

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Prediction of hyperaldosteronism subtypes when adrenal vein sampling is unilaterally successful

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1764443> since 2020-12-16T14:19:22Z

Published version:

DOI:10.1530/EJE-20-0656

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **Prediction of hyperaldosteronism subtypes when adrenal vein sampling is**
2 **unilaterally successful**

3 Jacopo Burrello^{1*}, Alessio Burrello^{2*}, Jacopo Pieroni¹, Elisa Sconfienza¹, Vittorio Forestiero¹,
4 Martina Amongero³, Denis Rossato⁴, Franco Veglio¹, Tracy A. Williams^{1,5}, Silvia Monticone^{1#},
5 Paolo Mulatero^{1#}.

6
7 (1) Division of Internal Medicine and Hypertension, Department of Medical Sciences, University of
8 Torino, Italy. (2) Department of Electrical, Electronic and Information Engineering "Guglielmo
9 Marconi" (DEI), University of Bologna, Italy. (3) Department of Mathematical Sciences G. L.
10 Lagrange, Polytechnic University of Torino, Italy. (4) AOU Città della Salute e della Scienza -
11 Service of Radiology, Torino, Italy. (5) Medizinische Klinik und Poliklinik IV, Klinikum der
12 Universität, Ludwig-Maximilians-Universität München, Munich, Germany.

13 * Contributed equally and should be considered as joint first authors.

14 # Contributed equally and should be considered as joint last authors.

15
16 Corresponding author: Prof. Paolo Mulatero - Division of Internal Medicine and Hypertension Unit,
17 Department of Medical Sciences, University of Torino, Città della Salute e della Scienza di Torino,
18 Via Genova 3, 10126 Torino, Italy. Telephone/Fax number: 0039.011.633.6959 / 0039.011.633.6931.
19 E-mail: paolo.mulatero@unito.it

20 Manuscript word count: 3,440 excluding tables, references and figure captions.

21 Abstract word count: 250.

22 Number of Tables: 3; Number of Figures: 4; 1 Supplementary file.

23 **Short title:** Subtype diagnosis in primary aldosteronism

24 **Keywords:** aldosterone; primary aldosteronism; contralateral suppression; adrenal venous sampling;
25 machine learning.

26 **ABSTRACT**

27 **Objective** – Adrenal venous sampling (AVS) is the gold standard to discriminate patients with
28 unilateral primary aldosteronism (UPA) from bilateral disease (BPA). AVS is technically-demanding
29 and in cases of unsuccessful cannulation of adrenal veins, the results may not always be interpreted.
30 The aim of our study was to develop diagnostic models to distinguish UPA from BPA, in cases of
31 unilateral successful AVS and the presence of contralateral suppression of aldosterone secretion.

32 **Design** – Retrospective evaluation of 158 patients referred to a tertiary hypertension unit who
33 underwent AVS. We randomly assigned 110 patients to a training cohort and 48 patients to a
34 validation cohort to develop and test the diagnostic models.

35 **Methods** – Supervised machine learning algorithms and regression models were used to develop and
36 validate two prediction models and a simple 19-point score system to stratify patients according to
37 their subtype diagnosis.

38 **Results** – Aldosterone levels at screening and after confirmatory testing, lowest potassium, ipsilateral
39 and contralateral imaging findings at CT scanning, and contralateral ratio at AVS, were associated
40 with a diagnosis of UPA and were included in the diagnostic models. Machine learning algorithms
41 correctly classified the majority of patients both at training and validation (accuracy 82.9-95.7%).
42 The score system displayed a sensitivity/specificity of 95.2/96.9%, with an AUC of 0.971. A flow-
43 chart integrating our score correctly managed all patients except 3 (98.1% accuracy), avoiding the
44 potential repetition of 77.2% of AVS procedures.

45 **Conclusions** – Our score could be integrated in clinical practice and guide surgical decision-making
46 in patients with unilateral successful AVS and contralateral suppression.

47 **ABBREVIATION LIST:** A/C, Aldosterone-to-Cortisol ratio; ARR, Aldosterone-to-Renin Ratio;
48 AVS, Adrenal Venous Sampling; BPA, Bilateral Primary Aldosteronism; CLR, Contralateral ratio;
49 CT, Computed Tomography; DDD, Defined Daily Dose; IVC, Inferior Vena Cava; LAV, Left
50 Adrenal Vein; LDA, Linear Discriminant Analysis; LI, Lateralization Index; PA, Primary
51 Aldosteronism; PRA, Plasma Renin Activity; RAV, Right Adrenal Vein; RF, Random Forest; SI,
52 Selectivity Index; UPA, Unilateral Primary Aldosteronism.

53

54 **INTRODUCTION**

55 Primary aldosteronism (PA) is a common secondary cause of arterial hypertension^{1,2}, associated with
56 an increased cardiovascular risk compared with patients with essential hypertension^{3,4}. This condition
57 may be determined either by unilateral or bilateral hypersecretion of aldosterone by the adrenal
58 glands, justifying the two major subtypes of PA, aldosterone producing adenoma, and bilateral
59 adrenal hyperplasia.

60 Patients with a diagnosis of unilateral PA (UPA) derive a clinical benefit after surgical adrenalectomy
61 in more than 80% of cases⁵⁻⁷. Medical therapy with mineralocorticoid receptor antagonists is
62 recommended for patients with bilateral disease⁷. The correct treatment leads to a significant
63 improvement of long-term outcomes, reverting the cardiovascular risk excess displayed by these
64 patients⁸. For these reasons, an early diagnosis of PA and subtype differentiation is fundamental.
65 Guidelines recommends adrenal venous sampling (AVS) as the gold standard to discriminate UPA
66 from bilateral PA (BPA)¹⁻³.

67 However, AVS may not be interpretable when cannulation is unsuccessful for one or both adrenal
68 veins. The cannulation of the left adrenal vein (LAV) is relatively straight forward, because it merges
69 with the inferior phrenic vein to form a common vessel draining into the left renal vein. Conversely,
70 the cannulation of the right adrenal vein (RAV), which is shorter and smaller, directly drains into the
71 inferior vena cava (IVC) at an acute angle and may thus be challenging^{9,10}. The correct cannulation

72 of RAV and LAV is assessed by the selectivity index (SI), which is calculated as the cortisol ratio
73 between adrenal veins and IVC. The ratio between the aldosterone-to-cortisol (A/C) ratio in the
74 dominant adrenal vein and the A/C ratio in the non-dominant vein is defined as lateralization index
75 (LI), and displays high sensitivity and specificity to distinguish UPA from bilateral forms^{3,10,11}.

76 Several studies have described the diagnostic performance of the contralateral ratio (CLR, defined as
77 A/C ratio in the non-dominant adrenal vein divided by A/C ratio in the IVC). Contralateral
78 suppression is defined in the presence of an A/C ratio in the non-dominant adrenal vein lower than
79 the A/C ratio in IVC, and a CLR lower than 1 is used in some centers to define lateralization when
80 only one adrenal vein is successfully cannulated^{12,13}. Patients with UPA should display a contralateral
81 normal adrenal gland with suppressed aldosterone secretion from the *zona glomerulosa*^{14,15}.
82 Nevertheless, the CLR cannot predict UPA by itself, because about one third of patients with BPA
83 also display contralateral suppression¹¹, and for the occurrence of apparent bilateral aldosterone
84 suppression, defined as adrenal A/C ratio bilaterally lower than that in IVC¹⁶. Thresholds to interpret
85 CLR and assess lateralization of aldosterone secretion have been not yet defined.

86 Therefore, the aim of our study was to develop and validate a diagnostic model able to discriminate
87 UPA from BPA, when the AVS is unilaterally successful and the adrenal vein A/C ratio is lower than
88 the A/C ratio in the IVC (so called contralateral suppression).

89

90 **METHODS**

91 ***Study cohort and data extraction***

92 We retrospectively evaluated 158 patients who underwent AVS in the Hypertension Unit of Torino
93 (Italy), between 2008 and 2019. All patients gave informed written consent to the study according to
94 Helsinki declaration. The study was approved by the local ethical committee of the hospital “Città
95 della Salute e della Scienza” of Torino. PA was diagnosed according with the Endocrine Society
96 Guideline¹. Patients were screened using the aldosterone-to-plasma renin activity (PRA) -ratio

97 (ARR); if possible, all interfering drugs were withdrawn for at least 4 weeks prior to analysis (6 weeks
98 for mineralocorticoid receptor antagonist and diuretics). The cut-off for a positive screening test was
99 an ARR greater than $30 \text{ ng/dL/ng} \cdot \text{mL}^{-1} \cdot \text{h}^{-1}$ together with an aldosterone concentration greater than
100 10 ng/dL . Patients with a positive screening test underwent confirmatory/exclusion testing by either
101 intravenous saline loading test or a captopril challenge test¹. Confirmed patients with PA underwent
102 multi-slice computed tomography (CT) scanning and AVS. An adrenal nodule was reported in the
103 presence of a mass equal or greater than 8 mm. AVS was performed under basal conditions or after
104 continuous cosyntropin (ACTH) infusion (76 vs. 82, respectively). The catheterization of adrenal
105 veins was sequential in both protocols. In case of cosyntropin stimulation, the infusion was performed
106 at 50 ug/h , starting 30 minutes before the procedure. AVS procedures were performed by a single
107 experienced interventional radiologist. The radiologist firstly cannulates the right adrenal vein and
108 subsequently the left adrenal vein (within 15 minutes). ACTH stimulation was necessary for patients
109 who need preparation with steroids before the procedure for a history of contrast allergy (or other
110 allergy; $n=13$), or when the procedure was not performed in the early morning ($n=69$)¹⁰. Adrenal vein
111 cannulation was considered successful in the presence of a SI equal or greater than 3 under basal
112 conditions, and of 5 under cosyntropin infusion. The diagnosis of UPA was established when the LI
113 was at least 4¹⁰.

114 Patients were included in the study if: 1) the AVS was bilaterally successful; 2) in presence of
115 contralateral suppression, that is when at least one adrenal vein A/C ratio was lower than the A/C
116 ratio in the IVC ($\text{CLR} < 1$). Hypercortisolism was excluded in all patients. Enrolled patients were
117 randomly assigned to a training cohort ($N = 110$) or to a validation cohort ($N = 48$), with a ratio 70:30.
118 Contralateral and ipsilateral adrenal glands were defined on the basis of AVS findings. In case of
119 UPA, the contralateral adrenal was the gland on the side opposite to the lateralization. In case of BPA
120 diagnosis, the contralateral adrenal was defined as the gland showing an A/C ratio lower than in the
121 IVC. Ipsilateral and contralateral adrenal imaging at CT scanning were defined accordingly. To build

122 our model, aldosterone and cortisol measurements from the IVC and from the adrenal vein with A/C
123 ratio lower than in the IVC were used. When UPA is predicted, the ipsilateral adrenal gland should
124 be removed. For all patients, antihypertensive medication was quantified as Defined Daily Dose
125 (DDD), which is the average maintenance dose per day for a drug used for its main indication in
126 adults.

127 ***Machine learning and statistical analysis***

128 IBM SPSS Statistics 22 (IBM Corp., Armonk, New York, USA) and Python 3.5 (library, scikit-learn)
129 were used for statistics. The study followed the TRIPOD statement for transparent reporting of a
130 multivariable prediction model for individual prognosis or diagnosis.

131 Sample size was calculated on the estimated variability for median value of CLR. With a mean effect
132 size (Cohen's d coefficient) of 1.24, a minimum power ($1 - \beta$ error probability) of 95%, and a
133 significance level (α error) of 0.05, the estimated total sample size was 44. Sample size calculation
134 was carried out with GPower 3.1.

135 Kolmogorov–Smirnov test was used to assess variable distribution. Normally and non-normally
136 distributed variables were reported as mean \pm standard deviation, or median and interquartile range,
137 and analyzed by T-student, or Mann-Whitney's test, respectively. Categorical variables were reported
138 as absolute number and percentage distribution and analyzed by Chi-square test. Logistic regression
139 analyses were used for univariate and multivariate models; an odds ratio (OR) greater than 1 was
140 associated with an increased likelihood of diagnosis of UPA. Supervised machine learning techniques
141 were used to develop the diagnostic models and the clinical score, as previously described¹⁷. Briefly,
142 linear discriminant analysis (LDA) and random forest (RF) algorithms were applied to develop two
143 different diagnostic models able to discriminate UPA vs. BPA using the best discriminant patients'
144 parameters, previously selected by univariate and multivariate regression analysis. The models were
145 developed after correction of dataset imbalance using a SMOTE algorithm (Synthetic Minority
146 Oversampling Technique). Briefly, SMOTE imputes new patient data (defined as “neighbour”), along

147 lines that link real patients from the training dataset in the virtual space created by patient parameters,
148 in order to balance the number of UPA and BPA patients at model training. A number of neighbours
149 equal to 3 (K=3), corresponded to the higher accuracy and was used in all analyses.

150 The LDA computes a set of coefficients for linear combination of each variable to classify patients
151 according with their diagnosis. The predicted diagnosis is derived from the following equation: UPA
152 = $LDAcoeff_1 * Variable_1 + LDAcoeff_2 * Variable_2 + \dots + LDAcoeff_n * Variable_n > 9.7425$. The RF
153 algorithm is composed by 20 classification trees with a maximum number of 8 splits. The predicted
154 diagnosis resulted from the outcome of each classification tree. If at least 11 of 20 trees of the RF
155 predicted UPA, then the patient is classified accordingly. Machine learning models were tested by a
156 10-fold validation algorithm: (i) the cohort was randomly divided into 10 groups; (ii) the model was
157 trained within the first 9 groups and validated with the remaining group; (iii) the process was repeated
158 10 times, rotating the validation group at each round. The accuracy at validation resulted from the
159 mean of the accuracy obtained at each round. A 19-point score based on the same discriminant
160 parameters was finally developed in the training cohort and tested by 10-fold cross validation (with
161 bootstrapping) and in both validation and combined cohorts. Points for each reference category and
162 relative cut-offs were automatically derived to achieve the best accuracy. The analysis of ROC curve
163 was used to assess the area under the curve (AUC) and derive the Youden Index ($J = \text{sensitivity} +$
164 $\text{specificity} - 1$). Accuracy was defined as the ratio between “true positive” plus “true negative” divided
165 by the total number of patients included in each cohort. An online tool was developed to calculate the
166 score and define the diagnosis (available at [https://github.com/ABurrello/CLR-](https://github.com/ABurrello/CLR-score/raw/master/CLR%20Score%20Calculator.xlsm)
167 [score/raw/master/CLR%20Score%20Calculator.xlsm](https://github.com/ABurrello/CLR-score/raw/master/CLR%20Score%20Calculator.xlsm)). All data supporting the findings of this study
168 are available from the corresponding author upon reasonable request.

169

170 **RESULTS**

171 *Patient characteristics*

172 One hundred and fifty-eight patients underwent AVS for PA subtyping and were included in the
173 analysis. The mean age at diagnosis of PA was 50 ± 10 , mean BP was 164/98, with a duration of
174 hypertension of 67 [23; 130] months. Seventy-six patients underwent AVS under basal conditions,
175 and 82 after ACTH stimulation. No differences were observed between patients who underwent AVS
176 with or without ACTH except for SI higher under ACTH stimulation ($P = 0.002$) (Supplementary
177 Table S1). For this reason, patients were grouped together for all subsequent analyses, irrespective of
178 the protocol used for AVS.

179 Clinical and biochemical characteristics of patients after subtype diagnosis are reported in Table 1;
180 126 patients were diagnosed as UPA, and 32 as BPA. The high prevalence of unilateral forms is
181 expected, because only patients with contralateral suppression at AVS were enrolled in this study.
182 Patients with a diagnosis of UPA were younger (49 ± 10.3 vs. 55 ± 7.1 ; $P = 0.001$), frequently females
183 (42.1% vs. 21.9%; $P = 0.036$), with a higher DDD (4 [2.5; 6] vs. 2.9 [1.4; 4.3] $P = 0.008$). The
184 prevalence of microalbuminuria, left ventricular hypertrophy, and prior cardiovascular events was
185 not different between groups.

186 UPA patients displayed lower potassium levels (3.1 ± 0.6 vs. 3.9 ± 0.4 mEq/L; $P < 0.001$) and higher
187 aldosterone, both at screening (38.2 [25.9; 49.8] vs. 30.2 [21.1; 41] ng/dL; $P = 0.044$) and after
188 confirmatory testing (21.9 [13.9; 35.5] vs. 11.4 [7.7; 19.9] ng/dL; $P < 0.001$). At CT scanning,
189 ipsilateral imaging was normal in 1.6% of patients with UPA, compared to 43.8% of BPA patients;
190 conversely, contralateral imaging was normal in 84.9% of UPA and 53.1% of BPA patients ($P <$
191 0.001 for both comparisons). After AVS, contralateral A/C and CLR were both lower in patients with
192 UPA (4.1 [1.5; 8.3] vs. 7.9 [3.2; 12] and 0.3 [0.2; 0.5] vs. 0.7 [0.5; 0.8], respectively).

193 Univariate logistic regression analysis confirmed the association with the diagnosis of UPA for all
194 these parameters (Supplementary Table S2). In particular, female sex (OR 2.59), age at diagnosis
195 (OR 0.94), DDD (OR 1.43), lowest potassium (0.07), aldosterone at screening (OR 1.01) and after
196 confirmatory testing (1.01), a normal ipsilateral and contralateral imaging (OR 0.02 and OR 4.97,

197 respectively), contralateral A/C (OR 0.91), and CLR (OR 0.01). Six of these 10 variables were
198 selected considering their discriminative performance and expert knowledge¹⁸, and were introduced
199 in the multivariate model (Table 2), which confirmed their independent association with UPA
200 diagnosis (female sex, age at diagnosis, DDD, and contralateral A/C were no longer associated with
201 UPA when introduced in the multivariate analysis; data not shown).

202

203 *Linear discriminant analysis and random forest models*

204 Patients included in the analysis were randomized into two subgroups: the training cohort (N = 110)
205 and the validation cohort (N = 48). No differences were found for all the evaluated variables
206 (Supplementary Table S3).

207 Supervised machine learning algorithms were used to develop two diagnostic models: an LDA model
208 and a RF classification algorithm. The models were developed in the training cohort and subsequently
209 tested by both internal validation (10-fold cross validation applied to the same training cohort) and
210 external validation (on the randomly selected validation cohort). Supplementary Table S4 reports the
211 diagnostic performance at training, internal and external validation, and on the combined cohort. The
212 linear combination of the 6 above-selected variables is represented in the canonical plot (Figure 1A);
213 each patient is represented by a point, and the clear separation according to their subtype diagnosis
214 indicates the high diagnostic performance of the LDA model (accuracy 92.3%, 88.9%, 84.6%,
215 respectively at training, internal, and external validation). In the combined cohort, 112 of 126 patients
216 with UPA and 30 of 32 with BPA, were correctly classified, resulting in a sensitivity and specificity
217 of 88.8% and 93.4%, respectively (Figure 1B). Internal and external validation confirmed the high
218 predictive performance, with a minimum overfitting effect (3.4-7.7%).

219 The RF classification algorithm (the first tree of the RF is reported in Figure 2A) correctly
220 discriminated 145 of 158 patients (accuracy 91.7%) from the combined cohort, with a sensitivity and
221 specificity of 92.1% and 90.3%, respectively (Figure 2B). Also in this case, the accuracy at external

222 and internal validation by 10-fold cross validation was high (accuracy 82.9% and 89.6%, respectively;
223 overfitting effect 6.0-12.7%; see Supplementary Table S4). Ipsilateral imaging and CLR were the
224 strongest predictors in the LDA model, whereas lowest potassium levels and aldosterone post-
225 confirmatory test in the RF (Figures 1C and 2C). Estimate predictor importance is reported in
226 Supplementary Table S5 for both LDA and RF models.

227

228 ***Prediction score and management of PA patients***

229 The 6 variables used in the LDA and RF models were used to develop the CLR score, a 19-points
230 scoring system to discriminate patients with a diagnosis of UPA vs. BPA. The CLR score was
231 developed in the training cohort and then tested by 10-fold cross validation, and in the validation and
232 combined cohorts. Figure 3A show how each parameter was categorized and points assigned. The
233 analysis of the ROC curve confirmed the reliable performance of CLR score, with an AUC of 0.971
234 (95% CI 0.947-0.994; Figure 3B). In the training cohort, a CLR score greater than 11 displayed the
235 higher accuracy (96.4%), with the correct classification of 84 of 88 UPA patients (sensitivity 95.5%),
236 whereas a score equal or lower than 11 classified all the BPA patients (specificity 100%). By 10-fold
237 cross validation, we confirmed a reliable accuracy (96.3%). The diagnostic performance of the CLR
238 score at external validation remained very high, with an accuracy, sensitivity, and specificity of
239 93.7%, 94.7% and 90.0%, respectively, higher than the machine learning algorithms (confusion
240 matrix for training, validation, and combined cohorts are reported in Supplementary Tables S4 and
241 S6). A cut-off of greater than 8 optimized sensitivity, correctly classified 86 of 88, and 37 of 38
242 patients with UPA, respectively at training and at validation, with a sensitivity of 97.8% and 97.4%.
243 A cut-off lower than 13 maximized specificity, and correctly identified all patients with a diagnosis
244 of BPA, both at training and at validation (specificity 100%).
245 Using 11 as cut-off, patients which underwent AVS under basal conditions were correctly
246 discriminated in 94.7% of cases, whereas after stimulation with ACTH in 96.3% of cases.

247 Figure 4A shows the stratification of patients with a diagnosis of UPA or BPA for the CLR score.
248 The scoring system was directly correlated with the proportion of patients with a diagnosis of UPA.
249 In addition, all patients with a score greater than 13 had UPA (N = 88), whereas all patients with a
250 score equal or lower than 3 had BPA (N = 6; Supplementary Table S7).

251 Overall, 102 of the 126 patients with UPA underwent adrenalectomy. According to the PASO
252 criteria⁵, at follow-up of 6-12 months after surgery, patients with UPA displayed complete clinical
253 and biochemical success in 50% and 96.1% of cases, respectively (Supplementary Table S8). All the
254 6 patients with UPA misclassified as BPA displayed partial or absent clinical outcome (4 with a
255 complete and 2 with a partial biochemical outcome). Forty three percent (41 of 96) of correctly
256 predicted patients with UPA displayed a partial clinical outcome and 4% absent clinical outcome;
257 biochemical outcome was complete in 94 of 96 correctly classified patients and partial in the
258 remaining 2 patients. No statistically significant differences were observed in the clinical and
259 biochemical outcomes for patients who underwent unstimulated *vs.* ACTH stimulated AVS
260 (Supplementary Table 8).

261 The CLR score was finally integrated into a flow chart for the management of patients with PA with
262 a unilateral successful AVS and contralateral suppression (CLR < 1; Figure 4B and 4C). Patients with
263 a score equal to or greater than 13 were classified as “probable UPA” with indication to surgical
264 intervention. Notably, all patients with UPA identified in this way (N = 104) correctly underwent
265 unilateral adrenalectomy. Patients with a score equal to or lower than 8 were classified as “probable
266 BPA” and treated with MRA (N = 18), resulting in 15 patients with BPA correctly managed and 3
267 UPA patients, who missed the possibility to be operated. All the other patients (N = 36), with a score
268 ranging between 8.5 and 12.5 should repeat AVS. The application of the prediction score in our
269 clinical context would result in the correct management of 155 of 158 patients (accuracy 98.1%),
270 allowing a subtype diagnosis with the repetition of only 22.8% of AVS procedures (sensitivity 97.6%
271 and specificity 100.0%).

272

273 **DISCUSSION**

274 We propose two prediction models based on supervised machine learning algorithms, and an easily
275 applicable scoring system, to discriminate UPA from BPA. An online free-downloadable tool is
276 provided and allows the application of a flow-chart for the management of patients undergoing
277 unilateral successful AVS in the presence of contralateral suppression.

278 Although the rate of bilaterally successful cannulation during AVS may be higher than 90% when
279 performed by an experienced radiologist¹⁹, several centers do not have a comparable reliability, with
280 a successful rate ranging between 8.0 and 30.5%, especially where a low number of AVS are
281 performed per year²⁰⁻²². Given its course and anatomical variability²³, the main reason for
282 unsuccessful AVS is the failed cannulation of RAV because of difficulties in identification of the
283 vessel. Therefore, over the last few years, some studies have tried to develop indices to predict
284 subtype diagnosis from incomplete AVS data²⁴⁻²⁸.

285 Kline et al. demonstrated a high performance of the CLR in the identification of UPA; sensitivity and
286 specificity were 90% and 94%, respectively, using a CLR < 1.4 as cut-off²⁴. However, the authors
287 did not validate their approach in a second cohort of patients and observed a high accuracy only in
288 patients who underwent cosyntropin-stimulated AVS, whereas in unstimulated procedures the
289 accuracy was lower. Many patients with bilateral disease may be misclassified using a CLR cut-off
290 of 1.4 that includes subjects without contralateral suppression of aldosterone secretion. On the other
291 hand, Lin et al. proposed very low cut-offs (CLR < 0.07 for left and 0.08 for right lateralisation) in a
292 cohort of 160 patients who underwent AVS in basal conditions. Specificity was 100%, but sensitivity
293 was unacceptable for clinical application, ranging between 27.5 and 40%, thus missing the majority
294 of patients with a diagnosis of UPA²⁸. In a subsequent study, Durivage et al. applied multinomial
295 regression modelling in patients with unsuccessful right adrenal vein cannulation. The authors
296 correctly identified 75.1% and 64.1% of UPA patients in cosyntropin-stimulated and unstimulated

297 AVS, respectively²⁶. In another study performed in 62 AVS procedures²⁵, a CLR equal or lower than
298 0.5 displayed a specificity and positive predictive value of 100% but missed about half of patients
299 with UPA (sensitivity 58%). Strajina et al. tested the same cut-off in a cohort of 150 patients; the
300 specificity was 100%, but 20% of patients with UPA were classified as BPA and missed the
301 possibility to be cured by adrenalectomy²⁷.

302

303 In our cohort, 79.7% of patients were diagnosed as UPA, thus being representative of the prevalence
304 of unilateral disease in patients with contralateral suppression²⁴⁻²⁸. With our machine learning-based
305 models and with the CLR score, we obtained a higher diagnostic performance compared to previous
306 methods. We achieved a 97.6% sensitivity and 100% specificity with the proposed flow-chart for
307 patient management. Combining the cut-offs which optimized sensitivity and specificity in our flow-
308 chart, all patients predicted as UPA by the CLR score were correctly diagnosed (positive predictive
309 value 100%), whereas only 3 patients with a diagnosis of UPA were misclassified as BPA and would
310 have missed the possibility of surgical treatment but would have a long-term pharmacological
311 treatment. The cut-off with the maximal specificity of the CLR score displayed a sensitivity higher
312 than 80% both at training and validation, outperforming previous scores. The accuracy was similarly
313 very high, independent of the AVS protocol (with or without cosyntropin stimulation).

314 In our study, 20.3% of patients had BPA and displayed a CLR lower than 1, thus confirming a high
315 prevalence of contralateral suppression in patients with bilateral disease¹¹. Using a CLR equal or
316 lower than 0.5^{25,27}, 8 of 32 patients with BPA (25%) still received a diagnosis of UPA and would be
317 operated inappropriately. Indeed, a large multicenter study demonstrated that contralateral
318 suppression was not associated with a better clinical and biochemical outcome after surgery¹³,
319 whereas the studies evaluating the diagnostic performance of CLR did not assess post-surgical
320 outcomes^{24,25,27,28}. With our flow-chart, only 3 UPA patients were classified as BPA would have
321 missed the possibility to be surgically treated. When assessed with the PASO criteria⁵, these 3 patients

322 displayed partial clinical success, without normalization of aldosterone levels in 2 cases (partial
323 biochemical success).

324

325 Contralateral suppression alone is not considered a reliable parameter to recommend
326 adrenalectomy¹⁰. Similarly, CT scanning and/or clinical and biochemical parameters do not allow to
327 predict subtype diagnosis in patients with PA^{7,29}.

328 Combining CLR with CT scanning findings and potassium and aldosterone levels, we considerably
329 improved the diagnostic performance described for the CLR alone²⁴⁻²⁸. To evaluate the net benefit of
330 our clinical score, compared to CLR alone, we assessed the respective diagnostic performance by
331 ROC curve analysis. The AUC for CLR was 0.836 (CI 95% 0.759-0.913), compared with 0.971 for
332 the clinical score (+16.1%). A CLR < 0.45 displayed an accuracy of 84.8% in the discrimination of
333 patients with UPA from those with BPA. In the combined cohort, the score, LDA and RF models
334 reached an accuracy of 95.5%, 90.0%, and 91.7% with a net benefit ranging between 6.1% and 12.6%.
335 Our score **could be** of interest for centers with a low rate of bilateral adrenal vein cannulation, since
336 it correctly classifies 98.1% of patients that display contralateral suppression and avoid the repetition
337 of AVS in 77.2% of the cases. This approach **would** allow to recommend adrenalectomy (100%
338 positive predictive value and specificity), without the repetition of the AVS in case of unsuccessful
339 right adrenal vein cannulation.

340

341 There are some limitations to this study. This was a retrospective analysis of patients referred to a
342 single center: **we validated our scoring system in a separate randomly selected population, but the**
343 **algorithm should be validated in independent prospective cohorts from other centers, to assess his**
344 **generalizability and exclude selection bias**. The strengths of our study include the high accuracy of
345 the proposed prediction models, integrating machine learning algorithms and a scoring-system,
346 allowing the correct management of patients with unilateral successful AVS and contralateral

347 suppression. Moreover, we developed an online tool to calculate the CLR score automatically,
348 allowing the easy application of our flow-chart in clinical practice.

349 In conclusion, AVS remains a technically demanding and invasive procedure but still essential to
350 distinguish UPA from BPA. Our findings suggest that integrating clinical and biochemical
351 parameters, CT scanning imaging, and CLR, partial AVS data could be used to define the subtype
352 diagnosis, facilitating surgical decision-making in case of missed cannulation of one adrenal vein.

353

354 **Declarations**

355 **Conflict(s) of Interest/Disclosure(s):** the authors declare no conflict of interest that could be
356 perceived as prejudicing the impartiality of the research reported.

357 **Source(s) of Funding:** This research did not receive any specific grant from any funding agency in
358 the public, commercial or not-for-profit sector.

359 **REFERENCES**

- 360 1. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M, Young WF
361 Jr. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An
362 ES Clinical Practice Guideline. *Journal of Clinical Endocrinology and Metabolism* 2016 **101**
363 1889-1916.
- 364 2. Mulatero P, Monticone S, Deinum J, Amar L, Prejbisz A, Zennaro MC, Beuschlein F, Rossi GP,
365 Nishikawa T, Morganti A, et al. Genetics, prevalence, screening and confirmation of primary
366 aldosteronism: a position statement and consensus of the Working Group on Endocrine
367 Hypertension of The European Society of Hypertension. *J Hypertens* 2020 [Epub ahead of print].
- 368 3. Mulatero P, Sechi LA, Williams TA, Lenders JWM, Reincke M, Satoh F, Januszewicz A, Naruse
369 M, Doumas M, Veglio F, et al. Subtype diagnosis, treatment, complications and outcomes of
370 primary aldosteronism and future direction of research: a position statement and consensus of
371 the Working Group on Endocrine Hypertension of the European Society of Hypertension. *J*
372 *Hypertens* 2020 [Epub ahead of print].
- 373 4. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, Mulatero P.
374 Cardiovascular events and target organ damage in primary aldosteronism compared with
375 essential hypertension: a systematic review and meta-analysis. *The Lancet Diabetes &*
376 *Endocrinology* 2018 **6** 41-50.
- 377 5. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, Satoh F, Amar
378 L, Quinkler M, Deinum J, et al. Outcomes after adrenalectomy for unilateral primary
379 aldosteronism: an international consensus on outcome measures and analysis of remission rates
380 in an international cohort. *The Lancet Diabetes & Endocrinology* 2017 **5** 689-699.
- 381 6. Williams TA, Burrello J, Sechi LA, Fardella CE, Matrozoza J, Adolf C, Baudrand R, Bernardi
382 S, Beuschlein F, Catena C, et al. Computed Tomography and Adrenal Venous Sampling in the
383 Diagnosis of Unilateral Primary Aldosteronism. *Hypertension* 2018 **72** 641-649.

- 384 7. Takeda M, Yamamoto K, Akasaka H, Rakugi H, Naruse M, Takeda Y, Kurihara I, Itoh H,
385 Umakoshi H, Tsuiki M, et al., JPAS Study Group. Clinical characteristics and postoperative
386 outcomes of primary aldosteronism in the elderly. *Journal of Clinical Endocrinology and*
387 *Metabolism* 2018 **103** 3620-3629.
- 388 8. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and
389 mortality in medically treated primary aldosteronism: a retrospective cohort study. *The Lancet*
390 *Diabetes & Endocrinology* 2018 **6** 51-59.
- 391 9. Daunt N. Adrenal vein sampling: how to make it quick, easy, and successful. *Radiographics*
392 2005 **25** S143-S158.
- 393 10. Monticone S, Viola A, Rossato D, Veglio F, Reincke M, Gomez-Sanchez C, Mulatero P. Adrenal
394 vein sampling in primary aldosteronism: towards a standardised protocol. *The Lancet Diabetes*
395 *& Endocrinology* 2015 **3** 296-303.
- 396 11. Young WF, Stanson AW, Thompson GB, Grant CS, Farley DR, van Heerden JA. Role for
397 adrenal venous sampling in primary aldosteronism. *Surgery* 2004 **136** 1227-1235.
- 398 12. Wolley MJ, Gordon RD, Ahmed AH, Stowasser M. Does contralateral suppression at adrenal
399 venous sampling predict outcome following unilateral adrenalectomy for primary aldosteronism?
400 A retrospective study. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** 1477-1484.
- 401 13. Monticone S, Satoh F, Viola A, Fischer E, Vonend O, Bernini G, Lucatello B, Quinkler M,
402 Ronconi V, Morimoto R, et al. Aldosterone suppression on contralateral adrenal during adrenal
403 vein sampling does not predict blood pressure response after adrenalectomy. *Journal of Clinical*
404 *Endocrinology and Metabolism* 2014 **99** 4158-4166.
- 405 14. Espiner EA, Ross DG, Yandle TG, Richards AM, Hunt PJ. Predicting surgically remedial
406 primary aldosteronism: role of adrenal scanning, posture testing, and adrenal vein sampling.
407 *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 3637-3644.

- 408 15. Auchus RJ, Wians FH Jr, Anderson ME, Dolmatch BL, Trimmer CK, Josephs SC, Chan D,
409 Toomay S, Nwariaku FE. What we still do not know about adrenal vein sampling for primary
410 aldosteronism. *Hormone and Metabolic Research* 2010 **42** 411-415.
- 411 16. Shibayama Y, Wada N, Naruse M, Kurihara I, Ito H, Yoneda T, Takeda Y, Umakoshi H, Tsuiki
412 M, Ichijo T, et al. The Occurrence of Apparent Bilateral Aldosterone Suppression in Adrenal
413 Vein Sampling for Primary Aldosteronism. *Journal of the Endocrine Society* 2018 **2** 398-407.
- 414 17. Burrello J, Burrello A, Stowasser M, Nishikawa T, Quinkler M, Prejbisz A, Lenders JWM, Satoh
415 F, Mulatero P, Reincke M, et al. The Primary Aldosteronism Surgical Outcome Score for the
416 Prediction of Clinical Outcomes After Adrenalectomy for Unilateral Primary Aldosteronism.
417 *Annals of Surgery* 2019 [Epub ahead of print].
- 418 18. Roe KD, Jawa V, Zhang X, Chute CG, Epstein JA, Matelsky J, Shpitser I, Taylor CO. Feature
419 engineering with clinical expert knowledge: A case study assessment of machine learning model
420 complexity and performance. *PLoS One* 2020 **15** e0231300.
- 421 19. Young WF, Stanson AW. What are the keys to successful adrenal venous sampling (AVS) in
422 patients with primary aldosteronism? *Clinical Endocrinology* 2009 **70** 14-17.
- 423 20. Vonend O, Ockenfels N, Gao X, Allolio B, Lang K, Mai K, Quack I, Saleh A, Degenhart C,
424 Seufert J, et al. Adrenal venous sampling: evaluation of the German Conn's registry.
425 *Hypertension* 2011 **57** 990-995.
- 426 21. Elliott P, Holmes DT. Adrenal vein sampling: substantial need for technical improvement at
427 regional referral centres. *Clinical Biochemistry* 2013 **46** 1399-1404.
- 428 22. Harsha A, Trerotola SO. Technical aspects of adrenal vein sampling. *Journal of Vascular and*
429 *Interventional Radiology* 2015 **26** 239.
- 430 23. Matsuura T, Takase K, Ota H, Yamada T, Sato A, Satoh F, Takahashi S. Radiologic anatomy of
431 the right adrenal vein: preliminary experience with MDCT. *AJR American journal of*
432 *roentgenology* 2008 **191** 402-408.

- 433 24. Kline GA, Chin A, So B, Harvey A, Pasiaka JL. Defining contralateral adrenal suppression in
434 primary aldosteronism: implications for diagnosis and outcome. *Clinical Endocrinology* 2015 **83**
435 20-27.
- 436 25. Pasternak JD, Epelboym I, Seiser N, Wingo M, Herman M, Cowan V, Gosnell JE, Shen WT,
437 Kerlan RK Jr, Lee JA, et al. Diagnostic utility of data from adrenal venous sampling for primary
438 aldosteronism despite failed cannulation of the right adrenal vein. *Surgery* 2016 **159** 267-273.
- 439 26. Durivage C, Blanchette R, Soulez G, Chagnon M, Gilbert P, Giroux MF, Bourdeau I, Oliva VL,
440 Lacroix A, Therasse E. Adrenal venous sampling in primary aldosteronism: multinomial
441 regression modeling to detect aldosterone secretion lateralization when right adrenal sampling is
442 missing. *Journal of Hypertension* 2017 **35** 362-368.
- 443 27. Strajina V, Al-Hilli Z, Andrews JC, Bancos I, Thompson GB, Farley DR, Lyden ML, Dy BM,
444 Young WF, McKenzie TJ. Primary aldosteronism: making sense of partial data sets from failed
445 adrenal venous sampling-suppression of adrenal aldosterone production can be used in clinical
446 decision making. *Surgery* 2018 **163** 801-806.
- 447 28. Lin L, Zhou L, Guo Y, Liu Z, Chen T, Liu Z, Wang K, Li J, Zhu Y, Ren Y. Can incomplete
448 adrenal venous sampling data be used in predicting the subtype of primary aldosteronism?
449 *Annales Endocrinology* 2019 **80** 301-307.
- 450 29. K Kempers MJ, Lenders JW, van Outheusden L, van der Wilt GJ, Schultze Kool LJ, Hermus
451 AR, Deinum J. Systematic review: diagnostic procedures to differentiate unilateral from bilateral
452 adrenal abnormality in primary aldosteronism. *Annals of Internal Medicine* 2009 **151** 329-337.

453 **FIGURE LEGENDS**

454 *Legend to Figure 1 – Diagnostic Modelling: Linear Discriminant Analysis.* The LDA model
455 included the 6 variables with the highest prediction power for subtype diagnosis in the training
456 cohort (N = 110). Contralateral adrenal gland corresponds to the adrenal with the A/C ratio lower
457 than the A/C ratio in the inferior vena cava, while ipsilateral gland corresponds to the opposite side.
458 When unilateral PA (UPA) is predicted, the ipsilateral adrenal gland should be removed. **Panel A**,
459 canonical plot representing diagnostic performance of LDA; each patient is indicated by a point and
460 subtype diagnosis are reported by colour (UPA, black; BPA, bilateral PA, grey). The axes (canonical
461 component 1 and 2) are calculated by weighted linear combination of the 6 variables included in the
462 model to maximize the separation between groups. The crosses indicate the means of (canonical 1;
463 canonical 2) for patients with UPA or BPA, the ellipse included patients with a linear combination
464 coefficient that falls within the mean \pm SD. **Panel B**, confusion matrix reporting real and predicted
465 diagnosis, accuracy, sensitivity, specificity in the combined cohort, and 10-fold cross validation.
466 **Panel C**, histogram representing normalized LDA coefficients for each variable included in the
467 model (see also Supplementary Table S5).

468
469 *Legend to Figure 2 – Diagnostic Modelling: Random Forest.* The RF algorithm included the 6
470 variables with the highest classification power for subtype diagnosis in the training cohort (N =
471 110). Contralateral adrenal gland corresponds to the adrenal with the A/C ratio lower than the A/C
472 ratio in the inferior vena cava, while ipsilateral gland corresponds to the opposite side. When
473 unilateral PA (UPA) is predicted, the ipsilateral adrenal gland should be removed. **Panel A**, the first
474 classification tree of the forest is shown for the prediction of UPA vs. BPA (bilateral PA). **Panel B**,
475 confusion matrix reporting real and predicted diagnosis, accuracy, sensitivity, specificity in the
476 combined cohort, and 10-fold cross validation. **Panel C**, histogram representing normalized
477 predictive coefficients for each variable included in the model (see also Supplementary Table S5).

478

479 *Legend to Figure 3 – Score development.* Univariate/multivariate regression analyses and
480 coefficients from the LDA and RF models were used to assign points to each variable according to
481 stratification level. The score was developed in the training cohort (N = 110) and tested on the
482 validation cohort (N = 48). Contralateral adrenal gland corresponds to the adrenal with the A/C ratio
483 lower than the A/C ratio in the inferior vena cava, while ipsilateral gland corresponds to the opposite
484 side. When unilateral PA (UPA) is predicted, the ipsilateral adrenal gland should be removed. **Panel**
485 **A**, table showing included variables and scoring-point system. **Panel B**, receiver operating
486 characteristics (ROC) curve to assess AUC (area under the curve) and the best cut-off for the score
487 in the combined cohort (N = 158).

488

489 *Legend to Figure 4 – Score performance and management of PA patients.* Flow chart for the
490 management of patients with PA, unilateral successful AVS, and CLR < 1. Contralateral adrenal
491 gland corresponds to the adrenal with the A/C ratio lower than the A/C ratio in the inferior vena
492 cava, while ipsilateral gland corresponds to the opposite side. When unilateral PA (UPA) is
493 predicted, the ipsilateral adrenal gland should be removed. **Panel A**, histogram showing the
494 proportion of patients (y-axis, %) for each subtype diagnosis (UPA, black; BPA, bilateral PA, grey),
495 stratified by score points (x-axis) on the combined cohort. The total number of patients (N) for each
496 AVS score level and their proportion (%) are reported in Table S5. **Panel B**, PA patient management
497 using our score; the number of patients is indicated in bold; cut-offs and misclassified patients are
498 indicated in grey. **Panel C**, confusion matrix representing real and predicted subtype diagnosis,
499 sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). CLR,
500 Contralateral Ratio; AVS, Adrenal Venous Sampling; MRA, Mineral Receptor Antagonist.

501

502 **TABLE LEGENDS**

503 *Legend to Table 1* – Characteristics of patients included in the analysis stratified for diagnosis:
504 unilateral PA (UPA; N=126) vs. bilateral PA (BPA; N=32). Antihypertensive medication is
505 expressed as Defined Daily Dose (DDD). DDD is the average maintenance dose per day for a drug
506 used for its main indication in adults. HTN, Hypertension; BP, Blood Pressure; PRA, Plasma Renin
507 Activity; LVH, Left Ventricular Hypertrophy; Echo, Echocardiography; CV, Cardiovascular; CT,
508 Computed Tomography; SI, Selectivity Index; A/C, Aldosterone to Cortisol ratio. Normally and
509 non-normally distributed variables were reported as mean \pm standard deviation or median
510 [interquartile range], respectively. Categorical variables were reported as absolute number (n) and
511 proportion (%).

512
513 *Legend to Table 2* – Logistic regression analysis was performed to assess the odds ratio (OR), β
514 estimate and the 95% confidence interval (CI) for each variable. Univariate and multivariate analysis
515 are shown as indicated. An OR greater than 1 indicates an increased likelihood of unilateral PA
516 (UPA), and an OR less than 1 a decreased likelihood (i.e. an OR of 1.01 for aldosterone means an
517 increase of 1% of the likelihood of UPA for each 1 ng/dL increase of aldosterone concentration).
518 Aldosterone at screening, lowest potassium, aldosterone post-confirmatory test, and contralateral
519 ratio were treated as continuous variables; ipsilateral and contralateral imaging (defined as normal
520 in absence of nodules, or with thickening $<$ 4 mm) were treated as categorical variables.

Table 1. Patient Characteristics of Study Cohort

Variable	UPA (N = 126)	BPA (N = 32)	P-value
Female sex, n (%)	53 (42.1)	7 (21.9)	0.036
Age at diagnosis (years)	49 ± 10.3	55 ± 7.1	0.001
Duration of HTN (months)	66 [22; 156]	75 [44; 124]	0.721
Systolic BP (mmHg)	164 ± 25.7	164 ± 20.3	0.934
Diastolic BP (mmHg)	97 ± 14.3	98 ± 12.7	0.824
Antihypertensive medication (DDD)	4.0 [2.5; 6.0]	2.9 [1.4; 4.3]	0.008
PRA at screening (ng/mL/h)	0.3 [0.20; 0.40]	0.20 [0.10; 0.22]	0.014
Aldosterone at screening (ng/dL)	38.2 [25.9; 49.8]	30.2 [21.1; 41.0]	0.044
Lowest potassium (mEq/L)	3.1 ± 0.6	3.9 ± 0.4	< 0.001
Aldosterone post-confirmatory test (ng/dL)	21.9 [13.9; 35.5]	11.4 [7.7; 19.9]	< 0.001
Microalbuminuria, n (%)	37 (29.6)	6 (19.0)	0.333
LVH at Echo, n (%)	70 (55.2)	24 (76.0)	0.058
CV events, n (%)	17 (13.6)	6 (18.5)	0.519
Largest nodule at CT scanning (diameter, mm)	13 [10; 18]	11 [9; 20]	0.433
Normal ipsilateral imaging, n (%)	2 (1.6)	14 (43.8)	<0.001
Normal contralateral imaging, n (%)	107 (84.9)	17 (53.1)	<0.001
Peripheral A/C ratio	15.9 [3.7; 26.2]	13.3 [7.2; 18.1]	0.145
Contralateral A/C ratio	4.1 [1.5; 8.3]	7.9 [3.2; 12.0]	0.012
Contralateral ratio	0.3 [0.2; 0.5]	0.7 [0.5; 0.8]	< 0.001

Legend to Table 1 – Characteristics of patients included in the analysis stratified for diagnosis:

unilateral PA (UPA; N=126) vs. bilateral PA (BPA; N=32). Antihypertensive medication is expressed as Defined Daily Dose (DDD). DDD is the average maintenance dose per day for a drug used for its main indication in adults. HTN, Hypertension; BP, Blood Pressure; PRA, Plasma Renin Activity; LVH, Left Ventricular Hypertrophy; Echo, Echocardiography; CV, Cardiovascular; CT, Computed Tomography; SI, Selectivity Index; A/C, Aldosterone to Cortisol ratio. Normally and non-normally distributed variables were reported as mean ± standard deviation or median [interquartile range], respectively. Categorical variables were reported as absolute number (n) and proportion (%).

Table 2. Selected discriminant variables for a diagnosis of unilateral PA

Variable (ref. BPA)	Univariate analysis			Multivariate Analysis		
	OR (CI 95%)	β	<i>P</i>	OR (CI 95%)	β	<i>P</i>
Aldosterone at screening (ng/dL)	1.01 (1.01 – 1.01)	0.003	0.046	1.01 (1.01-1.03)	0.001	0.032
Lowest potassium (mEq/L)	0.07 (0.03 – 0.20)	-2.639	< 0.001	0.04 (0.01 – 0.26)	-3.139	0.001
Aldosterone post-confirmatory test (ng/dL)	1.01 (1.01 – 1.02)	0.006	0.003	1.01 (1.01 – 1.02)	0.004	0.038
Normal ipsilateral imaging	0.02 (0.01 – 0.10)	-3.876	< 0.001	0.02 (0.01 – 0.19)	-4.120	0.001
Normal contralateral imaging	4.97 (2.13 – 11.61)	1.603	< 0.001	5.35 (1.02 – 33.11)	1.678	0.041
Contralateral ratio	0.01 (0.01 – 0.03)	-5.710	< 0.001	0.01 (0.01 – 0.08)	-6.532	0.001

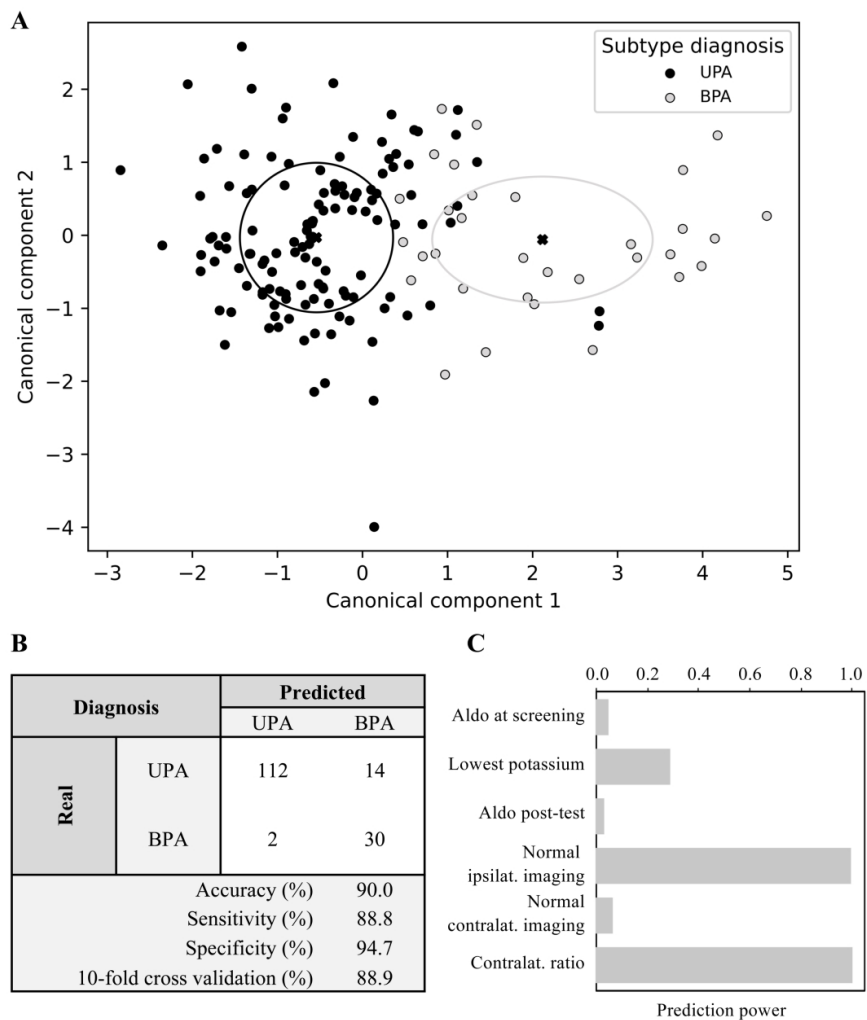


Figure 1. Diagnostic Modelling: Linear Discriminant Analysis.

126x141mm (600 x 600 DPI)

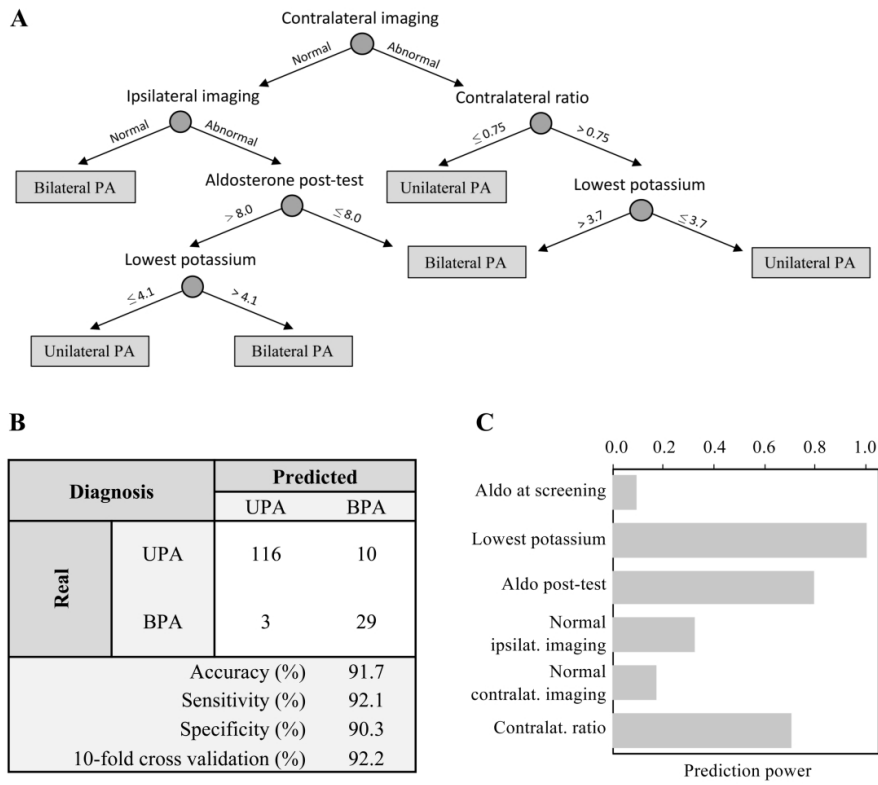


Figure 2. Diagnostic Modelling: Random Forest

127x110mm (600 x 600 DPI)

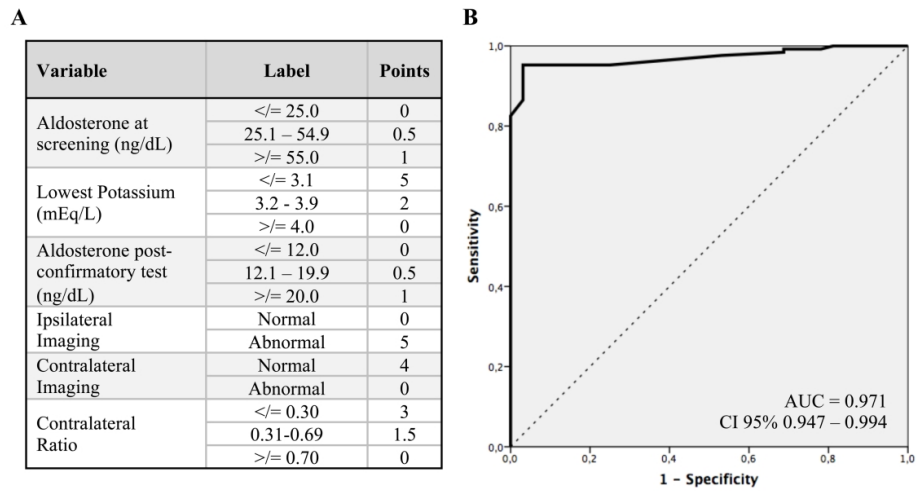


Figure 3. Score development

139x75mm (600 x 600 DPI)

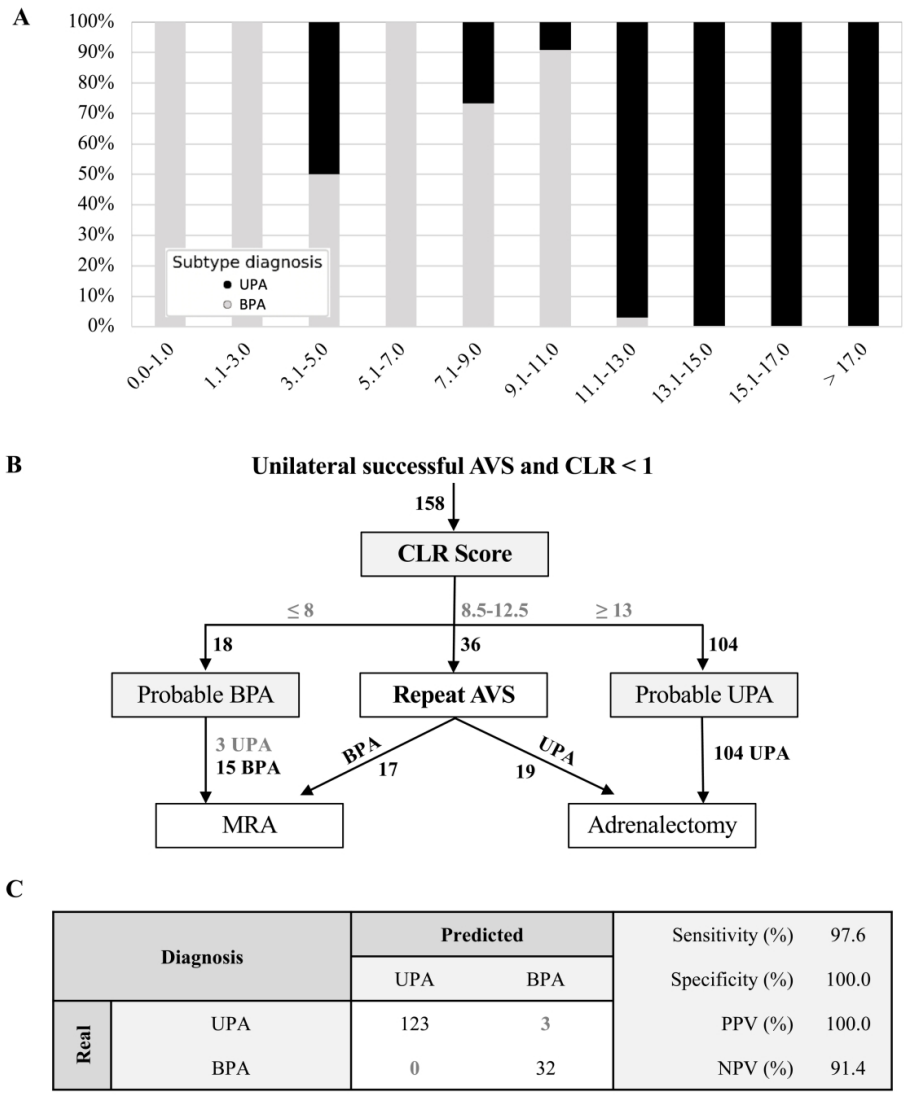


Figure 4. Score performance and management of PA patients

131x155mm (600 x 600 DPI)

Supplementary Table 1. Patient Characteristics of Study Cohort

Variable	Basal (N = 76)	ACTH (N = 82)	P-value
Diagnosis of UPA, n (%)	57 (75.0)	69 (84.1)	0.153
Female sex, n (%)	27 (35.5)	33 (40.2)	0.542
Age at diagnosis (years)	51 ± 9.3	49 ± 10.5	0.156
Duration of HTN (months)	69 [24; 176]	66 [22; 125]	0.342
Systolic BP (mmHg)	163 ± 25.2	164 ± 23.8	0.821
Diastolic BP (mmHg)	98 ± 13.0	97 ± 15.0	0.756
Antihypertensive medication (DDD)	4.0 [2.4; 5.7]	3.7 [2.2; 5.0]	0.671
PRA at screening (ng/mL/h)	0.20 [0.10; 0.30]	0.20 [0.15; 0.40]	0.656
Aldosterone at screening (ng/dL)	38.9 [25.8; 50.5]	34.8 [24.3; 45.3]	0.198
Lowest potassium (mEq/L)	3.3 ± 0.7	3.1 ± 0.7	0.085
Aldosterone post-confirmatory test (ng/dL)	19.6 [11.9; 32.7]	19.6 [10.8; 32.0]	0.738
Microalbuminuria, n (%)	23 (30.4)	21 (25.0)	0.541
LVH at Echo, n (%)	49 (65.1)	44 (53.7)	0.188
CV events, n (%)	10 (12.7)	13 (16.4)	0.549
Largest nodule at CT scanning (diameter, mm)	12 [10; 16]	14 [10; 20]	0.189
Normal ipsilateral imaging, n (%)	8 (10.5)	8 (9.8)	0.873
Normal contralateral imaging, n (%)	62 (81.6)	62 (75.6)	0.362
Contralateral SI	14.6 [7.5; 33.3]	23.0 [13.9; 48.6]	0.002
Peripheral A/C ratio	17.8 [3.4; 28.7]	14.4 [4.1; 19.7]	0.348
Contralateral A/C ratio	4.4 [1.2; 9.9]	4.9 [1.6; 8.6]	0.867
Contralateral ratio	0.3 [0.2; 0.6]	0.4 [0.3; 0.6]	0.274

Clinical characteristics of patients included in the analysis: patients were stratified according to the protocol of adrenal venous sampling (basal vs. ACTH). Antihypertensive medication is expressed as Defined Daily Dose (DDD). DDD is the average maintenance dose per day for a drug used for its main indication in adults. UPA, Unilateral Primary Aldosteronism; HTN, Hypertension; BP, Blood Pressure; PRA, Plasma Renin Activity; LVH, Left Ventricular Hypertrophy; Echo, Echocardiography; CV, Cardiovascular; CT, Computed Tomography; SI, Selectivity Index; A/C, Aldosterone to Cortisol ratio. Normally and non-normally distributed variables were reported as mean ± standard deviation or median [interquartile range], respectively. Categorical variables were reported as absolute number (n) and proportion (%).

Supplementary Table 2. Patient Characteristics of Study Cohort

Variable (ref. BPA)	OR (CI 95%)	β	P-value
Female sex, n (%)	2.59 (1.04 – 6.44)	0.943	0.040
Age at diagnosis (years)	0.94 (0.90 – 0.98)	-0.065	0.006
Duration of HTN (months)	1.01 (0.99 – 1.01)	0.002	0.236
Systolic BP (mmHg)	1.00 (0.98 – 1.02)	-0.001	0.933
Diastolic BP (mmHg)	1.00 (0.96 – 1.03)	-0.004	0.822
Antihypertensive medication (DDD)	1.43 (1.09 – 1.87)	0.357	0.009
PRA at screening (ng/mL/h)	4.40 (0.56 – 34.43)	1.480	0.159
Aldosterone at screening (ng/dL)	1.01 (1.01 – 1.01)	0.003	0.046
Lowest potassium (mEq/L)	0.07 (0.03 – 0.20)	-2.639	< 0.001
Aldosterone post-confirmatory test (ng/dL)	1.01 (1.01 – 1.02)	0.006	0.003
Microalbuminuria, n (%)	1.79 (0.54 – 5.88)	0.582	0.337
LVH at Echo, n (%)	0.39 (0.14 – 1.05)	-0.942	0.063
CV events, n (%)	0.69 (0.23 – 2.13)	-0.368	0.521
Largest nodule at CT scanning (diameter, mm)	0.99 (0.92 – 1.06)	-0.015	0.683
Normal ipsilateral imaging, n (%)	0.02 (0.01 – 0.10)	-3.876	< 0.001
Normal contralateral imaging, n (%)	4.97 (2.13 – 11.61)	1.603	< 0.001
Peripheral A/C ratio	1.03 (1.00 – 1.06)	0.028	0.071
Contralateral A/C ratio	0.91 (0.85 – 0.97)	-0.093	0.007
Contralateral ratio	0.01 (0.01 – 0.03)	-5.710	< 0.001

Univariate logistic regression analysis was performed to assess the odds ratio (OR), β estimate and the 95% confidence interval (CI) for each variable. An OR greater than 1 indicates an increased likelihood of unilateral primary aldosteronism (UPA), and an OR less than 1 a decreased likelihood (i.e. an OR of 1.01 for aldosterone means an increase of 1% of the likelihood of UPA for each 1 ng/dL increase of aldosterone concentration). Antihypertensive medication is expressed as Defined Daily Dose (DDD). DDD is the average maintenance dose per day for a drug used for its main indication in adults. HTN, Hypertension; BP, Blood Pressure; PRA, Plasma Renin Activity; LVH, Left Ventricular Hypertrophy; Echo, Echocardiography; CV, Cardiovascular; CT, Computed Tomography; SI, Selectivity Index; A/C, Aldosterone to Cortisol ratio. Ipsilateral and contralateral imaging were defined as “normal” in absence of nodules, or with thickening < 4 mm.

Supplementary Table 3. Patient Characteristics of Study Cohort

Variable	Combined Cohort (N = 158)	Training Cohort (N = 110)	Validation Cohort (N = 48)	P-value
ACTH protocol, n (%)	82 (51.9)	53 (48.2)	29 (60.4)	0.157
Diagnosis of UPA, n (%)	126 (79.7)	88 (80.0)	38 (79.2)	0.905
Female sex, n (%)	60 (38.0)	40 (36.4)	20 (41.7)	0.528
Age at diagnosis (years)	50 ± 10.0	50 ± 9.3	50 ± 11.5	0.949
Duration of HTN (months)	67 [23; 130]	66 [23; 128]	68 [24; 161]	0.825
Systolic BP (mmHg)	164 ± 24.5	163 ± 24.5	166 ± 24.6	0.534
Diastolic BP (mmHg)	98 ± 13.9	98 ± 13.8	97 ± 14.3	0.925
Antihypertensive medication (DDD)	3.8 [2.2; 5.0]	3.7 [2.4; 5.0]	4.0 [2.2; 5.5]	0.986
PRA at screening (ng/mL/h)	0.20 [0.12; 0.40]	0.29 [0.15; 0.40]	0.20 [0.10; 0.30]	0.166
Aldosterone at screening (ng/dL)	36.0 [25.5; 47.8]	35.9 [25.6; 47.3]	37.9 [25.0; 49.8]	0.867
Lowest potassium (mEq/L)	3.2 ± 0.7	3.2 ± 0.7	3.2 ± 0.7	0.658
Aldosterone post-confirmatory test (ng/dL)	19.6 [11.3; 32.0]	20.1 [13.1; 32.3]	16.4 [9.5; 33.0]	0.172
Microalbuminuria, n (%)	43 (27.5)	32 (29.1)	11 (21.9)	0.394
LVH at Echo, n (%)	94 (59.2)	65 (59.1)	29 (59.5)	0.963
CV events, n (%)	23 (14.6)	16 (14.4)	7 (15.0)	0.934
Largest nodule at CT scanning (diameter, mm)	13 [10; 19]	14 [10; 20]	12 [10; 18]	0.219
Normal ipsilateral imaging, n (%)	16 (10.1)	10 (9.1)	6 (12.5)	0.514
Normal contralateral imaging, n (%)	124 (78.5)	84 (76.4)	40 (83.3)	0.327
Contralateral SI	18.8 [10.2; 41.5]	18.8 [11.3; 47.5]	20.2 [7.9; 30.3]	0.463
Peripheral A/C ratio	17.8 [3.4; 28.7]	15.9 [3.7; 23.1]	14.3 [5.4; 26.0]	0.950
Contralateral A/C ratio	4.4 [1.2; 9.9]	4.4 [1.4; 9.1]	5.5 [1.8; 9.3]	0.456
Contralateral ratio	0.3 [0.2; 0.6]	0.3 [0.2; 0.6]	0.4 [0.2; 0.7]	0.393

Characteristics of patients included in the analysis: patients from the combined cohort (N = 158) were randomly assigned to training (N = 110), or validation cohort (N = 48). Antihypertensive medication is expressed as Defined Daily Dose (DDD). DDD is the average maintenance dose per day for a drug used for its main indication in adults. UPA, Unilateral Primary Aldosteronism; HTN, Hypertension; BP, Blood Pressure; PRA, Plasma Renin Activity; LVH, Left Ventricular Hypertrophy; Echo, Echocardiography; CV, Cardiovascular; CT, Computed Tomography; SI, Selectivity Index; A/C, Aldosterone to Cortisol ratio. Normally and non-normally distributed variables were reported as mean ± standard deviation or median [interquartile range], respectively. Categorical variables were reported as absolute number (n) and proportion (%).

Supplementary Table 4. Diagnostic performance

Score Performance	N	Cut-off	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Acc (%)
LDA Model (Training)	110		90.6 (88.2-93.0)	99.4 (96.4-100.0)	99.8 (99.0-100.0)	72.6 (67.4-77.8)	92.3 (90.1-94.5)
Internal Validation	110	N.A.	88.9 (85.6-92.2)	88.9 (77.7-100.0)	97.0 (94.1-99.9)	66.8 (59.5-74.1)	88.9 (85.3-92.5)
External Validation	48		84.7 (82.7-86.6)	84.2 (73.1-95.3)	95.4 (92.2-98.6)	59.1 (55.1-63.1)	84.6 (82.1-87.1)
Combined cohort	158		88.8 (86.8-90.8)	94.7 (91.1-98.3)	98.5 (97.5-99.5)	68.3 (64.7-71.9)	90.0 (88.5-91.6)
RF Model (Training)	110		95.8 (94.1-97.5)	94.9 (91.7-98.1)	98.7 (97.9-99.5)	85.1 (80.0-90.2)	95.6 (94.2-97.0)
Internal Validation	110	N.A.	92.2 (89.1-95.3)	79.5 (66.5-92.6)	94.7 (91.6-97.8)	71.9 (63.6-80.2)	89.6 (86.1-93.1)
External Validation	48		83.6 (79.0-88.2)	80.0 (77.8-82.2)	94.1 (93.8-94.4)	56.4 (49.2-63.6)	82.9 (79.3-86.5)
Combined cohort	158		92.1 (90.2-94.0)	90.3 (88.1-92.5)	97.4 (96.8-98.0)	74.5 (69.8-79.2)	91.7 (90.1-93.3)
CLR Score - Best Accuracy	110		95.5 (88.9-98.2)	100.0 (85.1-100.0)	100.0 (96.0-100.0)	84.7 (65.7-93.3)	96.4 (88.1-98.6)
Internal Validation	110	> 11	95.3 (83.9-100.0)	100.0 (97.5-100.0)	100.0 (98.4-100.0)	88.3 (59.9-95.4)	96.3 (87.7-100.0)
External Validation	48		94.7 (82.7-99.1)	90.0 (59.6-99.5)	97.3 (88.6-99.9)	81.7 (47.6-96.7)	93.7 (77.9-99.2)
Combined Cohort	158		95.2 (90.0-97.8)	96.9 (84.3-99.8)	99.2 (95.8-99.9)	83.7 (68.2-92.0)	95.5 (88.8-98.2)
CLR Score (Sensitivity Optimization)	110		97.8 (92.1-99.6)	45.5 (26.9-65.3)	87.8 (83.4-92.0)	83.8 (46.0-97.6)	87.3 (79.1-92.7)
Internal Validation	110	> 8	96.9 (87.6-100.0)	51.7 (28.0-75.4)	88.8 (72.0-100.0)	75.0 (47.0-96.9)	87.3 (71.0-94.7)
External Validation	48		97.4 (86.5-99.9)	50.0 (23.7-76.3)	88.1 (81.2-94.1)	83.5 (31.6-99.5)	87.5 (73.4-95.0)
Combined Cohort	158		97.6 (93.2-99.4)	46.9 (30.9-63.6)	87.9 (84.2-91.5)	83.2 (53.6-96.4)	87.3 (80.6-92.1)
CLR Score (Specificity Optimization)	110		81.8 (72.5-88.5)	100.0 (85.1-100.0)	100.0 (95.1-100.0)	57.9 (43.6-68.5)	85.4 (75.0-90.8)
Internal Validation	110	≥ 13	79.2 (51.4-93.2)	100.0 (97.8-100.0)	100.0 (97.3-100.0)	54.5 (24.8-83.1)	82.7 (59.5-95.8)
External Validation	48		84.2 (69.6-92.6)	100.0 (72.2-100.0)	100.0 (90.5-100.0)	62.5 (38.5-78.1)	87.5 (70.1-94.1)
Combined Cohort	158		82.5 (75.0-88.2)	100.0 (89.3-100.0)	100.0 (96.5-100.0)	59.2 (47.6-68.3)	86.0 (77.9-90.6)

Sensitivity (Sens), specificity (Spec), positive/negative predictive value (PPV/ NPV), and accuracy (Acc) for proposed models (each indicator is derived considering unilateral PA as referral diagnosis and reported together with its 95% binomial confidence interval; confidence intervals are derived by normal approximation method and capped to 100%). Diagnostic indices are provided at training, at internal validation by 10-fold cross validation, at external validation, and in the combined cohort. A cut-off of greater than 11 corresponded to the higher model accuracy, as assessed by ROC curve analysis. The cut-offs of > 8 and ≥ 13 increased sensitivity or specificity, respectively, providing an overall model accuracy of at least 85% at validation. LDA, Linear Discriminant Analysis; RF, Random Forest.

Supplementary Table 5. Estimate predictor importance for LDA and RF models

Variable	LDA	RF
Aldosterone at screening (ng/dL)	0.047626	0.093263
Lowest potassium (mEq/L)	-0.287831	1.000000
Aldosterone post-confirmatory test (ng/dL)	0.033038	0.797178
Normal ipsilateral imaging, n (%)	-0.994998	0.327111
Normal contralateral imaging, n (%)	0.064665	0.172839
Contralateral ratio	-1.000000	0.707113

Normalized LDA and RF importance coefficients computed at the training of the models. A highest absolute value corresponds to the best predictor in each model.

Supplementary Table 6. Score development and validation

AVS Score Accuracy		Predicted Diagnosis		Performance		
Real Diagnosis (Cut-off > 11)	Training cohort (N = 110)		UPA	BPA	Accuracy (%)	96.4
		UPA	84	4	Sensitivity (%)	95.5
		BPA	0	22	Specificity (%)	100.0
	Validation cohort (N = 48)		UPA	BPA	Accuracy (%)	93.7
		UPA	36	2	Sensitivity (%)	94.7
		BPA	1	9	Specificity (%)	90.0
	Combined cohort (N = 158)		UPA	BPA	Accuracy (%)	95.5
		UPA	120	6	Sensitivity (%)	95.2
		BPA	1	31	Specificity (%)	96.9
Real Diagnosis (Cut-off > 8)	Training cohort (N = 110)		UPA	BPA	Accuracy (%)	87.3
		UPA	86	2	Sensitivity (%)	97.8
		BPA	12	10	Specificity (%)	45.5
	Validation cohort (N = 48)		UPA	BPA	Accuracy (%)	87.5
		UPA	37	1	Sensitivity (%)	97.4
		BPA	5	5	Specificity (%)	50.0
	Combined cohort (N = 158)		UPA	BPA	Accuracy (%)	87.3
		UPA	123	3	Sensitivity (%)	97.6
		BPA	17	15	Specificity (%)	46.9
Real Diagnosis (Cut-off >= 13)	Training cohort (N = 110)		UPA	BPA	Accuracy (%)	85.4
		UPA	72	16	Sensitivity (%)	81.8
		BPA	0	22	Specificity (%)	100.0
	Validation cohort (N = 48)		UPA	BPA	Accuracy (%)	87.5
		UPA	32	6	Sensitivity (%)	84.2
		BPA	0	10	Specificity (%)	100.0
	Combined cohort (N = 158)		UPA	BPA	Accuracy (%)	86.0
		UPA	104	22	Sensitivity (%)	82.5
		BPA	0	32	Specificity (%)	100.0

The table shows the real and predicted subtype diagnosis, accuracy sensitivity, specificity for the training cohort (N = 110), the validation cohort (N = 48), and the combined cohort (N = 158). A cut-off of greater than 11 identifies patients with unilateral primary aldosteronism (UPA) with the higher accuracy. A cut-off of greater than 8 identifies patients with a diagnosis of UPA with an optimized sensitivity; cut-off of greater than or equal to 13 identifies patients with a diagnosis of UPA with an optimized specificity. BPA, Bilateral Primary Aldosteronism.

Supplementary Table 7. Distribution of PA patients according to the score

Score points	Total (N)	UPA		BPA	
		(N)	(%)	(N)	(%)
0.0-1.0	1	0	0.0	1	100.0
1.1-3.0	5	0	0.0	5	100.0
3.1-5.0	2	1	50.0	1	50.0
5.1-7.0	3	0	0.0	3	100.0
7.1-9.0	15	4	26.7	11	73.3
9.1-11.0	11	1	9.1	10	90.9
11.1-13.0	33	32	97.0	1	3.0
13.1-15.0	21	21	100.0	0	0.0
15.1-17.0	34	34	100.0	0	0.0
> 17.0	33	33	100.0	0	0.0
<i>Total</i>	158	126	N.A.	32	N.A.

The number (N) and the proportion (%) of patients stratified for subtype diagnosis (unilateral PA *versus* bilateral PA) is shown according to the score in the combined cohort (N = 158).

Supplementary Table 8. Sub-analysis according to patient outcome

Subgroup of patients	Clinical Outcome	Biochemical Outcome
<i>UPA predicted as UPA (true prediction)</i>		
Complete, n (%)	51 (53.1)	94 (97.9)
Partial, n (%)	41 (42.7)	2 (2.1)
Absent, n (%)	4 (4.2)	0 (0.0)
<i>UPA predicted as BPA (false prediction)</i>		
Complete, n (%)	0 (0.0)	4 (66.7)
Partial, n (%)	5 (83.3)	2 (33.3)
Absent, n (%)	1 (16.7)	0 (0.0)
<i>Unstimulated AVS</i>		
Complete, n (%)	21 (42.9)	48 (98.0)
Partial, n (%)	27 (55.1)	1 (2.0)
Absent, n (%)	1 (2.0)	0 (0.0)
<i>ACTH-stimulated AVS</i>		
Complete, n (%)	30 (56.7)	50 (94.3)
Partial, n (%)	19 (35.8)	3 (5.7)
Absent, n (%)	4 (7.5)	0 (0.0)

Clinical and biochemical outcomes according to the PASO criteria were reported for patients with unilateral primary aldosteronism (UPA) after a follow-up of 6-12 months. Patients were stratified according to the CLR score predicted diagnosis (true vs. false predictions) or AVS protocol (ACTH-stimulated vs. unstimulated procedures).