Plasma Metanephrine and Adrenal Venous Sampling

Plasma Metanephrine for Assessing the Selectivity of Adrenal Venous Sampling


Abstract—Adrenal vein sampling is used to establish the origins of excess production of adrenal hormones in primary aldosteronism. Correct catheter positioning is confirmed using adrenal vein measurements of cortisol, but this parameter is not always reliable. Plasma metanephrine represents an alternative parameter. The objective of our study was to determine the use of plasma metanephrine concentrations to establish correct catheter positioning during adrenal vein sampling with and without cosyntropin stimulation. We included 52 cosyntropin-stimulated and 34 nonstimulated sequential procedures. Plasma cortisol and metanephrine concentrations were measured in adrenal and peripheral venous samples. Success rates of sampling, using an adrenal to peripheral cortisol selectivity index of 3.0, were compared with success rates of metanephrine using a selectivity index determined by receiver operating characteristic curve analysis. Among procedures assessed as selective using cortisol, the adrenal to peripheral vein ratio of metanephrine was 6-fold higher than that of cortisol (94.0 versus 15.5; P<0.0001). There were significant positive relationships between adrenal to peripheral vein ratios of cortisol and metanephrine for cosyntropin-stimulated samplings but not for nonstimulated samplings. Receiver operating characteristic curve analysis indicated a plasma metanephrine selectivity index cutoff of 12. Using this cutoff, concordance in sampling success rates determined by cortisol and metanephrine was substantially higher in cosyntropin-stimulated than in nonstimulated samplings (98% versus 59%). For the latter procedures, sampling success rates determined by metanephrine were higher (P<0.01) than those determined by cortisol (91% versus 56%). In conclusion, metanephrine provides a superior analyte compared with cortisol in assessing the selectivity of adrenal vein sampling during procedures without cosyntropin stimulation. (Hypertension. 2013;62:1152-1157.) • Online Data Supplement

Key Words: cortisol ■ cosyntropin ■ hyperaldosteronism ■ metanephrine

Adrenal hypertension caused by primary aldosteronism comprises the most common curable form of secondary hypertension. In the analytic workup of patients with primary aldosteronism, adrenal venous sampling (AVS) is recommended for establishing the origins of excess production of hormones.1 AVS is a technically demanding procedure in which correct cannulation of the adrenal veins, especially the right, can pose significant difficulty.2,3 Correct positioning of the catheter is verified by measurement of plasma cortisol concentrations. High cortisol concentrations in adrenal blood compared with peripheral blood ascertain correct catheter placement and thus selective sampling. Because cortisol has a long circulating half-life (100 minutes), increases in adrenal vein (AV) blood above levels of peripheral venous (PV) blood are relatively minor and subsequently subject to interpretative error. Furthermore, as a result of physiological corticotropin fluctuations, cortisol is secreted in a variable fashion so that fluctuating levels can interfere with the interpretation of AVS selectivity.4-6 This problem can be overcome using cosyntropin stimulation.7 Cosyntropin stimulation, however, adds to the complexity of the procedure and for this reason is not always used.

With the above considerations in mind, there seems a need for more reliable parameters than cortisol in assessing the correct positioning of catheters during AVS.8 Plasma metanephrine, the O-methylated metabolite of epinephrine, represents one such alternative analyte. More than 90% of plasma metanephrine is produced within the adrenal medulla, with <10% produced from epinephrine after release from the adrenals.7 Compared with cortisol, plasma metanephrine has a short circulating half-life of 3 to 6 minutes, resulting in close to 90-fold increases of AV compared with PV concentrations in situations where catheters are correctly positioned.7 Such large

Received April 23, 2013; first decision May 9, 2013; revision accepted August 31, 2013.

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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.113.01601/-/DC1.

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Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.113.01601

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gradients should provide more accurate and sensitive means to detect the correct AV site of sampling than the smaller gradients of plasma cortisol. Importantly, adrenal production of metanephrine occurs as a result of leakage of adrenaline from storage vesicles into the cytoplasm where the amine is metabolized by catechol-O-methyltransferase. This process occurs continuously and independently of adrenaline release. Hence, plasma concentrations of metanephrine show relatively little increase in response to stress. We hypothesized that the continuous adrenal production and rapid circulatory clearance of metanephrine might provide advantages for measurements of the metabolite compared with cortisol in assessing the correct positioning of catheters during AVS. We further hypothesized that any advantage would be most apparent for procedures conducted without cosyntropin stimulation. The purpose of this study was to, therefore, determine the usefulness of AV measurements of metanephrine compared with cortisol concentrations to establish selective cannulation in AVS with and without cosyntropin stimulation.

Methods
An expanded Methods section is available in the online-only Data Supplement.

Subjects
We included 83 consecutive patients who underwent a total of 86 AVS procedures between 2010 and 2012 at the Radboud University Nijmegen Medical Center and the University Hospital Düsseldorf (Table 1). At the Radboud University Nijmegen Medical Center, all AVS procedures were performed under continuous cosyntropin stimulation of 50 μg/h with sequential catheterization of AVs (n=52). At the University Hospital Düsseldorf, all procedures were performed without cosyntropin stimulation with sequential catheterization of AVs. PV samples were collected simultaneously with each AV sample to account for cortisol fluctuations (n=34). Blood was collected with gentle negative pressure, and heparinized blood samples were directly stored on ice.

Informed consent was obtained under approved clinical protocols from all patients at Düsseldorf and 35 patients at Nijmegen. In 14 patients at Nijmegen, consent was waived by the local ethics committee. This was in accordance with the applicable rules on reviews by research ethics committees and informed consent.

Measurements of Cortisol, Metanephrines, and Catecholamines
At the Radboud University Nijmegen Medical Center, cortisol measurements were performed by electrochemiluminescence immunoassays using a Modular E170 analyzer (Roche diagnostics, Woerden, The Netherlands). At the University Hospital Düsseldorf, cortisol measurements were performed by an Elecsys analyzer (Roche Diagnostics, Mannheim, Germany). Plasma concentrations of metanephrines and catecholamines were measured at a single central laboratory (Hospital Carl Gustav Carus, Dresden) using liquid chromatography with tandem mass spectrometry or electrochemical detection.

Data and Statistical Analysis
The cortisol-derived selectivity index (SI) was calculated as the concentration of cortisol in AV samples divided by that in PV samples. A cortisol SI of ≥3.0 was used to determine successful catheterization. In addition, the effect of lowering this cutoff to ≥2.0 was analyzed. The metanephrine-derived selectivity index was calculated from the ratio of AV to PV plasma metanephrine concentrations and the selectivity index cutoff established by receiver operating characteristic curve analyses. Ratios of concentrations of metanephrine to normetanephrine and of epinephrine to norepinephrine in AV and PV plasma were also calculated to assess the use of these parameters for establishing correct AV catheter positioning. Data are expressed as means and SDs or, in case of skewed distributions, as medians and ranges. P<0.05 was considered significant.

Results

AV Cortisol, Metanephrine, and Epinephrine for Selective Samplings
With a cortisol-derived selectivity index of ≥3.0 to define selective samplings, plasma concentrations of metanephrine and epinephrine were considerably higher (P<0.0001) in right and left AV samples than in PV samples with and without cosyntropin stimulation (Table 2). AV and PV concentrations of cortisol and right AV and PV concentrations of epinephrine were higher (P<0.05) in samplings with than without cosyntropin stimulation. As indicated by ratios of AV to PV concentrations of cortisol, metanephrine, and epinephrine, PV to AV increases in plasma metanephrine and epinephrine were, respectively, 6.1- and 19.0-fold higher (P<0.0001) than those in cortisol (Table 2). The difference in combined left and right AV/PV ratios for metanephrine compared with cortisol was larger (P=0.001) in studies without than with cosyntropin stimulation (9.9 versus 5.4), whereas no difference was present for epinephrine (19.6 versus 18.5).

Ratios of Metanephrine to Normetanephrine and Epinephrine to Norepinephrine
Selective AV samples showed metanephrine to normetanephrine ratios and epinephrine to norepinephrine ratios that were, respectively, 10- and 41-fold higher (P<0.0001) than the ratios in PV samples (Figure S1 in the online-only Data Supplement). The 2.5 and 97.5 percentiles of these ratios in AV samples showed no overlap with those of PV samples.

AV Cortisol and Metanephrines for Nonselective Samplings
For AV samples in which a cortisol selectivity index of 3.0 did not confirm correct catheter positioning (Table S1), metanephrine to normetanephrine ratios were within the 2.5 and 97.5 percentiles of ratios for confirmed AV samples in more (P<0.0001) samplings without than with cosyntropin stimulation (89% versus 22%). Similarly, AV/PV ratios of metanephrine were on average 37-fold higher (P<0.0001) without than with cosyntropin stimulation.
In contrast, there were no relationships between AV/PV ratios for metanephrine and cortisol for right AV ($r = -0.040$; 95% confidence interval, $-0.30$ to $0.37$; $P = 0.41$) and left AV ($r = 0.229$; 95% confidence interval, $-0.13$ to $0.53$; $P = 0.096$) samplings without cosyntropin stimulation (Figure 2B).

**AVS Selectivity Determined by Plasma Cortisol**

AV/PV cortisol ratios ≥3.0 indicated successful final positioning of catheters at both AVS sites in 83% of studies with cosyntropin stimulation, substantially more ($P < 0.01$) than the 56% of studies without stimulation (Table 3). A lower SI cutoff of 2.0 increased ($P < 0.05$) success rates of selective AV catheterizations for nonstimulated samplings to 79% but was without significant effect for cosyntropin-stimulated samplings.

**Receiver Operating Characteristic Curve Analysis to Determine the AVS Selectivity Index of Metanephrine**

Receiver operating characteristic curve analyses exploring the performance of cosyntropin-stimulated AV/PV ratios of metanephrine to assess the selectivity of AVS sampling, with a cortisol selectivity index of 3.0 as the reference index, established an area under the curve of 0.999 (Figure S3). In contrast, the area under the curve for nonstimulated samplings was only 0.673 and not significantly improved using a cortisol selectivity index of 2.0 (0.702). Using the receiver operating characteristic curve for stimulated samplings, an AV/PV selectivity index of between 11.3 and 15.3 for metanephrines provided optimal sensitivity (99%) and specificity (100%), with no difference in either sensitivity or specificity within this selectivity index range to establish selective sampling. A selectivity index of 12 was therefore chosen to maintain high sensitivity (Figure S3).

**AVS Selectivity Determined by Plasma Metanephrine Versus Cortisol**

Using the selectivity index cutoffs of ≥3 for cortisol and ≥12 for metanephrine, there was disagreement in the assessment of correct catheter positioning in only 1 of the total 113 left and right AV samples obtained with cosyntropin stimulation (Figure 2A). This translated to a concordance rate for bilateral successful catheterization of 98% (51/52), reflecting no difference in the overall success of AV samplings determined by cortisol (83%) or metanephrine (83%) for studies with cosyntropin stimulation (Table 3). For procedures without cosyntropin stimulation, there was disagreement in the assessment of catheter positioning according to cortisol and metanephrine in 17 of the 68 samplings (Figure 2B); in all except 1 case, this involved AV/PV ratios below the cutoff of 3.0 for cortisol and >12.0 for metanephrine. This translated to a concordance rate of only 59% (20/34) for establishing bilateral success of AVS, substantially lower ($P = 0.0001$) than that of 98% for cosyntropin-stimulated samplings. Using metanephrine, AVS was assessed as bilaterally successful in 91% of samplings, considerably more ($P < 0.01$) than the 56% (19/34) using the cutoff of 3.0 for cortisol (Table 3). Using a lower cutoff of 2.0 improved successful bilateral selectivity to 79%; nevertheless, for 5 of the 7 samplings with AV/PV ratios of cortisol <2.0, AV/PV ratios of metanephrine

### Table 2. Adrenal and Peripheral Venous Plasma Concentrations and Adrenal Venous to Peripheral Venous Ratios of Cortisol, Metanephrine, and Epinephrine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cosyntropin stimulated</th>
<th>Nonstimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol, µg/dL</td>
<td>n=34 (3.4–5.2)</td>
<td>n=24 (3.4–5.2)</td>
</tr>
<tr>
<td>PV</td>
<td>31 22 (14–36)*</td>
<td>26 21 (11–31)*</td>
</tr>
<tr>
<td>RAV</td>
<td>42 25 (12–45)†</td>
<td>31 20 (9–40)†</td>
</tr>
<tr>
<td>Metanephrine, pg/mL</td>
<td>n=10 (3.4–5.2)</td>
<td>n=6 (3.4–5.2)</td>
</tr>
<tr>
<td>PV</td>
<td>31 20 (14–36)*</td>
<td>26 15 (9–30)*</td>
</tr>
<tr>
<td>RAV</td>
<td>42 25 (12–45)†</td>
<td>31 20 (9–40)†</td>
</tr>
<tr>
<td>Epinephrine, pg/mL</td>
<td>n=10 (3.4–5.2)</td>
<td>n=6 (3.4–5.2)</td>
</tr>
<tr>
<td>PV</td>
<td>31 20 (14–36)†</td>
<td>26 15 (9–30)†</td>
</tr>
<tr>
<td>RAV</td>
<td>42 25 (12–45)†</td>
<td>31 20 (9–40)†</td>
</tr>
<tr>
<td>Cortisol AV/PV ratios</td>
<td>n=10 (3.4–5.2)</td>
<td>n=6 (3.4–5.2)</td>
</tr>
<tr>
<td>RAV</td>
<td>42 25 (12–45)†</td>
<td>31 20 (9–40)†</td>
</tr>
<tr>
<td>Metanephrine AV/PV ratios</td>
<td>n=10 (3.4–5.2)</td>
<td>n=6 (3.4–5.2)</td>
</tr>
<tr>
<td>RAV</td>
<td>42 25 (12–45)†</td>
<td>31 20 (9–40)†</td>
</tr>
</tbody>
</table>

*P<0.0001 different from RAV and LAV.
†P<0.05 different from corresponding sampling site in cosyntropin-treated patients.
‡P<0.016 different from LAV.

To convert to SI units of nmol/L, multiply by 27.59 for cortisol and divide by the molecular weight for metanephrine (197.2) and epinephrine (183.2). Data are shown for both with and without cosyntropin stimulation for selective samplings according to a cortisol-derived SI of 3.0. AV indicates adrenal vein; LAV, left adrenal vein; PV, peripheral vein; and RAV, right adrenal vein.

### Relationships of Plasma Cortisol, Metanephrine, and Epinephrine

There were positive ($P < 0.01$) relationships of right and left AV plasma cortisol concentrations with both metanephrine and epinephrine concentrations in respective right and left AV samples for procedures with cosyntropin stimulation (Figure 1A and 1B). In contrast, there were no relationships between plasma cortisol with metanephrine or epinephrine for procedures without cosyntropin stimulation (Figure 1C and 1D). Nevertheless, for both procedures, positive relationships were observed between plasma epinephrine and metanephrine (Figure S2).

### Relationships of AV/PV Ratios for Plasma Metanephrine Versus Cortisol

Significant positive relationships between AV/PV ratios for metanephrine and cortisol were observed for right AV ($r = 0.764$; 95% confidence interval, 0.62–0.86; $P < 0.001$) and left AV ($r = 0.577$; 95% confidence interval, 0.36–0.73; $P < 0.001$) samplings with cosyntropin stimulation (Figure 2A).
were between 36 and 244, well above the cutoff of 12 (Figure 2B and Table S1).

**Discussion**

This study establishes novel use of plasma metanephrine as a more sensitive alternative to cortisol to assess the selectivity of AVS. Plasma metanephrine is particularly useful during AVS performed without cosyntropin stimulation for several reasons: (1) excellent agreement between use of cortisol and metanephrine in samplings performed with but not without cosyntropin stimulation; (2) larger step-ups in PV to AV plasma concentrations of metanephrine relative to cortisol; and (3) higher rates of success for establishing AVS selectivity using metanephrine than cortisol in nonstimulated samplings.

In agreement with emerging findings from other groups,5,6,13,14 the above considerations conversely imply that cortisol provides a less than optimal parameter to establish selectivity of AVS are further indicated by the complete lack of relationships between AV plasma cortisol with metanephrine or epinephrine during procedures without cosyntropin stimulation.

There are several reasons why the advantages of plasma metanephrine compared with cortisol for confirming correct positioning of AV catheters are most apparent for procedures without cosyntropin stimulation. First, as demonstrated by others,4–6 adrenal secretion of cortisol fluctuates so that AV plasma concentrations during periods of low secretion may be only slightly higher than those in peripheral plasma, providing the rationale for cosyntropin stimulation. In contrast, metanephrine is produced continuously within adrenal medullary cells from epinephrine leaking from storage vesicles, a process that is independent of fluctuations in epinephrine release.7,9,10 Second, without cosyntropin stimulation, up to a third of circulating cortisol may be produced and released designated nonselective, based on a cortisol-derived selectivity index of 3.0, were well above the range for ratios in PV samples and within the range for the AV samples designated as selective. These shortcomings in use of cortisol to indicate the selectivity of AVS are further indicated by the complete lack of relationships between AV plasma cortisol with metanephrine or epinephrine during procedures without cosyntropin stimulation.

Figure 1. Correlation of plasma metanephrine, plasma epinephrine, and plasma cortisol for cosyntropin-stimulated (upper row: A and B) and nonstimulated (bottom row: C and D) adrenal vein sampling. Spearman correlation coefficient $r_s$ is given for each sampling location. Conversion factor to SI units—cortisol (nmol/L): 27.59; epinephrine (pmol/L): 5.454; metanephrine (pmol/L): 5.07. LAV indicates left adrenal vein; PV, peripheral vein; and RAV, right adrenal vein.

Figure 2. Correlation between cortisol ratio and metanephrine ratio for the cosyntropin-stimulated (A) and nonstimulated (B) samplings. The cutoff for the cortisol ratio ($\geq 2$ and $\geq 3$) and the metanephrine ratio ($\geq 12$) is represented by the vertical and horizontal dashed lines, respectively. AV indicates adrenal vein; LAV, left adrenal vein; PV, peripheral vein; and RAV, right adrenal vein.
from extra-adrenal locations, particularly hepatosplanchnic sites.\textsuperscript{15,16} This extra-adrenal source contributes to peripheral cortisol levels and potentially affects the selectivity index. Furthermore, a previous study showed that admixture of blood from the accessory hepatic veins into AVs lowers the selectivity index of cortisol.\textsuperscript{17} In contrast, >90% of all circulating metanephrine is produced within the adrenals, with <10% produced from epinephrine after release.\textsuperscript{7,10} Third, cortisol is cleared from the circulation slowly, resulting in high peripheral plasma concentrations relative to rates of secretion and consequently relatively small step-ups in concentrations from PV to AV sites of release that are more easily detected by stimulating secretion with cosyntropin after release.\textsuperscript{7,10} In contrast, metanephrine is cleared rapidly from the circulation so that PV concentrations are maintained at much lower levels compared with those at AV sites where most of the metabolite enters the systemic circulation.\textsuperscript{18,19} All the above factors likely contribute to the consistently high gradients in PV to AV plasma concentrations of metanephrine, which provide an opportunity for more accurate and sensitive detection of selective AV catheterization than the smaller gradients for cortisol or other substances evaluated for this purpose, such as chromogranin.\textsuperscript{8,20} Additional consideration of the much higher ratios of metanephrine to normetanephrine in AV than PV plasma provides a further means for confirming correct positioning of AV catheters. Because measurements of metanephrine are commonly performed together with normetanephrine, the additional use of metanephrine to normetanephrine ratios offers another advantage of measuring these metabolites not possible with measurements of cortisol.

Although others have proposed measurements of epinephrine to assess the selectivity of AVS and although PV to AV gradients in plasma epinephrine are larger than those in metanephrine, we nevertheless recommend metanephrine for two reasons. First, epinephrine, like cortisol, is a stress hormone that exhibits extreme physiological fluctuations, whereas metanephrine does not.\textsuperscript{5,8,10,21} Second, metanephrines are more stable than catecholamines, so that more care must be taken with blood collections for the latter than the former.\textsuperscript{22,23}

In addition to stimulating release of cortisol, cosyntropin increases adrenal blood flow and release of epinephrine,\textsuperscript{24,25} which could influence adrenal medullary and cortical-derived indices of AV selectivity. Our findings of higher plasma concentrations of epinephrine with cosyntropin stimulation are consistent with effects on adrenal medullary function. Nevertheless, lack of influence of cosyntropin on metanephrine indicates that the influence does not extend to the metabolite, an expected observation given the independent nature of chromaffin cell epinephrine metabolism and exocytotic release.

The present study had some methodological limitations. First, the study did not incorporate a prospective, randomized design comparing therapeutic outcomes according to cortisol with metanephrine because this was not possible without proof that metanephrine was at least as good as cortisol in indicating the selectivity of AVS. Second, stimulated and nonstimulated AVS procedures were performed at two different centers with measurements of cortisol by different methods. Nevertheless, this was unlikely to have influenced results because the two methods yield comparable results.\textsuperscript{26} A third limitation was the use of plasma cortisol selectivity ratios as the reference standard. All AV selectivity indices used in both research and clinical practice are arbitrary because they have not been formally linked to outcome data in an evidence-based manner. However, the cutoffs used in our study are commonly used and recommended in the literature.\textsuperscript{2,27}

**Perspectives**

In view of the importance of primary aldosteronism as a cause of hypertension, an accurate diagnosis of the site of excess aldosterone production is pivotal. AV sampling is recommended as the reference test to differentiate between unilateral and bilateral excess aldosterone production. However, technical success depends on correct positioning of sampling catheters in AVs, verified using measurements of cortisol. This study shows that these measurements fail to verify correct positioning of catheters in a substantial number of procedures performed without cosyntropin, a failing that can be overcome by measurements of plasma metanephrine. Should improved therapeutic outcomes using metanephrine be established in a prospective study, cosyntropin stimulation may become redundant and AV sampling less laborious and more diagnostically accurate than currently practiced. Nevertheless, in this event it must be recognized that the wider availability of measurements of plasma metanephrines is required for their routine use in AVS to be fully realized.

**Acknowledgments**

We express our gratitude to Daniela Pelzel for technical assistance. We also thank Martin Reincke and the Else-Kröner-Fresenius German Conn Registry.

**Sources of Funding**

This study was supported by a grant from the Deutsche Forschungsgemeinschaft to H.S. Willenberg, J.W.M. Lenders, and G. Eisenhofer (WI 3660/1-1, KFO252) and a grant from ZonMW DoelmatigheidsOnderzoek 2010–2012 (E&K (171002102) to J. Deinum. Grant providers had no influence on the content of the article.

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**Table 3. Success Rates of Selective AV Samplings With and Without Cosyntropin Stimulation According to Cortisol-Derived and Metanephrine-Derived SI Cutoffs**

<table>
<thead>
<tr>
<th>AVS Procedures</th>
<th>No. (%) Based on Cortisol (Cutoff 3.0)</th>
<th>No. (%) Based on Cortisol (Cutoff 2.0)</th>
<th>No. (%) Based on Metanephrine (Cutoff 12.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cosyntropin stimulated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAW</td>
<td>44/52 (85)</td>
<td>46/52 (89)</td>
<td>43/52 (83)</td>
</tr>
<tr>
<td>LAV</td>
<td>51/52 (98)</td>
<td>52/52 (100)</td>
<td>51/52 (98)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>43/52 (83)</td>
<td>46/52 (89)</td>
<td>43/52 (83)</td>
</tr>
<tr>
<td>Nonstimulated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAW</td>
<td>26/34 (76)</td>
<td>31/34 (91)*</td>
<td>32/34 (94)*</td>
</tr>
<tr>
<td>LAV</td>
<td>24/34 (71)†</td>
<td>29/34 (85)**</td>
<td>33/34 (97)*</td>
</tr>
<tr>
<td>Bilateral</td>
<td>19/34 (56)†</td>
<td>27/34 (79)*</td>
<td>31/34 (91)*</td>
</tr>
</tbody>
</table>

AV indicates adrenal vein; AVS, AV sampling; LAV, left adrenal vein; and RAV, right adrenal vein.

*P<0.05 higher than corresponding success rates determined by a cortisol-derived cutoff of 3.0.

†P<0.05 lower than corresponding success rate in cosyntropin-stimulated samplings.
Disclosures

None.

References


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Hypertension. 2013;62:1152-1157; originally published online September 30, 2013;
doi: 10.1161/HYPERTENSIONAHA.113.01601
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/62/6/1152

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Plasma Metanephrine for Assessing Selectivity of Adrenal Venous Sampling

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Expanded methods

Subjects
We included 83 consecutive patients who underwent a total of 86 AVS procedures between 2010 and 2012 at the Radboud University Nijmegen Medical Centre and the University Hospital Düsseldorf. AVS was performed to differentiate between unilateral and bilateral primary aldosteronism (n=77), to examine the functional state of an incident aloma (n=4), to evaluate bilateral adrenal masses in subclinical Cushing’s syndrome (n=1) and to assess non-classic (late-onset) congenital adrenal hyperplasia (n=1). Informed consent was obtained under approved clinical protocols from all patients at Düsseldorf and 35 patients at Nijmegen. In 14 patients at Nijmegen consent was waived by the local ethics committee. This was in accordance with the applicable rules concerning reviews by research ethics committees and informed consent.

Adrenal venous sampling
Prior to AVS, interfering medications were discontinued in accordance with current guidelines. AVS was performed after an overnight fast and at least three hours of bed rest. In cases of primary aldosteronism, hypokalemia, if present, was corrected with oral or intravenous potassium supplementation before AVS. At the Radboud University Nijmegen Medical Centre all AVS procedures were performed under continuous ACTH stimulation of 50 μg/hr with sequential catheterization of both adrenal veins (N=52). At the University Hospital Düsseldorf all procedures were performed without ACTH stimulation, with sequential catheterization of adrenal veins and simultaneous collection of PV and AV samples (N=34).

Blood was collected by gravity or with gentle negative pressure. Cortisol assays were performed during procedures using rapid measurements to confirm correct catheter placement, with measurements subsequently repeated according the methods outlined below for more accurate measurement. Blood samples were immediately stored on ice in lithium-heparin tubes and within 1 hour centrifuged at 3500g at 4°C for 10 minutes. Thereafter plasma was stored at -80°C.

Measurements of cortisol, metanephrines and catecholamines
At the Radboud University Nijmegen, cortisol measurements were performed by electrochemiluminescence immunoassays using a Modular E170 analyzer (Roche diagnostics Woerden, the Netherlands). Inter-assay coefficients of variation (CVs) were 2.3-3.8%. At the UHD cortisol measurements were performed by an Elecsys analyzer (Roche Diagnostics, Mannheim, Germany) with an inter-assay CV 6.1%. Sample dilutions, performed to bring cortisol concentrations within the assay range, were carried out using the kit buffers with maintained CVs.

Plasma concentrations of metanephrine and normetanephrine were measured by liquid chromatography with tandem mass spectrometry following sample purification using a solid phase extraction 96 well plate format. Inter-assay CVs for metanephrines ranged from 3.7% at high plasma concentrations to 13.5% at low concentrations. Sample dilutions were not required for these measurements.

Plasma concentrations of norepinephrine and epinephrine — along with additional measurements of the catecholamine precursor, dihydroxyphenylalanine (DOPA), and the metabolites dihydroxyphenylglycol (DHPG) and dihydroxyphenylacetic acid (DOPAC) — were measured by liquid chromatography with electrochemical detection after batch alumina extraction. DHPG and DOPAC are present in plasma at higher concentrations than the catecholamines, have a relatively narrower concentration range, and are similarly sensitive to oxidative degradation as the catecholamines. Their measurement in this study thereby enabled assessment of this potential source of artifact and exclusion of catecholamine measurements in 2 out of 52 cosyntropin-stimulated and 17 out of 34 non-stimulated samplings. Inter-assay CVs for plasma catecholamines ranged from 2.5% to 11.0%. Sample dilutions were also not required for these measurements.

In studies involving cosyntropin stimulation, additional data from 9 non-selective (SI < 3.0) blood samples (8 right AV and 1 left AV) obtained from AVS procedures in which further searching for the adrenal vein subsequently yielded selective sampling results, were included in the analysis. These additional non-selective samplings were included to delineate analyte concentrations at non-
selective sampling sites and establish relationships between AV concentrations and AV:PV ratios of cortisol and metanephrine.

**Data analysis**
The cortisol-derived selectivity index was calculated as the concentration of cortisol in AV samples divided by that in PV samples. For both cosyntropin-stimulated and non-stimulated samplings, a cortisol selectivity index of ≥ 3.0 was used to determine successful catheterization. In addition, the effect of lowering this cut-off to ≥ 2.0 was analyzed.

The metanephrine-derived selectivity index was similarly calculated as the ratio of the AV to PV plasma concentrations of metanephrine, with the selectivity index cutoff cut-off established by receiver operating characteristic (ROC) curve analyses according to established procedures. A cortisol derived SI of ≥3.0 was utilized as the gold standard to establish which samples were taken from correctly positioned catheters.

Ratios of concentrations of metanephrine to normetanephrine and of epinephrine to norepinephrine in AV and PV plasma were also calculated to assess use of these parameters for additionally establishing correct AV catheter positioning.

**Statistical analysis**
Data are expressed as means and standard deviations or, in case of skewed distributions, as medians and ranges. Mann-Whitney U, Kruskal-Wallis or Wilcoxon matched paired sign-rank tests were used to assess significance of differences in variables at the three sampling sites, or between groups. A Bonferroni-adjusted P-value ($P_{adjusted} = 0.05/3 = 0.0167$) was used to determine significance for differences among the three sampling sites. For other differences, a $P<0.05$ was considered significant. Relationships between cortisol, metanephrine and epinephrine were assessed by one-tailed Spearman’s correlation coefficient ($r_s$).

Differences between AVS success rates determined by cortisol and metanephrine derived SIs in cosyntropin-stimulated and non-stimulated AVS were determined by McNemar and Chi-square tests according to whether comparisons were paired or non-paired. Corresponding 95% confidence intervals (95% CI) for non-paired comparisons were calculated using the Wilson Score Method without continuity correction. Statistical analyses utilised the JMP statistics software package (SAS Institute Inc, Cary, NC), GraphPad Prism 4.0 and SPSS 18.0 for windows.

**References**


### Supplemental table S1. Adrenal Venous to Peripheral Venous Cortisol and Metanephrine Ratios and Adrenal Venous Metanephrine to Normetanephrine Ratios for Non-selective Samplings (AV:PV cortisol ratio <3.0)

<table>
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<tr>
<th>Subject</th>
<th>AV sampling side</th>
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<th>AV MN:NMN ratio</th>
<th>AV:PV metanephrine ratio</th>
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<tr>
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<tr>
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<td>R</td>
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</table>

Abbreviations: AV, adrenal venous; PV, peripheral venous; MN, metanephrine; NMN, normetanephrine, R, right; L, left. * Indicates an AV MN:NMN ratio within the 2.5 to 97.5 percentiles of those determined for selectively positioned AV catheters (see Figure S1). † Indicates a subject in whom initial non-selective sampling was followed by a selective sampling result;
Legend to supplemental figure S1
Peripheral venous (PV) and adrenal venous (AV) plasma metanephrine to normetanephrine ratios (panel A) and plasma epinephrine to norepinephrine ratios (panel B) for selective samplings. Dotted line: 2.5 percentile of the AV samples. Dashed line: 97.5 percentile of the PV samples. The 2.5 and 97.5 percentiles of these ratios in adrenal venous samples showed no overlap with those for peripheral venous samples.
Legend to supplemental figure S2
Correlation of plasma metanephrine and plasma epinephrine for cosyntropin-stimulated (panel A) and non-stimulated (Panel B) adrenal venous samplings. Spearman correlation coefficients ($r_s$) are shown for each sampling location. RAV = right adrenal vein; LAV = left adrenal vein; PV = peripheral vein. Conversion factor to SI units: Epinephrine (pmol/l): 5.45; Metanephrine (pmol/l): 5.07.
Legend to supplemental figure S3. ROC curve analysis exploring the diagnostic performance of cosyntropin-stimulated (Panel A) and non-stimulated (Panel B) AV:PV ratios of metanephrine to assess selectivity of AVS sampling, according to a cortisol selectivity index of ≥ 3.0. The AV:PV metanephrine ratio most appropriate to indicate selective adrenal venous sampling was established for the point on the ROC curve for cosyntropin-stimulated sampling that provided both optimal diagnostic sensitivity (99%) and specificity (100%). This point corresponded to an AV:PV ratio between 11.3 and 15.3.