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Subtyping of patients with primary aldosteronism: an update

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Running title: Subtyping of primary aldosteronism

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27 **Abstract**

28 Primary aldosteronism (PA) comprises two main subtypes: unilateral aldosteronism, mainly caused by
29 aldosterone-producing adenoma; and bilateral adrenal hyperplasia. Establishing the correct subtype in
30 patients with PA is indispensable for choice of treatment. In addition to established methods, alternative
31 tests are evolving for subtyping. Computed tomography (CT) and adrenal venous sampling (AVS) are
32 currently recommended in the guidelines for the diagnostic work-up of patients with PA. CT cannot be
33 used as a stand alone test for subtyping because of its limited accuracy but may be used in combination
34 with other tests such as AVS or functional imaging. Nevertheless CT remains mandatory to exclude
35 adrenocortical carcinoma. AVS provides the most accurate test to detect excessive secretion of
36 aldosterone from an adrenal mass but has several practical limitations and disadvantages. Therefore
37 alternative non-invasive and patient-friendly methods are required to determine the need for
38 adrenalectomy. Functional imaging with specific molecular positron emission tomographic ligands is a
39 potential alternative method that may replace AVS for patients with PA. The results of preliminary
40 studies of ¹¹C-metomidate are promising but ligands incorporating radionuclides with longer half-lives
41 that selectively bind to CYP11B2 are needed. Steroid profiling provides another method for subtyping
42 and selecting patients for adrenalectomy but this technology is in its infancy and prospective outcome-
43 based studies are required to determine if this technique may provide an alternative to AVS.

44

45 **Key words:** adrenal, adenoma, hyperplasia, sampling, steroid, imaging

46

47 **Introduction**

48 Among patients with primary aldosteronism (PA) excessive aldosterone secretion from a single adrenal
49 may be caused by a unilateral adrenocortical aldosterone-producing adenoma (APA) (~40% of all cases
50 of PA) or more rarely by unilateral hyperplasia. Bilateral aldosterone excess results from micronodular
51 or macronodular hyperplasia and is designated as bilateral adrenal hyperplasia (BAH) (~60% of all cases
52 of PA) [1, 2]. Due to differential expression of steroidogenic enzymes there can be variable secretion of
53 steroids other than aldosterone. In particular, APAs often secrete higher amounts of corticosterone,
54 deoxycorticosterone and the hybrid steroids, 18-hydroxycortisol and 18-oxocortisol, compared to
55 adrenals of BAH patients [3].

56 Although the concept of the distinct nature of both subtypes is controversial, definitive and reliable
57 distinction of unilateral and bilateral disease is imperative for therapeutic management of patients with
58 PA. Unilateral disease is usually treated by adrenalectomy (ADX) whereas bilateral disease is treated
59 using mineralocorticoid receptor antagonists. Therefore the primary goal of adequate subtyping is not
60 just to determine the correct diagnosis but to select correctly the patients with unilateral disease who can
61 be expected to benefit from surgery (i.e. those with the best chance of complete clinical and biochemical
62 remission). As a histological diagnosis is only possible for the removed adrenal, the diagnosis in the
63 other gland remains obscure.

64 A conclusive final diagnosis of unilateral disease is feasible when the post-surgical follow-up data
65 show complete biochemical cure [4]. Patients with bilateral disease (BAH) will not be intentionally
66 operated and therefore a conclusive diagnosis can never be established for this group. A glimpse into
67 the histopathology of BAH may be provided from patients operated for supposedly unilateral disease
68 who subsequently show persistent aldosteronism following ADX. In addition some patients with BAH
69 and 'asymmetric' aldosterone secretion may also undergo ADX as a consequence of their severe
70 phenotype and a last resort for treatment [5].

71 There are several methods potentially useful for differentiating unilateral (APA) from bilateral
72 disease (BAH): 1. adrenal computed tomography (CT) or magnetic resonance imaging (MRI); 2. adrenal
73 venous sampling (AVS); 3. targeted functional imaging using a radiolabeled metomidate PET tracer;

74 and 4. measurements of peripheral venous steroid profiles. Currently AVS is the only method
75 recommended according to the Endocrine Society guideline to distinguish both subtypes and for
76 selecting patients eligible for ADX [6]. Functional imaging and steroid profiling are evolving and
77 promising methods but their true value for reliable subtyping requires validation. In this article we
78 summarize these four methods and review their pro's and con's for their role in subtyping patients with
79 PA.

80

81 **Anatomical imaging by CT and MRI**

82 Starting in the 1970's when CT became available, this anatomic imaging modality enjoyed continuous
83 use for locating adrenal adenomas in patients with a biochemical diagnosis of PA. CT is relatively
84 inexpensive and non-invasive and provides high spatial resolution for imaging an adrenal mass.
85 Subsequently MRI has provided an alternative anatomical imaging modality but apart from lack of
86 radiation exposure carries no advantages over CT for subtyping PA patients [6].

87 Multidetector CT scanning has several serious drawbacks for selecting PA patients for ADX (Table
88 1). First, CT (and also MRI) provide an anatomical and not a functional diagnosis, so that these imaging
89 modalities cannot establish whether an adrenal adenoma is actually the source of any excessive
90 aldosterone production. This is important since non-functional adenomas are not uncommon,
91 particularly with advancing age when prevalences increase to 10% in patients older than 50 years [7].
92 For this reason CT suffers from limited specificity. Diagnostic sensitivity remains also far from optimal.
93 Although CT is generally reported to be unable to detect microadenomas (< 1 cm) with any degree of
94 certainty, modern CT scans using thinner slices may detect microadenomas of less than 1 cm. Many
95 APAs have a size of 1-2 cm but it has also been shown that adenomas of less than 1 cm account for
96 nearly 25-50% of all APAs [8, 9]. Thus, a substantial number of patients with APAs who might be cured
97 by ADX can be missed by CT.

98

99 The value of CT for subtype diagnosis has been addressed in numerous studies, but most have been
100 retrospective with poorly standardized clinical and biochemical follow-up criteria to classify outcomes.

101 Additionally, most studies used AVS as the reference standard. A systematic review showed that in
102 nearly 38% of PA patients, results from CT or MRI indicated the incorrect APA or BAH subtype and
103 would have resulted in inappropriate treatment [10]. Nevertheless, this high proportion was obtained
104 under the assumption that AVS provides the perfectly correct subtype classification. As outlined later,
105 this assumption is not fully justified. Nevertheless, in contrast to the outlined retrospective studies, a
106 recent prospective study showed persistent PA in 9 of 46 patients (20%) who underwent ADX based on
107 CT scan [11]. Volumetric analysis of the adrenal glands demonstrated, that mean adrenal gland volume
108 in APA patients was not significantly different from BAH adrenal volume [12]. Moreover, volumes of
109 the contralateral adrenal in APA patients were significantly larger in comparison to non-PA control
110 adrenals.

111 Despite all above considerations, CT should still be carried out as the initial imaging test in PA
112 patients for two reasons: a CT scan helps to rule out the occasional adrenocortical carcinoma that
113 produces aldosterone [6]. Second, contrast enhanced CT provides useful information on the venous
114 anatomy, which facilitates cannulation in those patients who need to undergo AVS [13, 14].

115

116 **Adrenal venous sampling**

117 Adrenal venous sampling (AVS) is recommended by the Endocrine Society guideline to distinguish
118 APA and BAH subtypes [6]. Except for young patients (<35 years) with clear-cut PA and an unilateral
119 adenoma, all patients should undergo AVS to select those amenable for unilateral ADX. AVS is a
120 functional test as opposed to CT and the concept that this technique is able to establish the source of
121 aldosterone excess is intuitively very appealing. It is not surprising that use of AVS has shown a steep
122 increase in major referral centres worldwide. This technique involves selective cannulation of the
123 adrenal veins and one peripheral vein for measurements of plasma aldosterone and cortisol
124 concentrations [15-17]. The technical success rates of correct adrenal vein cannulation is over 90% in
125 hands of experienced radiologists and the reported complication rate is less than 2.5% [6].

126 Accurate placement of catheters is essential for reliable assessment of adrenal aldosterone
127 secretion. However, selective cannulation, particularly of the right adrenal vein, is notoriously difficult

128 and complicated by considerable anatomic variations [14]. Commonly, the ratio of plasma
129 concentrations of cortisol in the adrenal vein (AV) to a peripheral vein (PV) is used to verify and
130 document accurate catheter placement. This ratio, also called selectivity index (SI), is calculated as
131 cortisol AV/PV. An additional purpose of measuring cortisol is to correct for dilution from non-adrenal
132 blood contamination. There are several drawbacks of using cortisol for these purposes. First, in
133 comparison to aldosterone, cortisol is protein-bound (transcortin) and has a long circulating half-life.
134 Thus, the step-ups in peripheral to adrenal vein cortisol concentrations are relatively modest and subject
135 to interpretative error. An additional disadvantage is that some APAs can co-secrete cortisol [18, 19].
136 Potentially this may further invalidate the use of cortisol to determine the correct catheter position.
137 Finally, cortisol is subject to pulsatile stress-mediated increments, thus impairing its usefulness for
138 correct catheter positioning.

139 To minimize stress induced variations in cortisol secretion, cosyntropin stimulation is used at many
140 centers [20]. There are two advantages of cosyntropin stimulation: it increases the selectivity index and
141 thereby the technical success rates of AVS and it obviates the need for simultaneous adrenal vein blood
142 sampling. However, cosyntropin stimulation makes the procedure more complex, there is no consensus
143 about the optimal stimulation protocol and in terms of outcome it is still controversial whether its use
144 affects the accuracy of subtyping [16, 17].

145 Since 2015 several alternatives to cortisol have been identified to provide improved assessment of
146 of selectivity of adrenal vein sampling. Measurements of plasma metanephrine, the O-methylated
147 metabolite of epinephrine, in particular show much larger step-ups from peripheral to adrenal venous
148 plasma than cortisol and in many cases have clearly indicated selective sampling when measurements
149 of cortisol suggested incorrect catheter placement [20]. Measurements of metanephrine is also
150 particularly useful in for APAs that cosecrete cortisol [21, 22]. Since metanephrine is produced
151 continuously within adrenal medullary cells from epinephrine leaking from chromaffin storage granules,
152 a process that is independent of catecholamines secretion, these measurements should also not be
153 affected by stress. Several steroids, including as dehydroepiandrosterone, androstenedione, 11-
154 deoxycortisol and 17-hydroxyprogesterone also produce much larger step-ups from peripheral to adrenal

155 venous plasma than cortisol and as such also provide superior biomarkers than cortisol to assess the
156 selectivity of adrenal vein sampling [23-25]. It is time to give up on use of cortisol as an indicator of
157 adrenal vein sampling selectivity. Apart from metanephrine and the four steroids outlined above, almost
158 all steroids produced as part of the adrenal biosynthetic backbone produce higher selectivity indices than
159 cortisol and are thereby also likely to offer superior assessments of correct catheter placement than
160 cortisol [23].

161 Lateralization of aldosterone hypersecretion is currently commonly assessed by calculation of the
162 lateralization index (LI) using the higher ratio of aldosterone to cortisol in one vein compared to the
163 other. The cut-off level of the LI depends on whether cosyntropin stimulation is used or not but most
164 centres use an LI of >4 to diagnose unilateral PA and of <3 to diagnose bilateral PA [16]. In patients
165 with an LI between 3.0-4.0, lateralization can be diagnosed when the contralateral suppression index
166 (defined as the ratio of aldosterone/cortisol between the non-dominant adrenal vein and a peripheral
167 vein) is ≤ 1.0 . Many centres use the contralateral suppression index as an extra criterium for lateralization
168 but there are no outcome studies to show unequivocally that contralateral suppression is a useful
169 diagnostic criterium [16, 20].

170 There are several limitations to the AVS technique that need to be considered (Table 1). It is a
171 complex, technically demanding, time consuming and an invasive technique that is not convenient for
172 patients or medical staff [16, 17]. Invasiveness includes not only catheter manipulations but also
173 exposure to radiation, although this depends on the proficiency of the interventional radiologist. More
174 importantly, the procedure is not standardized and this applies in particular to the use of the cut-off
175 levels to determine selectivity and lateralization. Lack of medical staff with the necessary technical
176 expertise is one of the main reasons why AVS is insufficiently available to accommodate all patients
177 that should undergo AVS according to the Endocrine Society guideline. Moreover, currently only a
178 small fraction of hypertensive patients with PA are identified and if all patients with PA were actually
179 identified through hypertensive population screening it is highly unlikely that any centre could cope
180 with the subsequent demand for AVS.

181 An major consideration to all the above concerns the accuracy of AVS to establish the correct
182 subtype of PA. The increasing use has gradually led to the contention that AVS is the ‘gold’ standard
183 test but AVS has never been scrutinized according to the recommendations of the GRADE consortium
184 [26]. Under conditions in which no ‘gold’ standard test is available, a prespecified outcome representing
185 the benefits for the patients should be determined. In cases of patients who undergo ADX for unilateral
186 disease (APA) the biochemical and clinical remission rates reflect the endpoints that determine the long-
187 term outcome for patients. The many published studies that reported on the diagnostic accuracy of AVS
188 have a retrospective design with an inherent potential for bias. In addition, most studies suffered from
189 incomplete or non-standardized follow-up data to assess outcome. Even more importantly, management
190 of patients in comparative studies was determined by the result of AVS. That AVS is not a ‘gold’
191 standard test can also be derived from recently published data. An international multicentre study using
192 standardized follow-up data showed that the biochemical cure of 94% (range: 83-100) was lower than
193 the 96-100% as published in a recent systematic review [27, 28]. A recently published randomized
194 controlled outcome trial comparing management based on AVS versus CT could not establish a better
195 clinical and biochemical outcome after one year of follow-up [11]. Although AVS is currently still the
196 best performing test for subtyping of patients with PA, further and better designed studies are required
197 to determine its true value for selecting patients with PA for ADX.

198

199 **Functional imaging using radiolabeled metomidate PET tracers**

200 Prior to the development of new radiolabeled tracers for molecular imaging, one of the first agents used
201 for functional imaging of APAs was the norcholesterol derivative, ¹³¹I-6β-iodomethyl-19-
202 norcholesterol, also named NP-59 [29]. One retrospective study suggested that NP-59 might be helpful
203 as functional imaging agent to select patients for ADX [30]. However, this was a small group of selected
204 patients with PA in whom CT and AVS were inconclusive. This technique has major disadvantages that
205 explain why this technique has been abandoned for subtyping patients with PA: it is time consuming, it
206 is associated with a high radiation exposure (30 mSv) to the adrenal glands, the tracer is not widely

207 available and most importantly, uptake of the tracer is poor in small APAs and the spatial resolution to
208 detect these small adenomas is insufficient, even if SPECT/CT imaging is used.

209 In more recent years an extensive search has evolved for agents suitable for highly specific
210 molecular PET imaging of adrenocortical tissue. One of the first agents was ^{11}C -metomidate that has the
211 capacity to bind with high specificity and avidity to the enzymes exclusively expressed in the adrenal
212 cortex and involved in steroid synthesis, including CYP11B1 and CYP11B2 [31]. However ^{11}C -
213 metomidate has a low selectivity for CYP11B2 over CYP11B1. This agent was shown to be potent for
214 differentiating adrenocortical from non-adrenocortical tissue and has been used in several studies to
215 identify the adrenal lesion [32].

216 Several studies have assessed the diagnostic value of ^{11}C -metomidate in patients with PA, but most
217 studies were not designed to evaluate its role in subtyping [33]. One study addressed the value ^{11}C -
218 metomidate PET/CT in patients with PA in a head-to-head design using AVS as the reference test [34].
219 The sensitivity and specificity of ^{11}C -metomidate PET/CT, employing a ratio of tumor SUVmax
220 (standardized uptake value) to normal adrenal background SUV of >1.25 for diagnosing APA, was 76%
221 and 87% respectively. This small series of patients is promising but the applicability of this tracer is
222 limited because one needs an on-site cyclotron due to the short 20 minute half-life of ^{11}C . Radioisotopes
223 with a longer half-life are more desirable. For subtyping of patients with PA, one would need a molecular
224 tracer capable of binding specifically to CYP11B2 (aldosterone synthase). This is not only a crucial
225 enzyme in aldosterone synthesis but its expression in smaller APAs is increased as compared to
226 CYP11B1 (Table 1). Potential fluorine-18 labeling of such compounds will make them of practical use
227 for PET scanning. Several new promising compounds for this purpose are currently under investigation
228 in *in vitro* and in animal studies. A preliminary report of such compound (CDP2230) showed a more
229 than 15 times higher selectivity for CYP11B2 over CYP11B1 compared to ^{11}C -metomidate [35]. These
230 data suggest that this compound has the potential to become a promising new agent for subtyping
231 patients with PA.

232

233 **Steroid measurements**

234 A new emerging method for subtyping of patients with PA involves measurements of additional steroids
235 to aldosterone, including the hybrid steroids 18-hydroxycortisol and 18-oxocortisol. This has been
236 fostered by the development of liquid chromatography with tandem mass spectrometry (LC-MS/MS)
237 technology [36]. This technology is not only more accurate than immunoassays but enables
238 simultaneous measurements of multiple steroids in a single sample [23].

239 Several studies have examined whether adrenals from APAs and BAH are associated with a specific
240 steroid signature as measured in urine or in adrenal venous plasma while more recently a few studies
241 addressed the value of steroid profiling in a peripheral venous blood sample for subtyping. Some studies
242 focused on one hormone, others used a panel of steroids.

243 The first evidence that patients with APAs differed from patients with BAH in steroid secretion
244 came from the group of Biglieri in 1979, showing that the use of a peripheral venous plasma 18-
245 hydroxycorticosterone level of >100ng/dL was highly predictive for an APA [37]. This could be
246 confirmed several years later by other groups [38, 39]. More recently the basis for this finding was
247 provided by a small study reporting a nearly 3-fold higher concentration of 18-hydroxycorticosterone in
248 adrenal veins draining from APAs than in those from BAH [40]. The additional finding of a high
249 concordance between the 18-hydroxycorticosterone/cortisol ratio and the aldosterone/cortisol ratio in
250 the APA patients indicated however that measurement of 18-hydroxycorticosterone was not more useful
251 for lateralization than aldosterone itself. Given the lack of larger prospective studies, measurement of
252 18-hydroxycorticosterone in adrenal or peripheral venous plasma has not made its way into routine
253 clinical practice and cannot be recommended for selecting patients for ADX.

254 Most recently we carried out a retrospective study in a large group of 216 patients with PA,
255 comprising 126 patients with an APA and 90 patients with BAH [41]. Aldosterone, 18-oxocortisol and
256 18-hydroxycortisol were all higher in adrenal venous samples from APAs compared to BAH. The hybrid
257 steroids were not overly helpful for lateralization as they showed lateralization in only 76% (n=90) (18-
258 oxocortisol) and 35% (18-hydroxycortisol) of cases where lateralization was apparent using aldosterone.
259 Another retrospective study confirmed also higher 18-oxocortisol levels in adrenal blood from APAs

260 than BAH but found no evidence that the use of the 18-oxocortisol/cortisol ratio was superior to the
261 aldosterone/cortisol ratio for subtyping patients with PA [42].

262 As far back as 1992 Ulick and co-workers established that patients with APA showed higher urinary
263 excretion of the hybrid steroids, 18-hydroxycortisol and 18-oxocortisol, than patients with BAH [43]. A
264 later study that assessed both plasma and urinary steroids came to a similar conclusion although they
265 noticed considerable overlap between both groups [39]. Although the sensitivity of the urinary 18-
266 hydroxycortisol excretion for the APA diagnosis was only 30%, an excretion rate of > 510 ug/day was
267 diagnostic for APA, suggesting that such patients with proven PA and a unilateral adenoma on CT could
268 be sent straight for ADX.

269 There are a few retrospective studies that aimed to evaluate the role of steroids in a peripheral
270 venous blood sample (Table 1). Satoh et al reported on 18-hydroxycortisol and 18-oxocortisol levels
271 measured in peripheral venous samples using LC-MS/MS [44]. They established much higher plasma
272 levels of both steroids in patients with APA than in those with BAH with relatively stronger increments
273 for 18-oxocortisol (12.5 fold) than for 18-hydroxycortisol (2.5 fold). Plasma 18-oxocortisol displayed a
274 sensitivity and specificity of 83% and 99% using a cut-off level of 4.7 ng/dL. All patients with an APA
275 showed 18-oxocortisol levels of >1.2 ng/dL while no patient with BAH showed a level of >6.1 ng/dL.
276 If combined with plasma aldosterone concentration, 84% of the patients with APA with a unilateral
277 adenoma had a plasma aldosterone concentration of >32.7 ng/dL and a plasma 18-oxocortisol
278 concentration of >6.1 ng/dL. These results suggest that in nearly 50% of all patients with PA, AVS could
279 be circumvented because these patients could have been sent straight for ADX.

280 The value of a peripheral venous steroid profile for predicting the correct subtypes (APA vs BAH)
281 was reported in a recent large retrospective study of 216 patients with PA [41]. In 80% (172/216) of
282 patients it was possible to classify the PA subtype correctly (based on post-ADX outcome as reference
283 method) using a panel of 12 steroids (aldosterone, 18-oxocortisol, 18-hydroxycortisol, 11-dexycortisol,
284 21-deoxycortisol, corticosterone, 11-deoxycorticosterone, progesterone, 17-hydroxyprogesterone,
285 androstenedione, DHEA and DHEAS). This classification, shows promise but requires further

286 improvements. It should be noted however that that study used an AVS procedure with cosyntropin
287 stimulation in some patients, thus potentially introducing bias.

288 In a related study we measured a panel of 15 steroids in 79 patients with PA who were genotyped
289 for mutations in the *KCNJ5*, *ATPIA1*, *ATP2B3* and *CACNAID* genes [45]. A remarkable finding was
290 that the APAs with *KCNJ5* mutations showed the highest peripheral plasma levels of 18-oxocortisol
291 compared with all other mutations and the wild-type group. A subpanel of 7 steroids (aldosterone, 18-
292 oxocortisol, 18-hydroxycortisol, 11-deoxycorticosterone, corticosterone, cortisol, and 21-deoxycortisol)
293 measured in peripheral venous plasma correctly predicted the genotype in 73 of 79 patients with APAs
294 (92%). In 26/27 patients the presence of a somatic *KCNJ5* mutation could be reliably predicted by the
295 venous steroid profile. If validated this indicates that measurement of such a panel of 7 steroids may
296 circumvent the need for AVS in patients with BAH because only patients with APA would require AVS
297 to determine which adrenal gland overproduces aldosterone for selecting for ADX. The relatively small
298 size and the retrospective nature of this study precludes any definite conclusions until these results are
299 confirmed by a larger prospective study with standardized follow-up for outcome to verify the final
300 subtype diagnosis.

301

302 **Conclusions and Perspectives**

303 Of the four discussed methods, CT and AVS are recommended for the diagnostic work-up of patients
304 with PA. CT with its limited sensitivity and specificity cannot be used as a stand alone test but only in
305 combination with other techniques such as AVS or functional imaging (PET scanning). CT scanning is
306 nonetheless mandatory in every patient to exclude a potential adrenocortical carcinoma. AVS is a highly
307 attractive test because it tests the functionality of an adrenal mass, even if small. This technique has
308 however several limitations and disadvantages, urging the development and investigation of alternative
309 non-invasive patient-friendly methods to determine which patients should be sent for ADX. Functional
310 imaging with highly specific molecular PET ligands is a potentially promising method that may replace
311 AVS in some or even all patients. Although the results of preliminary studies of ¹¹C-metomidate are
312 encouraging, more specific and fluorinated ligands that selectively bind to CYP11B2 are needed. Steroid

313 profiling as a method for subtyping and selecting patients for ADX is still in its infancy and more
314 prospective outcome-based studies are required to see if this technique is a better alternative to AVS.

315

316 **Acknowledgements**

317 JL and GE were supported by the Deutsche Forschungsgemeinschaft ((DFG) within the
318 CRC/Transregio 205/1 „The Adrenal: Central Relay in Health and Disease“, B15). JL and GE were also
319 supported by a grant from the Deutsche Forschungsgemeinschaft (LE3660/1-2, KFO252). MR was
320 supported by a grant from the European Research Council (ERC) under the European Union’s Horizon
321 2020 research and innovation programme (grant agreement No [694913]), by a grant of the Else Kröner-
322 Fresenius Stiftung in support of the German Conn’s Registry-Else-Kröner Hyperaldosteronism Registry
323 (2013_A182 and 2015_A171) and by grants from the Deutsche Forschungsgemeinschaft (RE 752/20-
324 1).

325

326

327 **Table 1.**

328 Advantages and disadvantages of the methods for subtyping of patients with primary aldosteronism

	Advantages	Disadvantages
Computed tomography	<ul style="list-style-type: none"> - widely available - non-invasive - high spatial resolution - relatively cheap 	<ul style="list-style-type: none"> - not specific: anatomical information - limited sensitivity - radiation exposure moderate - contraindication if contrast allergy
Adrenal venous sampling	<ul style="list-style-type: none"> - functional test - highly predictive of outcome 	<ul style="list-style-type: none"> - limited availability - laborious and technically demanding - not-standardized procedure - radiation exposure significant - contraindication if contrast allergy
Metomidate imaging	<ul style="list-style-type: none"> - proof of principle - specific binding to CYP11B2 - non-invasive technique 	<ul style="list-style-type: none"> - limited availability (cyclotron needed) - radiation exposure moderate - lower selectivity of binding to CYP11B2 than to CYP11B1 - very limited data available
Steroid profiling	<ul style="list-style-type: none"> - non-invasive - high specificity (LC-MS/MS) - multiple hormones (one assay) - convenient: one blood sample 	<ul style="list-style-type: none"> - LC-MS/MS: expensive - LC-MS/MS expertise required - no prospective data available - interpretation of results: expertise needed

329

330 LC-MS/MS, liquid chromatography with tandem mass spectrometry

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