Primary Aldosteronism

Measurement of Peripheral Plasma 18-Oxocortisol Can Discriminate Unilateral Adenoma From Bilateral Diseases in Patients With Primary Aldosteronism


Abstract—Adrenal venous sampling is currently the only reliable method to distinguish unilateral from bilateral diseases in primary aldosteronism. In this study, we attempted to determine whether peripheral plasma levels of 18-oxocortisol (18oxoF) and 18-hydroxycortisol could contribute to the clinical differentiation between aldosteronoma and bilateral hyperaldosteronism in 234 patients with primary aldosteronism, including computed tomography (CT)–detectable aldosteronoma (n=113) and bilateral hyperaldosteronism (n=121), all of whom underwent CT and adrenal venous sampling. All aldosteronomas were surgically resected and the accuracy of diagnosis was clinically and histopathologically confirmed. 18oxoF and 18-hydroxycortisol were measured using liquid chromatography tandem mass spectrometry. Receiver operating characteristic analysis of 18oxoF discrimination of adenoma from hyperplasia demonstrated sensitivity/specificity of 0.83/0.99 at a cut-off value of 4.7 ng/dL, compared with that based on 18-hydroxycortisol (sensitivity/specificity: 0.62/0.96). 18oxoF levels above 6.1 ng/dL or of aldosterone >32.7 ng/dL were found in 95 of 113 patients with aldosteronoma (84%) but in none of 121 bilateral hyperaldosteronism, 30 of whom harbored CT-detectable unilateral nonfunctioning nodules in their adrenals. In addition, 18oxoF levels below 1.2 ng/dL, the lowest in aldosteronoma, were found 52 of the 121 (43%) patients with bilateral hyperaldosteronism. Further analysis of 27 patients with CT-undetectable micro aldosteronomas revealed that 8 of these 27 patients had CT-detectable contralateral adrenal nodules, the highest values of 18oxoF and aldosterone were 4.8 and 24.5 ng/dL, respectively, both below their cut-off levels indicated above. The peripheral plasma 18oxoF concentrations served not only to differentiate aldosteronoma but also could serve to avoid unnecessary surgery for nonfunctioning adrenocortical nodules concurrent with hyperplasia or microadenoma. (Hypertension. 2015;65:1096-1102. DOI: 10.1161/HYPERTENSIONAHA.114.04453.) • Online Data Supplement

Key Words: 1-(2-(18F)fluoro-3-pyridyl)-4-(2-isopropyl-1-oxo-isoindoline-5-yl)-5-methyl-1H-1,2,3-triazole

Primary aldosteronism (PA) is the most frequent form of secondary hypertension.1-12 Patients with PA present cardiovascular and cerebrovascular complications more frequently than those with essential hypertension (EH).3,4 Therefore, appropriate diagnosis and treatment of PA has become important for individual patients. In addition, results of our recent study indicated that PA should be detected and treated as early as possible to prevent chronic kidney disease.3 However, it is also true that its final diagnosis requires relatively long procedures, such as detection, confirmation testing and subtype diagnosis comprising computed tomography (CT) scans and adrenal venous sampling (AVS).5-7 AVS has been clearly established as the only reliable method for differential diagnosis between surgically curable unilateral aldosterone-producing adenoma (APA) and bilateral hyperaldosteronism (BHA).8-12 Nowadays, an increased number of specialized centers perform AVS in the world,10,11 but this test is still time-consuming, labor-intensive, and costly, which unfortunately prevents a wider application. Therefore, a much simpler, noninvasive and less expensive diagnostic method has been in demand by clinicians. 18-hydroxycortisol (18OHF) and

Received August 24, 2014; first decision September 22, 2014; revision accepted February 11, 2015.

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The online-only Data Supplement is available with this article at http://hyper.ahajournals.org lookup/suppl/doi:10.1161/HYPERTENSIONAHA.114.04453/-/DC1.

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Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.114.04453

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Peripheral Plasma 18-Oxocortisol in PA

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18-oxocortisol (18oxoF), which have structural characteristics of both aldosterone and cortisol in their chemical structures, are both synthesized from 11-deoxycortisol as substrate by aldosterone synthase (CYP11B2), although 18OHF can also be produced by 11β-hydroxylase (CYP11B1). Their production is known to be highly elevated in glucocorticoid-remediable aldosteronism. Furthermore, production of 18OHF and 18oxoF is elevated in patients with PA, especially in APA. The 24-hour urine excretion of these steroids is higher in patients with PA than that in normal subjects and patients with EH. Urinary 18OHF is considered more reliable in terms of subclassification of PA than urinary 18oxoF provided that they have been accurately evaluated by enzyme-linked immunoassay. We recently developed a highly sensitive test to measure 18oxoF based on liquid chromatography tandem mass spectrometry (LC–MS/MS). In this study, we examined the clinical significance of peripheral plasma concentrations of 18OHF and 18oxoF in patients with APA and underwent unilateral adrenalectomy with their diagnosis being CT-undetectable micro-APA were also diagnosed by AVS. Twenty-seven patients with CT-undetectable micro-APA were also diagnosed by AVS and underwent unilateral adrenalectomy with their diagnosis being confirmed histologically after surgery. Our preliminary study revealed that p18oxoF and 18OHF of patients with micro-APA were as low as those of patients with BHA (data not shown) and therefore, we evaluated mainly patients with APA and BHA in this study. This study was approved by the ethics committee of Tohoku University School of Medicine (number. 2010-359 and number 2010-360) and written informed consent was obtained from all participants. The patients with a plasma aldosterone concentration (PAC)/plasma renin activity ratio (ARR) of >20 (ng/dL per ng/mL per h) before challenge with 50 mg captopril were diagnosed with PA as previously reported. Patients were treated with calcium channel blockers and β-blockers during the study period. None of the 234 enrolled patients showed autonomous cortisol secretion, which was confirmed by cortisol concentrations >3.0 μg/dL after an overnight 1-mg dexamethasone suppression test. Among the 249 (234+15) patients with PA studied, 15 (6.0%) with 1-mg dexamethasone suppression test–positive cortisol/cortisol was considered to fall between 21% and 3.9% according to previously reported studies. Blood pressure was measured with Omron HEM 907 (Omron Healthcare Co Ltd, Kyoto, Japan) after >15-minutes rest in a sedentary position, and the average of 3 consecutive measurements was recorded. Peripheral blood samples were collected after the patient stayed in the recumbent position for 30 minutes between 8 and 10 AM.

The imaging procedure for CT scanning and its detailed interpretation is described in the online-only Data Supplement.

Methods

Patients and Imaging

From October 2010 to September 2013, 234 patients consisting of 113 patients with CT-detectable macro-APA and 121 patients with BHA, underwent computed tomographic scanning and successful cosyntropin-stimulated AVS. All of the 113 patients with APA underwent adrenalectomy, and diagnostic accuracy was further confirmed by postoperative measurement of plasma aldosterone concentration (PAC) and histopathologic evaluation, including immunohistochemical analysis of steroidogenic enzymes. Twenty-seven patients with CT-undetectable micro-APA were also diagnosed by AVS and underwent unilateral adrenalectomy with their diagnosis being confirmed histologically after surgery. Our preliminary study revealed that p18oxoF and 18OHF of patients with micro-APA were as low as those of patients with BHA (data not shown) and therefore, we evaluated mainly patients with APA and BHA in this study. This study was approved by the ethics committee of Tohoku University School of Medicine (number. 2010-359 and number 2010-360) and written informed consent was obtained from all participants. The patients with a plasma aldosterone concentration (PAC)/plasma renin activity ratio (ARR) of >20 (ng/dL per ng/mL per h) before challenge with 50 mg captopril were diagnosed with PA as previously reported. Patients were treated with calcium channel blockers and β-blockers during the study period. None of the 234 enrolled patients showed autonomous cortisol secretion, which was confirmed by cortisol concentrations >3.0 μg/dL after an overnight 1-mg dexamethasone suppression test. Among the 249 (234+15) patients with PA studied, 15 (6.0%) with 1-mg dexamethasone suppression test–positive cortisol/cortisol was considered to fall between 21% and 3.9% according to previously reported studies. Blood pressure was measured with Omron HEM 907 (Omron Healthcare Co Ltd, Kyoto, Japan) after >15-minutes rest in a sedentary position, and the average of 3 consecutive measurements was recorded. Peripheral blood samples were collected after the patient stayed in the recumbent position for 30 minutes between 8 and 10 AM.

The imaging procedure for CT scanning and its detailed interpretation is described in the online-only Data Supplement.

Reagents and Measurement

Fusaric acid was obtained from Sigma-Aldrich (St. Louis, MO). 18oxoF was kindly provided by Dr. Gomez-Sanchez. 18OHF was purchased from Steraloids Inc. (Newport, RI). Cortisol-3H (F-d4) and aldosterone-3H (Aldo-d7) were purchased from Isosiences (King of Prussia, PA) and C/D/N Isotopes (Pointe-Claire, Canada), respectively. InertSep Pharma cartridges and InertSep SI cartridges were obtained from GL Sciences (Tokyo, Japan). 4-Dimethylaminopyridine and 2-methyl-6-nitrobenzoic anhydride were purchased from Tokyo Chemical Industry (Tokyo, Japan). LC–MS/MS grade acetonitrile was obtained from Wako Pure Chemicals (Osaka, Japan). All other reagents and solvents were of analytic grade. Plasma renin activity and PAC were measured using commercially available kits as previously reported. We used SPAC-S Aldosterone Radioimmunoassay Kit (TBF, Inc, Tokyo, Japan) for measuring PAC in this study. The intra-assay variability of this assay was 1.8% to 8.3%. This radioimmunoassay of PAC had been standardized (Figure S1 in the online-only Data Supplement; Spearman r=0.9055; P<0.0001) using 90 plasma samples with different aldosterone concentrations confirmed by liquid chromatography tandem mass spectrometry (LC–MS/MS), as we previously reported. 18oxoF was measured by LC–MS/MS as previously reported. Sample preparation for LC–MS/MS measurement of 18OHF was described in the online-only Data Supplement. The intra-assay accuracy and precision were 90% to 111% and 3% to 9%, respectively, for both 18OHF and 18oxoF. Those of the interassay of their steroid metabolites were 87% to 108% and 1% to 10%, respectively. The lower limits of quantification for 18OHF and 18oxoF were 2.5 and 0.25 ng/dL, respectively.

Statistical Analysis

Normality of the collected data was analyzed by Kolmogorov–Smirnov test, and variables between groups were analyzed by Kruskal-Wallis test with Dunn multiple comparison test as a post hoc test. Receiver operating characteristic (ROC) analysis was performed to evaluate diagnostic ability. ROC curves were compared with the area under the curve (AUC). A linear regression model was used to analyze the correlation between 2 numeric variables, and their correlation was evaluated by Spearman correlation coefficient. Statistical significance was set at P<0.05.

Results

Clinical Characteristics, CT Imaging, and AVS in Patients With PA

We studied 113 patients with APA and 121 patients with BHA. As demonstrated in Table, baseline aldosterone concentration, baseline ARR, and captopril-challenged ARR were all significantly higher in those with APA (46.6 ng/dL, 363 ng/dL per ng/mL per hour and 233 ng/dL per ng/mL per hour) than those with BHA (18.3 ng/dL, 87.1 ng/dL per ng/mL per hour and 63.0 ng/dL per ng/mL per hour). CT scanning detected 15 APA cases with bilateral adrenal nodules and 30 BHA cases with unilateral nonfunctioning adrenocortical nodules, which also did confirm the superior diagnostic ability of AVS compared with imaging modalities (Table). Furthermore, AVS findings enabled us to diagnose 27 additional patients with micro-APA (CT-undetectable) during this study. Eight of them turned out to harbor unilateral nonfunctioning nodules in the contralateral adrenal. Thus, CT imaging findings were in agreement with those of AVS in 189 (ie, 261-15-30-27) of 261 patients with PA, that is a little >72% of the study population. This discriminatory value of only CT imaging was not so different from that found in previous studies. We also performed ROC analysis to compare the discriminating ability of serum potassium between APA (CT-detectable) and BHA. Those with BHA were tentatively regarded as control and those with APA as the unilateral tumor group. The value of serum potassium had a significant discriminating ability with an AUC of 0.78 using a cut-off value of 3.85 mmol/L, associated with a
sensitivity of 0.785 and specificity of 0.785. The serum potassium concentrations in patients with micro-APA were not significantly different from those in patients with BHA.

Peripheral Levels of 18oxoF and 18OHF
Both p18oxoF and p18OHF were significantly elevated in patients with APA (23.6 and 357 ng/dL) compared with those with BHA (1.89 and 129 ng/dL; Table). When comparing the averaged peripheral plasma concentrations of these 2 steroids between APA and BHA, the APA/BHA ratio of p18oxoF (12.5) was 5× higher than that of p18OHF (2.77). Comparison between PAC and p18oxoF levels subsequently demonstrated a statistically significant correlation between the above 2 groups, and those with APA showed the most marked correlation (Spearman $r=0.5336$; $P<0.05$) compared with those with BHA (Spearman $r=0.1987$; $P<0.05$; Figure 2SA and 2SB). In addition, linear regression analysis between peripheral aldosterone and 18oxoF levels also revealed the model was most fitted in those with APA ($R^2=0.6488$) compared with those with BHA ($R^2=0.0367$; Figure S2A and S2B). In contrast, comparison between peripheral PAC and p18OHF demonstrated a significant correlation in the APA group (Spearman $r=0.4886$; Figure S2C) but not in the BHA group (Figure S2D).

ROC Analyses Using p18oxoF, p18OHF, Aldosterone, and ARR
ROC analyses were performed to compare the diagnostic abilities of p18oxoF, p18OHF, PAC, and ARR (Figure 1A–1D) in terms of differentiation between unilateral neoplastic lesions and BHA. Those with BHA were regarded as control and those with APA as a unilateral tumor group. The value of p18oxoF was demonstrated to have the highest diagnostic ability with an AUC of 0.956 at a cut-off value of 4.7 ng/dL showing a sensitivity of 0.83 and specificity of 0.99 (Figure 1A). The value of PAC had the second highest ability with an AUC of 0.917 based on a cut-off level of 21.5 ng/dL showing a sensitivity of 0.81 and specificity of 0.93 (Figure 1C). Similarly, the value of p18OHF was third and that of ARR was fourth, with the AUC areas being 0.85 and 0.82 at cut-off values of 234 ng/dL and 152 ng/dL per ng/mL per hour and showing a sensitivity of 0.62 and 0.67 and specificity of 0.96 and 0.90, respectively (Figure 1B and 1D).

The distribution of p18oxoF showed that the highest level in the BHA group was 6.1 ng/dL, and the minimal value of p18oxoF in the APA group was 1.2 ng/dL (Figure 1E). The highest levels of p18OHF, PAC, and ARR in the BHA group were 345 ng/dL, 32.7 ng/dL, and 254 ng/dL per ng/mL per hour, respectively, and their minimal values were 5.7 ng/dL, 8.6 ng/dL, and 20 ng/dL per ng/mL per hour, respectively (Figure 1F, 1G, and 1H). In 86 of the 113 patients with APA (76%), p18oxoF concentration was above the maximum level (6.1 ng/dL) observed in patients with BHA. In 61 (54%), 56 (50%), and 47 (42%) of patients with APA, PAC, ARR, and p18OHF were higher than the maximum levels observed in patients with BHA. Therefore, p18oxoF was considered to be of the highest diagnostic value for PA subtyping. Furthermore,

<table>
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<tr>
<th>Parameters/Patients</th>
<th>APA</th>
<th>BHA</th>
<th>$P$ Value</th>
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<tbody>
<tr>
<td>Number</td>
<td>113</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
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<td>54.0±1.0</td>
<td>NS</td>
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<td>Sex, M and F</td>
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<td>40, 81</td>
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<tr>
<td>Body mass index, kg/m$^2$</td>
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<td>24.9±0.41</td>
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<td>SBP, mmHg</td>
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<td>160.8±7.0</td>
<td>NS</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>99.1±6.3</td>
<td>97.2±4.5</td>
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<td>Serum Na, mmol/L*</td>
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<td>142.3±0.18</td>
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<tr>
<td>Serum K, mmol/L*</td>
<td>3.4±0.058</td>
<td>4.1±0.033</td>
<td>$P&lt;0.05$</td>
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<td>Proportion of diabetes mellitus, %</td>
<td>11.5</td>
<td>11.6</td>
<td>NS</td>
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<tr>
<td>18-oxocortisol, ng/dL*</td>
<td>23.6±3.4</td>
<td>1.89±0.14</td>
<td>$P&lt;0.05$</td>
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<tr>
<td>18-hydroxycortisol, ng/dL*</td>
<td>357.1±34.6</td>
<td>128.6±7.3</td>
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<td>Aldosterone, ng/dL*</td>
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<td>PRA, ng/mL per hour</td>
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<td>0.23±0.021</td>
<td>NS</td>
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<td>ARR, ng/dL per ng/mL per hour*</td>
<td>363.3±38.7</td>
<td>87.1±4.50</td>
<td>$P&lt;0.05$</td>
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<td>Cortisol after 1-mg DST, μg/dL</td>
<td>1.19±0.059</td>
<td>0.94±0.035</td>
<td>$P&lt;0.05$</td>
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<td>Captopril-challenged ARR, ng/dL per ng/mL per hour*</td>
<td>232.5±33.4</td>
<td>63.0±3.70</td>
<td>$P&lt;0.05$</td>
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<td>30, 6, 85</td>
<td>NS</td>
</tr>
<tr>
<td>Size of CT-detected adrenal nodules, mm</td>
<td>15.3±2.9</td>
<td>19.6±6.1</td>
<td>NS</td>
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</tbody>
</table>

Data are shown as mean±SEM, unless otherwise stated. Proportion of diabetes mellitus shows percentage of patients with glycated hemoglobin of ≥6.5%; APA indicates aldosterone-producing adenoma; ARR, aldosterone:renin activity ratio; BHA, bilateral hyperaldosteronism; Bi, bilateral; CT, computed tomography; DBP, diastolic blood pressure; DST, dexamethasone suppression test; F, female; M, male; NS, not significant; PRA, plasma renin activity; SBP, systolic blood pressure; and Uni, unilateral.

* $P<0.05$.
we examined whether p18oxoF concentration served to differentiate 52 patients with APA whose PAC were ≤32.7 ng/dL, which was the maximum level in patients with BHA. Of particular interest, 34 (65%) of these 52 patients with APA who had p18oxoF concentrations >6.1 ng/dL were considered to require surgery (Figure 2). Therefore, 95 (84%) of the 113 patients with APA could be differentiated by the values of PAC and p18oxoF.

Moreover, we measured p18oxoF in patients with EH by excluding PA and any other cause of secondary hypertension. The p18oxoF (1.3±0.2 ng/dL) in 79 patients with EH was significantly lower than that in patients with APA but not significantly different from that in patients with BHA. The highest concentration of p18oxoF in patients with EH was 5.6 ng/dL, which was lower than the highest value in patients with BHA (Figure S3). Interestingly, there were 52 (43%) of 121 patients with BHA (Figure 2) and 52 (66%) of 79 patients with EH (Figure S3) whose p18oxoF was <1.2 ng/dL, the lowest 18oxoF concentration in patients with APA.

In addition, we measured p18oxoF and p18OHF in 27 patients with micro-APA who underwent adrenalectomy based on their AVS findings and who were clinically and histopathologically confirmed. The levels of both p18oxoF (1.80±0.26 ng/dL) and p18OHF (147±21.4 ng/dL) in these 27 patients with micro-APA were significantly lower than those patients with CT-detectable APA but not significantly different from those in patients with BHA. The highest levels of p18oxoF, p18OHF, PAC, and ARR in patients with micro-APA were 4.8 ng/dL, 483 ng/dL, 24.5 ng/dL, and 245 ng/dL per ng/mL per hour, respectively, and their distributions were almost overlapped with those in patients with BHA (Figure S4). None of these patients with micro-APA with or without contralateral unilateral nonfunctioning adrenocortical nodules had a p18oxoF level >6.1 ng/dL, which has been considered as the cut-off value to recommend adrenalectomy.

Furthermore, we added analyses about the relationship among p18oxoF, AVS lateralization index and somatic KCNJ5 mutations, and we summarized these results in the online-only Data Supplement.
Discussion

In this study, we measured p18oxoF and p18OHF in 261 (234+27) patients with PA, who were subsequently classified based on the results of AVS into 113 patients with APA, 121 patients with BHA, and 27 patients with micro-APA. In all the 113 patients with APA and 27 patients with micro-APA who underwent adrenalectomy, the diagnosis was further confirmed postoperatively based on PAC and immunohistochemical analysis of steroidogenic enzymes in resected adrenocortical tissue.25-27 We found that p18oxoF was a more reliable diagnostic parameter to differentiate between APA and BHA than p18OHF. The possibility of measuring 18oxoF by LC–MS/MS (lower limit: 0.25 ng/dL) has enabled us to detect a low concentration in peripheral plasma,21 and to differentiate APA from BHA (or EH) without the need to have patients to collect the 24-hour urine. Thus, this is the first study to demonstrate that the peripheral blood concentrations of 18oxoF measured by LC–MS/MS can clinically differentiate between APA and BHA with a reasonable precision. In particular, 84% of patients with APA had a p18oxoF concentration above 6.1 ng/dL or PAC of >32.7 ng/dL. In this study, patients with APA represented only those with a CT-detectable aldosterone-producing adenoma and did not include any of the patients with micro-APA. Moreover, this accuracy rate of 84% was obtained based on p18oxoF and CT findings. This is the primary reason why this accuracy rate of 84% was much higher than that found in previous studies.8-12 The discriminatory value of CT imaging alone was 72% as described above, which was not so different from that obtained in previous studies.8-12 Besides, in 43% of patients with BHA, p18oxoF was <1.2 ng/dL, which was the lowest value found in patients with APA. In addition, further analysis of the 27 patients with micro-APA revealed that 8 (30%) of them harbored contralateral adrenocortical nodules on CT images and the highest p18oxoF and PAC were 4.8 and 24.5 ng/dL, respectively. These values were significantly lower than their respective cut-off values of 6.1 and 32.7 ng/dL. Also, 30 of 121 patients with BHA had CT-detectable unilateral nonfunctioning adrenocortical nodules. Consequently, the evaluation of p18oxoF in such patients might contribute to avoid an unnecessary surgery in an institution where AVS is not available for preoperative routine evaluation of PA patients with unilateral adrenal nodules.

On the basis of the results of this present study, we recommend adding p18oxoF to the diagnostic workup for PA (Figure 3). Throughout this systematic clinical workup, we might have been able to limit the use of AVS in 87 patients with PA whose p18oxoF was between 1.2 and 6.1 ng/dL, a range in which patients with APA and BHA were found to overlap after selecting the patients highly suspected of having APA and those with BHA (Figures 2 and 3).

In this study, the LC–MS/MS intra-assay accuracy and precision were 90% to 111% and 3% to 9% for 18OHF and 18oxoF, respectively. Those of interassay were 87% to 108% and 1% to 10% for 18OHF and 18oxoF, respectively. The lower limits of quantification of 18OHF and 18oxoF were 2.5 and 0.25 ng/dL, respectively. The 3% to 9% precision of LC–MS/MS indicated, therefore, that this method was considered as a reliable mode of measurement in this setting. Further to this, the cut-off value was higher than the lower limit of quantification. Thus, we think that precision of the measurement method was demonstrated by the results obtained. Likewise, the sensitivity and the specificity of the cut-off value were influenced by the precision of the measurement method. Nevertheless, it is entirely true that the 3% to 9% precision of this method indicated that the clinical diagnosis of AP should be carefully performed in patients whose 18OHF and 18oxoF values are around the cut-off value. 18-Hydroxycorticosterone was also reported to help discriminate APA from BHA, especially after posture test.28 However, Mulatero et al29 reported that basal levels of 18-hydroxycorticosterone did not help to differentiate PA subtypes in their study and because we wanted to find surrogate markers to discriminate PA subtypes by their basal peripheral blood levels, we selected 18OHF and 18oxoF. Because

Figure 3. Diagnostic flow model based on peripheral aldosterone and 18-oxocortisol levels. Those with confirmed primary aldosteronism (PA) and both an aldosterone level >32.7 ng/dL and a unilateral nodule by computed tomography (CT) undergo adrenalectomy without adrenal venous sampling (AVS). Those with an aldosterone level ≤32.7 ng/dL are divided into 3 subgroups depending on their peripheral 18-oxocortisol level to determine subsequent diagnostic workup steps. APA indicates aldosterone-producing adenoma; ADX, adrenalectomy; BHA, bilateral hyperaldosteronism; GRA, glucocorticoid-remediable aldosteronism; MRA, mineralocorticoid receptor antagonist; p18oxoF, peripheral 18-oxocortisol; PAC, plasma aldosterone concentration; and PCR, polymerase chain reaction.
Peripheral Plasma 18-Oxocortisol in PA

18oxoF production was reported to be small but could be localized with CYP11B2, we thought that if we developed a sensitive LC–MS/MS method, we could expect to measure even basal p18oxoF and be able to differentiate APA from BHA. In contrast, 18OHF can be produced by both CYP11B2 and CYP11B1 in the zona glomerulosa and the zona fasciculata, respectively, resulting in higher secretion of 18OHF compared with 18oxoF. In this study, p18OHF was also higher than p18oxoF. However, the ratio of p18OHF between APA and BHA was merely one fifth that of p18oxoF, indicating that the sensitivity of differentiation by p18OHF (0.62) is lower than that by p18oxoF (0.83). Specificity of p18OHF (0.96) about subtyping was reasonably high as same as that by p18oxoF (0.99), and therefore, p18OHF can also be a reliable diagnostic tool to discriminate APA from BHA.

A major drawback of measuring p18oxoF by LC–MS/MS is the cost but this (≈150 US dollars per sample) is far less expensive than that of AVS, which amounts to ≈10000 US dollars in the United States. Moreover, we propose a diagnostic strategy for APA by precisely measuring p18oxoF after selecting patients with APA according to PAC from among confirmed patients with PA (Figure 3). Through this approach, we should be able to reduce the medical cost by not only omitting an unnecessary AVS procedure but also by limiting p18oxoF measurement. Therefore, we could reconfirm the usefulness of this strategy, which might have enabled 95 (ie, 61 + 34) of the 113 patients with APA (84%) to undergo surgery after the detection of unilateral adrenal nodules by CT scanning without subjecting them to AVS, and of the 121 patients with BHA (43%; p18oxoF < 12 ng/dL) to receive pharmacological treatment with mineralocorticoid receptor antagonists without performing AVS. Furthermore, the highest concentration of p18oxoF in patients with EH was 5.6 ng/dL (<6.1 ng/dL), and in 52 (66%) of 79 patients with EH p18oxoF was < 1.2 ng/dL, the lowest p18oxoF in patients with APA. Therefore, these results indicated that higher or lower levels of 18oxoF could serve to predict the presence or absence APA without further confirmatory evaluation in the patients with high ARR, but a further larger study will be required to confirm this interesting hypothesis.

Perspectives

The results of the large study revealed that measurement of 18oxoF in peripheral blood by LC–MS/MS should enable us to improve the clinical algorithm for PA, potentially omitting unnecessary AVS, which is a expensive and invasive procedure, and requires the dedicated work of experienced radiologists. Yet, AVS is still the only reliable method for discriminating the cause of hyperaldosteronism at specialized institutions where skillful radiologists make efforts to perform successful catheterizations. In particular, AVS is the only way to distinguish a CT-undetectable micro-APA from BHA even though no standardization of protocols has been performed yet. In this study, p18oxoF levels in patients with micro-APA were completely overlapped with those in patients with BHA, and AVS is therefore considered critical in differentiating these 2 subtypes. This was a monocentric, retrospective study in Japanese patients. A prospective, multicentric study in centers treating different ethnicities is necessary to confirm the results.

Acknowledgments

We thank Akane Sugawara and Yasuko Tsukada for their secretarial assistance, and also Kumi Kikuchi for laboratory assistance.

Sources of Funding

Y. Nakamura was partly supported by Takeda Science Foundation. W.E. Rainey was partly supported by grants from the National Institutes of Health (DK069950). The other authors report no conflicts.

Disclosures

None.

References


### Summary

**What Is New?**

- This is the first study to demonstrate that the peripheral blood concentration of 18-oxocortisol measured by liquid chromatography tandem mass spectrometry can be a clinically significant discriminating marker between aldosterone-producing adenoma and bilateral hyperaldosteronism.

**What Is Relevant?**

- This much simpler, noninvasive and less expensive measurement of 18-oxocortisol by liquid chromatography tandem mass spectrometry might allow us to assign patients with aldosteronoma for surgery without undergoing adrenal venous sampling.

**Novelty and Significance**

**Discrimination by the peripheral plasma level of 18-oxocortisol >6.1 ng/dL or aldosterone >32.7 ng/dL allowed classification of 95 of 113 (84%) patients with aldosteronoma, and 18-oxocortisol <1.2 ng/dL allowed differentiation of 52 of 121 (43%) patients with bilateral hyperaldosteronism. This noninvasive measurement of peripheral plasma 18-oxocortisol concentration by liquid chromatography tandem mass spectrometry might contribute to further subclassification of primary aldosteronism.**
Measurement of Peripheral Plasma 18-Oxocortisol Can Discriminate Unilateral Adenoma From Bilateral Diseases in Patients With Primary Aldosteronism


Hypertension. 2015;65:1096-1102; originally published online March 16, 2015; doi: 10.1161/HYPERTENSIONAHA.114.04453

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/65/5/1096

Data Supplement (unedited) at:
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http://hyper.ahajournals.org/content/suppl/2016/04/11/HYPERTENSIONAHA.114.04453.DC2

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Peripheral Plasma 18-Oxocortisol Can Discriminate Unilateral Adenoma from Bilateral Diseases in Primary Aldosteronism Patients

Brief title: Peripheral Plasma 18-Oxocortisol in Aldosteronoma

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Supplemental Methods

Methods of computed tomography scanning:
The computed tomography (CT) scanner used in this population of the patients was an Aquilion 64-detector row helical CT scanner (Toshiba, Tokyo, Japan). Four-phase dynamic scans were obtained with the following parameters: 0.5 second per rotation, 0.5 mm collimation, and 20.5 mm/sec table increment (pitch factor = 0.64). Before scanning, 100 mL of a non-ionic contrast media containing 300 mg of iodine per milliliter was injected into an antecubital vein of the patients at a rate of 4.0 mL/sec. The scan delay was set by means of an automatic triggering system (SureStart; Toshiba). When the attenuation value at the level of the ascending aorta reached the preset threshold (CT value of the abdominal aorta on plain CT plus 100HU {Hounsfield Unit}), early-arterial-phase scanning was automatically initiated. Late-arterial-phase scanning also started at 13 seconds after the completion of the early arterial scanning. Venous phase and delayed-phase scanning followed at 70 seconds and 3 minutes after the start of the contrast injection, respectively. Transverse sections were reconstructed with a 0.5-mm section thickness at 0.5-mm intervals. When evaluating the right adrenal vein (RAV), the reconstruction field of view was set to the area around the aorta, inferior vena cava (IVC) and both kidneys. This methodology has been used in our other publications.1,2

Methods of CT interpretation:
In this study, images were interpreted using a stand-alone workstation (AQ station; Terarecon, Tokyo, Japan). We used a multiplanar reformation (MPR) display mode which could allow simultaneous cine-mode observation of axial, coronal, and saggital MPR images to evaluate the RAV. The image analysis is based on Radiology report formulated before the analysis of the hormone level. At least two board certified radiologists were involved in interpretation of the findings in a blind fashion and when interobserver differences occurred, these diagnostic radiologists evaluated the images together to reach the consensus. Moreover, they carefully reanalyzed the images of the contralateral adrenal of the cases with the radiology reports of unilateral nodules in the blind fashion but there were no differences between the first and second reports. This is because at least two radiologists were involved in formulating the reports of adrenal images when generating the first report through reconstituting continual images by the latest imaging software. This methodology has been used in our other publications.1-3

Sample preparation for LC-MS/MS measurement of 18OHF
The plasma samples were prepared as previously reported.4 F-d4 (0.25 ng) and Aldo-d7 (0.05 ng) were added to plasma (0.1 mL) as internal standard. Acetonitrile (0.2 mL) was added to the
sample and the mixture was centrifuged to precipitate proteins. The diluted supernatant was applied to an InertSep Pharma cartridge, and the steroidal fraction was eluted with 80% acetonitrile (1 mL). After the eluate was evaporated to dryness, the residue was treated with 0.25 ml of 35% HCl in ethanol (1:5, vol/vol) at room temperature for 30 min. Derivatization of 18O HF, F-d4, 18oxoF, and Aldo-d 7 was performed according to the mixed anhydride method using fusaric acid. The resulting mixture was transferred to an InertSep SI cartridge and then washed successively with 50% hexane-ethyl acetate and 50% ethyl acetate-acetonitrile. The desired fraction was eluted with 50% ethyl acetate-acetonitrile (1.5 mL) and evaporated. The residue was dissolved in 40% acetonitrile (0.1 mL) and subjected to LC/MS/MS.

**Supplemental Results**

**The relationship among p18oxoF, AVS lateralization index (LI) and somatic KCNJ5 mutations**

LI (16.5 ± 2.0, mean ± SEM) after ACTH stimulation in 113 APA cases was significantly correlated with p18oxoF levels (Spearman’s r = 0.2681, \( P < 0.05 \)). We compared the p18oxoF levels of thirty-four somatic-KCNJ5-mutated patients with those of fourteen wild type patients in our study. The results demonstrated that the p18oxoF levels of somatic-KCNJ5-mutated cases (35.3 ± 9.1 ng/dL, mean ± SEM) were not significantly different from those of wild type patients (20.3 ± 5.4 ng/dL, mean ± SEM) \( (P=0.090 \) by unpaired t test with Welch’s correction), possibly due to the relatively small number of patients examined in this particular study. To our great regret, we could secure the informed consent for evaluating somatic KCNJ5 mutations from only 48 APA of the 113 APA patients examined in this study. Yet, the p18oxoF level in patients bearing a somatic-KCNJ5 mutation tended to be higher than that in wild type patients but further studies are required to examine whether p18oxoF can predict somatic-KCNJ5 mutations of individual patients.

**References**


Supplemental Figure S1  Comparison between aldosterone measured by LC-ESI-MS/MS and that measured by SPAC-S RIA demonstrated a statistically significant correlation
(Spearman’s r = 0.9055, P < 0.0001); LC-ESI-MS/MS, liquid chromatography-electrospray ionization- tandem mass spectrometry; RIA, radioimmunoassay.
Supplemental Figure S2  2SA, 2SB, Comparison between peripheral PAC and 18oxoF levels subsequently demonstrated a statistically significant correlation between APA and BHA groups, those with APA showed the most marked correlation (Spearman’s $r = 0.5336$, $R^2 = 0.6488$, $P < 0.05$) (2SA), compared to those with BHA (Spearman’s $r = 0.1987$, $R^2 = 0.0367$, $P < 0.05$) (2SB); 2SC, 2SD, In contrast, comparison between peripheral PAC and 18OHF levels demonstrated a significant correlation in the APA group (Spearman’s $r = 0.4886$, $R^2 = 0.4886$, $P < 0.05$) (2SC) but not in the BHA group (2SD); PAC, plasma aldosterone concentration; 18oxoF, 18oxo-cortisol; 18OHF, 18-hydroxycortisol; APA, aldosterone producing adenoma; BHA, bilateral hyperaldosteronism.
Supplemental Figure S3. Closed circles mean peripheral plasma aldosterone concentrations of 79 EH patients, and closed triangles mean peripheral 18oxoF concentrations of those patients. The highest concentration of p18oxoF in EH patients was 5.6 ng/dL, which was lower than the highest value (6.1 ng/dL) in BHA patients; 18oxoF, 18oxo-cortisol; EH, essential hypertension; BHA, bilateral hyperaldosteronism.
**Supplemental Figure S4.** Closed circles mean peripheral plasma aldosterone concentrations of 27 microAPA patients, and closed triangles mean peripheral 18oxoF concentrations of those patients. The highest concentration of p18oxoF in microAPA patients was 4.8 ng/dL, which was lower than the highest value (6.1 ng/dL) in BHA patients; 18oxoF, 18oxo-cortisol; microAPA, CT-undetectable micro aldosterone producing adenoma; BHA, bilateral hyperaldosteronism.
微生物与高血压（摘要）

肠道微生态失调与高血压有关

Gut Dysbiosis Is Linked to Hypertension

Tao Yang, Monica M. Santisteban, Vermaite Rodriguez, Eric Li, Niousha Ahmari, Jessica Marulanda Carvajal, Meigan Zadéh, Minghao Gong, Yanfei Qi, Jasenka Zubcevic, Bikash Sahay, Carl J. Pepine, Mohan K. Raizada, Mansour Mohamadzadeh

新的证据表明，肠道菌群对维持生理动态平衡至关重要。由于遗传、环境和饮食因素对肠道菌群和高血压都有明显影响，这项研究的目的旨在检验肠道菌群生态失调与高血压是否相关。这项研究对2个高血压大鼠模型和一个小的队列患者的粪便样本的细菌DNA进行细菌基因组分析。研究观察到自发性高血压大鼠的微生物丰富度、多样性和均匀度都显著减少，厚壁菌门/拟杆菌的比率增高。这些变化伴随着醋酸和乳酸菌的减少。另外还发现，小鼠模型中大鼠的微生物群具有类似的人群失调模式，即群组的人群菌群丰富度和多样性都较低。在慢性血管紧张素II灌注大鼠模型中观察到类似的肠道菌群变化：微生物丰富度显著减少和厚壁菌门/拟杆菌门比率升高。在这一模型中，我们评估了口服二甲胺四环素恢复肠道菌群的效率。除了减轻高血压之外，二甲胺四环素能够通过降低硬壁菌门/拟杆菌门的比率重新平衡高血压肠道菌群失调。这些观察结果表明，无论在动物实验还是人群研究中高血压和肠道菌群失调都存在相关性。研究认为，通过饮食干预来改善肠道菌群可能是一种创新性的高血压营养治疗策略。

(Hypertension. 2015;65:1331-1340)

原发性醛固酮增多症（摘要）

外周血18-氧皮质醇的测定可区分原发性醛固酮增多症患者的单侧腺瘤及双侧病变

Measurement of Peripheral Plasma 18-Oxocortisol Can Discriminate Unilateral Adenoma From Bilateral Diseases in Patients With Primary Aldosteronism


肾上腺静脉血取样是当前区分原发性醛固酮增多症（primary aldosteronism）患者为单侧还是双侧病变的唯一可靠方法。在本研究中，我们试图对已经确诊的234例原发性醛固酮增多症患者（primary aldosteronism）的患者113例中计算机断层扫描（computed tomography, CT）可探测到醛固酮腺瘤（aldosteronoma）的患者及121例双侧病变型醛固酮增多症（bilateral hyperaldosteronism）的患者，评估外周血18-氧皮质醇（18-oxocortisol, 18oxof）及18-氧皮质醇（18-hydroxycortisol）的水平有助于鉴别单侧醛固酮腺瘤及双侧醛固酮增多症。所有患者进行了CT检查及肾上腺静脉血取样。所有的醛固酮腺瘤均进行了手术切除，并以临床特征及组织病理学检查证实诊断的准确性。18-氧皮质醇及18-氧皮质醇通过两相色谱串联质谱法（liquid chromatography tandem mass spectrometry）测定。18-氧皮质醇的腺瘤及双受体为4.7 ng/dl，敏感度/特异度为0.83/0.99，与之相比，18-氧皮质醇腺瘤及双受体为0.62/0.96。在113例醛固酮腺瘤患者中，95例（84%）的18-氧皮质醇水平为6.1 ng/dl，醛固酮水平为32.7 ng/dl；而在121例双侧病变型醛固酮增多症患者中，没有1例患者达到这一水平，这121例双侧病变的醛固酮增多症患者中，有32例患者CT可探测其单侧肾上腺有功能性结节。另外，在121例双侧病变型醛固酮增多症患者中，有52例（43%）患者的18-氧皮质醇水平低干醛固酮腺瘤的最低水平（1.2 ng/dl）。进一步分析发现，27例CT未探测到单侧醛固酮腺瘤的患者中，8例患者CT可探测到对侧肾上腺结节，其18-氧皮质醇水平分别为4.8 ng/dl及24.5 ng/dl，分别低于前述的生理值。结论：外周血18-氧皮质醇浓度不仅有助于区分醛固酮瘤和双侧醛固酮增多症，还有助于避免对同时伴有增生性或微结节的无功能性肾上腺皮质结节行不必要的手术治疗。

(Hypertension. 2015;65:1096-1102.)