol/mU</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15 days</td>
<td>128</td>
<td>6.2</td>
<td>1,906</td>
<td>9,686</td>
<td>0.1968</td>
<td>homozygous mutation pThr185Ile in the CYP1182 gene</td>
</tr>
<tr>
<td>2</td>
<td>4 weeks</td>
<td>125</td>
<td>6.1</td>
<td>217</td>
<td>74,423</td>
<td>0.0029</td>
<td>homozygous mutation pThr185Ile in the CYP1182 gene</td>
</tr>
<tr>
<td>3</td>
<td>12 weeks</td>
<td>121</td>
<td>5.9</td>
<td>801</td>
<td>256,041</td>
<td>0.0031</td>
<td>homozygous mutation pThr185Ile in the CYP1182 gene</td>
</tr>
<tr>
<td>4</td>
<td>3 months</td>
<td>120</td>
<td>8.4</td>
<td>947</td>
<td>95,258</td>
<td>0.0099</td>
<td>homozygous mutation pThr185Ile in the CYP1182 gene</td>
</tr>
<tr>
<td>5</td>
<td>4 months</td>
<td>124</td>
<td>5.8</td>
<td>89</td>
<td>1,390,330</td>
<td>0.0001</td>
<td>homozygous mutation pThr185Ile in the CYP1182 gene</td>
</tr>
<tr>
<td>6</td>
<td>2 weeks</td>
<td>117</td>
<td>6.2</td>
<td>470</td>
<td>3,634</td>
<td>0.1293</td>
<td>homozygous mutation pThr185Ile in the CYP1182 gene</td>
</tr>
<tr>
<td>7</td>
<td>6 months</td>
<td>122</td>
<td>5.4</td>
<td>&gt;626</td>
<td>&lt;1.3387(^f)</td>
<td></td>
<td>homozygous mutation pThr185Ile in the CYP1182 gene</td>
</tr>
</tbody>
</table>

\(^a\) Reference range 134–144 mmol/l. \(^b\) Reference range 3.5–5 mmol/l. \(^c\) Reference range 27.7–4,995 pmol/l. \(^d\) Reference range 2.8–39.9 mU/l. \(^e\) Reference range 1–71 pmol/mU. \(^f\) The PRC was given as a minimal value and therefore the PAC/PRC ratio is the highest assumable value.
The PAC/PRC ratio was determined for each patient (1–6, in pmol/mU) and showed strongly decreased values clearly <1 pmol/mU (mean 0.0737 pmol/mU, range 0.0001–0.1968 pmol/mU) (table 1). In all 6 patients, mutations in the \( \text{CYP11B2} \) gene that are correlated with aldosterone synthase deficiency were detected. In patients 1, 2, 3, 4, and 6, homozygous C-to-T transversion in codon 185 in the \( \text{CYP11B2} \) gene was found (table 1). It causes an amino acid substitution of threonine by isoleucine (p.Thr185Ile).

Patient 5 was compound heterozygous for the p.Thr185Ile and c.2798 + 1 G>A (IVS8 + 1 G>A) mutations. The latter is located in a highly conserved area of the donor splice site of intron 8 of the \( \text{CYP11B2} \) gene (table 1). It was described in connection with hypoaldosteronism by Merakou et al. [16].

In patient 7, the diagnosis was genetically confirmed by detection of the homozygous mutation p.Thr185Ile in the \( \text{CYP11B2} \) gene (table 1). The PRC was only noted as a minimal value as the material was not sufficient for dilution of the sample to measure the exact concentration. After correlating the primary values to reference methods, the resulting PAC was 838 pmol/l and the PRC was >626 mU/l. The maximum credible value for the PAC/PRC ratio would be <1.3387 pmol/mU.

In patients 8 and 9, renin was measured as PRA. In patient 8, the PAC was given as a range and was correlated to the Nichols Advantage. The corrected values (172–283 pmol/l) remained in the reference range. In contrast, the PRA was highly increased [i.e. 37 ng/ml × h; reference range 0.2–2.8 ng/ml × h (supine), 1.5–7.7 ng/ml × h (upright)]. The PAC/PRC ratio resulted in a decreased range of 4.6486–7.6486 (pmol/l)/(ng/ml × h) [reference: >28 (pmol/l)/(ng/ml × h)]. Genetic analysis was performed but could not detect any mutation within the exonic area (table 2).

In patient 9, comparison with the other measurements was not possible as there were no correlation studies available. Nevertheless, the PRA (66 ng/ml × h) was strongly elevated in comparison to the provided reference range [0.2–2.0 ng/ml × h (supine), 1.5–4.5 ng/ml × h (upright)]. The PAC was normal [i.e. 300 pmol/l; reference range 80–11 pmol/l (supine), 166–665 pmol/l (upright)]. The PAC/PRA ratio was decreased [i.e. 4.5455 (pmol/l)/(ng/ml × h)]. The diagnosis of ASD was genetically confirmed in patient 9 by detection of the homozygous mutation p.Thr185Ile in the \( \text{CYP11B2} \) gene (table 2).

### Discussion

In newborns and infants, the diagnosis of primary hypoaldosteronism is based on clinical and laboratory results. That is how all 9 patients in this study were diagnosed initially.

Diagnosing ASD can be difficult due to the fact that the PAC may appear in the normal range like in all of our patients. In these cases the PAC/PRC ratio, which is already an accepted screening tool for hyperaldosteronism, can be useful. A PAC/PRC ratio <1 pmol/mU indicates an inappropriately low PAC for the stimulus. Therefore, a decreased aldosterone/renin ratio is suggestive of hypoaldosteronism as has been shown in adults [9, 17]. The benefit of using the PAC/PRA ratio to diagnose various disorders of the renin-angiotensin-aldosterone axis was described in 1991 by McKenna et al. [9], who described subnormal PAC/PRA ratios in the context of primary adrenal insufficiency in adults. In 2007,
Diederich et al. [17] revolutionized the approach by using the PRC instead of the PRA as the PRC became the international standard to measure renin values. A subnormal PAC/PRC ratio <1 pmol/mU (reference range 1–72 pmol/mU) was found in 14 adults with primary hypoaldosteronism mostly due to adrenal insufficiency. Based on our study of 9 newborns and infants with aldosterone synthase deficiency, the PAC/PRC ratio (pmol/mU) seems to be a reliable diagnostic parameter in this age group as well (table 1). The pathophysiology, of course, differs in adults with primary adrenal insufficiency compared to infants and newborns with ASD, but the hormone constellation with a high PRC and a low PAC is the same.

Based on correlation studies, we were able to correlate PAC and PRC to a reference method in 7 patients (1–7). In these patients, PAC and PRC values were correlated to Nichols Advantage (Material and Methods) as this was the method used by Diederich et al. [17], who suggested a PAC/PRC ratio <1 pmol/mU as a predictor of primary hypoaldosteronism in adults.

In 6 patients (1–6), the PAC/PRC ratio was clearly <1 pmol/mU (mean 0.0737 pmol/mU) even though the PAC values remained within the normal range, as is typical for ASD II.

In 5 (patients 1, 2, 3, 4, and 6) of these 6 patients the diagnosis was genetically confirmed by detection of the well-known homozygous p.Thr185Ile mutation, which is correlated with ASD II [18, 19]. Patient 5 was compound heterozygous. The well-known p.Thr185Ile mutation was found here as well in the heterozygote form. The second mutation c.2798 + 1 G>A (IVS8 + 1) lies within the donor splice site of intron 8 of the CYP11B2 gene. This compound-heterozygous mutation was described in connection with hypoaldosteronism by Merakou et al. [16] (table 1).

In patient 7, the PRC was only noted as a minimal value as the material was not sufficient for serial dilution analysis to determine the exact value. Therefore, the exact PAC/PRC ratio could not be determined.

In patients 8 and 9, renin was noted as PRA, which could not be correlated to PRC as no correlation studies for the used methods were available. Due to the fact that nanograms per millilitre × hour could not be converted into milliunits per liter, a direct comparison was not possible. Based on McKenna et al. [9], who used the PAC/PRA ratio as a diagnostic tool and associated values <28 (pmol/l)/(ng/ml × h) with hypoaldosteronism, the patients fulfilled this criterion as well. Both patients showed PAC/PRA ratios which were clearly lower than 28 (pmol/l)/(ng/ml × h) [patient 8: 4.6486–7.6486 (pmol/l)/(ng/ml × h), patient 9: 4.5455 (pmol/l)/(ng/ml × h)]. It is most likely that a PAC/PRC ratio measured in these patients (8 and 9) would have been <1 pmol/mU, too.

It is suspicious that 7 (patients 1, 2, 3, 4, 6, 7 and 9) of our 9 patients showed the same homozygous mutation (p.Thr185Ile). Even though patients 3 and 4 are second cousins to each other, we did not find any other family relationship between these study patients. Moreover, direct consanguinity could have been excluded within all of these families over 3 generations (see online suppl. fig. 1, 2, 3, 5, 6, and 8 showing the pedigree of patients 1, 2, 3, 4, 6, 7, and 9). It is conspicuous that 5 of these families belong to an ethnic minority group (i.e. Catholic Kosovo Albanian: patients 3, 4, and 7; Catholic Serb: patient 6, and Muslim Macedonian: patient 2). Moreover, the remaining patients (1 and 9) with the homozygous mutation also originated from the Balkan region (Macedonia and Albania). Due to this fact, we presume a founder effect.

Interestingly, in patient 8 no mutation was detected. Assuming the parents were cousins to each other, it is presumable that this patient is homozygous for a mutation, which could not be detected by the genetic analysis used (see online suppl. fig. 7 showing the pedigree of patient 8).

In summary, the data of our patients along with the given aldosterone and renin values strongly indicate that a PAC/PRC ratio <1 pmol/mU is a reliable marker to diagnose ASD in newborns and infants. Thus, we are convinced that the PAC/PRC ratio is a feasible and cost-saving tool for the diagnosis of primary hypoaldosteronism in this age group. We recommend using the PAC/PRC ratio in infants and newborns who present with the typical electrolyte constellation and clinical signs of hypoaldosteronism to verify the diagnosis. The PAC/PRC ratio can help to diagnose this life-threatening disease faster and consequently initiate life-saving therapy earlier.

Acknowledgements

We would like to thank Prof. Anna Lauber-Biason for her support and helpful advice. We also thank Harald Schmidt (Department of Endocrinology, Limbach Laboratory, Heidelberg, Germany), Thomas Frei, and Elke Rauhut (DiaSorin Switzerland AG) for providing correlation studies based on which we were able to trace back the values of our study patients to one method.

Aldosterone/Renin Ratio and Primary Hypoaldosteronism

DOI: 10.1159/000381852
References


