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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1742456> since 2022-06-13T06:40:02Z

Published version:

DOI:10.1210/clinem/dgaa379

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1 **Development and validation of prediction models for subtype diagnosis of**
2 **patients with primary aldosteronism.**

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15 **Short title:** Diagnostic modelling for PA subtyping

16 **Keywords:** aldosterone; primary aldosteronism; adrenal venous sampling; machine learning.

17

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23 **Source(s) of Funding:** this research did not receive any specific grant. CA and MR are supported
24 by the Else Kröner-Fresenius Stiftung (2019_A104) and by the Deutsche Forschungsgemeinschaft
25 within the CRC/Transregio 205/1 "The Adrenal: Central Relay in Health and Disease".

26 **Conflict(s) of Interest/Disclosure(s):** the authors have nothing to disclose.

27 **ABSTRACT**

28 **Context.** Primary aldosteronism (PA) comprises unilateral (lateralized, LPA) and bilateral disease
29 (BPA). The identification of LPA is important to recommend potentially curative adrenalectomy.
30 Adrenal venous sampling (AVS) is considered the gold standard for PA subtyping, but the procedure
31 is available in few referral centers.

32 **Objective.** To develop prediction models for subtype diagnosis of PA using patient clinical and
33 biochemical characteristics.

34 **Design, Patients and Setting.** Patients referred to a tertiary hypertension unit. Diagnostic
35 algorithms were built and tested in a training (N=150) and in an internal validation cohort (N=65),
36 respectively. The models were validated in an external independent cohort (N=118).

37 **Main outcome measure.** Regression analyses and supervised machine learning algorithms were
38 used to develop and validate two diagnostic models and a 20-point score to classify patients with
39 PA according to subtype diagnosis.

40 **Results.** Six parameters were associated with a diagnosis of LPA (aldosterone at screening and after
41 confirmatory testing, lowest potassium value, presence/absence of nodules, nodule diameter, and
42 computed tomography results) and were included in the diagnostic models. Machine learning
43 algorithms displayed high accuracy at training and internal validation (79.1% to 93%), whereas a
44 20-point score reached an AUC of 0.896, and a sensitivity/specificity of 91.7/79.3%. An integrated
45 flow-chart correctly addressed 96.3% of patients to surgery and would have avoided AVS in 43.7%
46 of patients. The external validation on an independent cohort confirmed a similar diagnostic
47 performance.

48 **Conclusions.** Diagnostic modelling techniques can be used for subtype diagnosis and guide surgical
49 decision in patients with PA in centers where AVS is unavailable.

50 INTRODUCTION

51 Primary aldosteronism (PA) accounts for 3-13% of primary care hypertensive patients [1-3] and is
52 associated with an increased cardio- and cerebrovascular risk compared with patients affected by
53 essential hypertension [4;5]. The two major subtypes of PA are unilateral primary aldosteronism
54 (lateralized, LPA), mainly due to an aldosterone producing adenoma (APA), and bilateral primary
55 aldosteronism (BPA). The treatments of choice are unilateral adrenalectomy, or medical therapy
56 with a mineralocorticoid receptor antagonist, respectively [6]. A timely and accurate subtype
57 diagnosis is critical to recommend the appropriate treatment and improving the outcomes of these
58 patients [7;8].

59 Over the last decades, many procedures have been proposed to differentiate LPA from BPA,
60 including posture testing, functional imaging (using 11-C-metomidate or 68-Ga-pentixafor tracers
61 [9;10] and steroid profiling [11-13]. Nevertheless, technical issues and/or the lack of sensitivity and
62 specificity hampered the introduction of these tests in the routine management of PA patients [6].

63 Adrenal venous sampling (AVS) is currently considered the gold standard for subtype diagnosis [6].
64 Nevertheless, several concerns prevent its widespread use: AVS is an invasive, time-consuming,
65 and relatively expensive procedure, requiring a high level of technical skill and is available only in
66 a limited number of referral centers [14]. Beside AVS, adrenal computed tomography (CT) scanning
67 is widely available in most centers and performed in all patients with confirmed PA [6]. Even if
68 several studies reported unreliable diagnostic performance of CT in PA subtyping [15;16], score-
69 based algorithms combining imaging findings with clinical and biochemical parameters have been
70 developed [17-23]. Küpers et al. first proposed a prediction score to bypass AVS; patients with a
71 potassium lower than 3.5 mmol/L, an estimated glomerular filtration rate (eGFR) more than 100
72 ml/min and a typical adenoma at CT imaging could avoid AVS. Sensitivity and specificity were
73 53.1% and 100%, respectively [17]. Other clinical scores were subsequently developed in the
74 attempt of differentiating LPA from BPA, with an accuracy ranging from 58.2% to 86.3% [18-23].
75 Only 2 of these scores were also validated in independent cohorts [17;22], 5 included less than 100

76 patients in the development cohort [17-20;23], and the majority of these scores was applicable only
77 in selected cohorts of patients with PA [18-21;23].

78 Considering the high prevalence of PA and the limited availability of AVS, an alternative method
79 that reduces the number of requested AVS is highly desirable. Our objective was to develop and
80 validate clinical models to discriminate LPA from BPA, which can bypass AVS for bilateral disease,
81 and indicating unilateral adrenalectomy for patients with high probability of LPA who cannot
82 undergo AVS. We propose herein two advanced diagnostic models based on supervised machine
83 learning algorithms and a flow-chart integrating our score-system (the SPACE score, Subtyping
84 Primary Aldosteronism by Clinical Evaluation) in PA patient management. Validation of previously
85 described score-based algorithms is also provided and demonstrates the superiority of our prediction
86 models.

87

88 **METHODS**

89 Data analyzed during the current study are not publicly available but are available from the
90 corresponding author on reasonable request. A detailed description of patient management and data
91 extraction, statistical analyses, and diagnostic modelling is provided as Supplemental Data [24]
92 (available at <https://github.com/ABurrello/SPACE-score>).

93 ***Study cohort and data extraction***

94 We retrospectively evaluated a cohort of 215 patients referred to the hypertension unit of the
95 University of Torino between 2008 and 2019, to train and test the diagnostic models. Eligible
96 patients were randomly assigned to the training cohort (N = 150) or to the internal validation cohort
97 (N = 65). An independent cohort of 118 consecutive patients from the Munich Klinikum der
98 Universität treated between 2008 and 2014 was used for external validation. PA was diagnosed
99 according to the Endocrine Society Guideline [6]. Inclusion criteria were: (1) confirmed diagnosis
100 of PA; (2) successful AVS for subtype diagnosis.

101 Unilateral PA mainly depends on unilateral aldosterone-producing adenoma. However, a
102 lateralization at AVS may also occur also in the presence of a dominant lesion with asymmetrical
103 autonomous aldosterone production in the context of bilateral adrenal alterations, including
104 aldosterone producing cell clusters, or diffuse/nodular hyperplasia. For this reason, the AVS-based
105 term of “lateralized PA” was used to indicate a prevalently unilateral disease throughout the present
106 study.

107 ***Statistical analysis***

108 IBM SPSS Statistics 22 (*IBM Corp., Armonk, New York, USA*) was used for statistical analyses.
109 Data distribution was assessed by the Kolmogorov–Smirnov test. Normally distributed variables
110 were analyzed by T-student test and reported as mean \pm standard deviation. Non-normally
111 distributed variables were analyzed by Mann-Whitney’s test and reported as median [interquartile
112 range]. Categorical variables were analyzed by Chi-square test and reported as absolute number and
113 proportion (%). Univariate logistic regression analysis was used to define the odds ratios (ORs) for
114 each analyzed parameter. Six selected variables were included in the multivariate logistic regression
115 analysis. An OR greater than 1 is associated with an increased likelihood of the defined outcome
116 (diagnosis of LPA), an OR less than 1, a decreased likelihood. A *P* value of less than 0.05 was
117 considered significant.

118 ***Diagnostic modelling***

119 Python 3.5 (library, scikit-learn) was used for the development and validation of diagnostic models
120 by machine learning techniques. Supervised machine learning algorithms are widely used in clinical
121 research to formulate predictions about possible outcomes based on a pre-defined set of labeled
122 paired input-output training sample data [13;25]. Supervised machine learning and in particular
123 linear discriminant analysis (LDA) and random forest (RF) algorithms were applied on the
124 combined cohort to develop diagnostic models able to discriminate patients with LPA vs. BPA. Six
125 selected variables were used in both models (aldosterone at screening and after confirmatory testing,

126 lowest potassium recorded in the absence of diuretic therapy, presence/absence of a nodule at CT
127 scanning imaging, nodule diameter, and descriptive CT scanning findings).

128 LDA maximize the separation between groups by increasing precision estimates by variance
129 reduction. The algorithm computes a set of coefficients for linear combination of each variable to
130 classify patients according to their diagnosis; a canonical plot was used to represent diagnostic
131 performance of the LDA model. The RF model was composed of 20 classification trees with a
132 maximum number of eight splits for each tree. The predicted diagnosis was defined on the basis of
133 the outcome of each classification tree of the RF: if at least 11 of 20 trees of the forest predict the
134 diagnosis of lateralized PA, the patient is classified as LPA. The RF model was integrated in a free-
135 downloadable tool which allows the application of the algorithm in clinical practice (available at
136 https://github.com/ABurrello/SPACE-score/raw/master/Random_Forest_model.zip).

137 Performance and generalizability of both LDA and RF models were evaluated by a 10K-cross
138 validation algorithm (see extended methods - Supplemental Data) [24].

139 The 6 variables were used to develop a 20-point score to predict the diagnosis of LPA. Variables
140 were categorized, points were assigned to each reference interval, and cut-offs were derived to
141 achieve the best accuracy in an automated way. The SPACE score was generated using the training
142 cohort and tested with both internal and external validation cohorts. ROC curve analysis was used
143 to assess the area under the curve and derive the best cut-off to discriminate patients with LPA by
144 evaluation of the Younden Index ($J = \text{sensitivity} + \text{specificity} - 1$). A second online tool was
145 developed to automatically calculate the score and the predicted diagnosis (available at
146 <https://github.com/ABurrello/SPACE-score/raw/master/SPACE%20Score%20Calculator.xlsm>).

147

148 **RESULTS**

149 *Patient characteristics*

150 Two hundred and fifteen patients were included in the analyses from the developmental cohort of
151 Torino, 133 with a diagnosis of LPA and 82 with BPA. Clinical and biochemical characteristics are

152 reported in Table 1. The mean age at diagnosis was 49 ± 9.5 years, mean BP was 164/99 mmHg,
153 with a duration of hypertension of 68 [27; 128] months. Patients with a diagnosis of LPA were more
154 frequently females (42.1% vs. 23.2%; $P = 0.005$), had a higher DDD (3.8 [2.2; 5.7] vs. 3.0 [1.3; 4.7]
155 $P = 0.027$) and a lower potassium level (3.1 ± 0.6 vs. 3.8 ± 0.4 ; $P < 0.001$). At the diagnostic workup,
156 patients with LPA displayed higher levels of aldosterone, both at screening (38.0 [25.7; 49.7] vs.
157 28.7 [19.8; 37.9] ng/dL; $P < 0.001$) and post-confirmatory testing (20.5 [13.3; 32.9] vs. 11.5 [8.2;
158 17.7] ng/dL; $P < 0.001$). To confirm PA diagnosis, 165 patients underwent saline infusion testing
159 (76.7%), and 50 had captopril challenge testing (23.3%). CT scanning demonstrated the presence
160 of a defined nodule in 85.7% of patients with LPA; a nodule was also detected in 41.5% of patients
161 with bilateral disease (unilateral nodule in 29 of 34 cases, bilateral in 5). In patients with BPA, CT
162 scanning was bilaterally normal in 24.4% of patients, bilaterally abnormal in 22%, and with a
163 unilateral abnormality in 53.7% of the cases (see the Supplemental Data for details on adrenal CT
164 scanning interpretation and definition of nodule) [24]. Among the 37 patients with bilateral
165 abnormalities at CT scanning, 40.6% displayed a unilateral nodule in the context of bilateral adrenal
166 thickening or contralateral thickening, 37.8% bilateral nodules, and 21.6% bilateral hyperplasia.
167 Prevalence of target organ damage (eGFR, microalbuminuria, and LVH at echocardiography) and
168 prior cardiovascular events was not significantly different between groups. As expected, the LI at
169 AVS was significantly higher in LPA than BPA patients (12.0 [6.9; 21.3] vs. 1.8 [1.3; 2.6]; $P <$
170 0.001). According to the PASO criteria [7], after a follow-up of 6-12 months from unilateral
171 adrenalectomy, patients with LPA displayed complete clinical and biochemical success in 54.1%
172 and 98.5% of cases, respectively.

173 Univariate logistic regression analysis was performed including all parameters (Table S1) [24],
174 showing a significant association with a diagnosis of LPA of female sex (OR 2.41), duration of
175 HTN (OR 1.01), DDD (Defined Daily Dose; OR 1.18), potassium (OR 0.10), aldosterone at
176 screening (OR 1.01) and after confirmatory testing (OR 1.01), presence of a nodule at CT scanning
177 (OR 8.33), nodule diameter (OR 1.12), and CT findings (OR 9.91). Six out of these 9 variables were

178 selected considering their discriminative performance and introduced in the multivariate model,
179 which confirmed a highly significant independent association with the diagnosis of LPA for all
180 parameters (Table 2).

181 ***Linear discriminant analysis model***

182 The 6 selected variables confirmed by the multivariate regression analysis were used in an LDA
183 model. The linear combination of variables included in the LDA is shown in the canonical plot
184 (Figure 1A). Each point represents a patient and the clear separation according to their subtype
185 diagnosis indicates that the model can discriminate LPA from BPA with reliable accuracy. In
186 particular, 175 of 215 patients (accuracy 81.4%) were correctly classified, with a sensitivity and
187 specificity for LPA detection of 86.5% and 73.2%, respectively (Figure 1B). To exclude overfitting
188 bias and assess how the model could generalize in an independent cohort, the LDA was validated
189 by a 10K-cross validation algorithm (see extended methods - Supplemental Data) [24]. The cross
190 validation showed a high predictive performance with an accuracy of 79.1%, compared with the
191 81.4% (at training), thus confirming a negligible overfitting bias (overfitting effect = 2.3%). In the
192 LDA model, the stronger predictor was the lowest potassium level (normalized LDA coefficient =
193 1.0), followed by presence of a defined nodule and CT findings (0.8 and 0.4, respectively; Figure
194 1C and Table S2) [24].

195 ***Random forest model***

196 Besides LDA, we also developed a non-linear classification model, exploiting RF classification
197 algorithms. The same 6 selected variables were combined in a RF model comprising 20
198 classification trees (a representative tree is reported in Figure 2A), and were able to correctly
199 discriminate 132 of 133 patients with LPA (sensitivity 99.2%), and 68 of 82 patients with BPA
200 (specificity 82.9%), resulting in an overall accuracy of 93% at the training and of 87% after 10K-
201 cross validation (overfitting effect 6%; Figure 2B). In this case, the stronger predictor was nodule
202 diameter, followed by the lowest potassium level and by the presence of a nodule at CT scanning
203 (Figure 2C).

204 **Prediction score**

205 Patients included in the described models (combined cohort; N = 215) were randomly assigned to a
206 training cohort (N = 150) or internal validation cohort (N = 65). No differences were found for all
207 evaluated parameters between the two groups (Table S3) [24]. The same 6 variables used in the
208 LDA and RF models were then used to develop the SPACE score, a 20-point score to discriminate
209 patients with LPA vs. BPA. The SPACE score was developed in the training cohort and then tested
210 in the internal validation cohort. Figures 3A and 3C report the categorization of the 6 different
211 variables and assignment of points. The analysis of the ROC curve demonstrated a high diagnostic
212 performance (Figure 3B). The area under the curve was 0.896 (95% CI 0.852-0.940) and the cut-
213 off with the higher accuracy was 12. At the training, a score greater than 12 correctly identified a
214 diagnosis of LPA in 87 of 93 patients (sensitivity 93.5%), whereas a score equal or lower than 12
215 identified a diagnosis of BPA in 47 of 57 patients (specificity 82.5%), with an overall accuracy of
216 89.3%. Of note, the prediction score displayed a very high performance with an accuracy of 81.5%
217 at validation, and a sensitivity and specificity of 87.5% and 72%, which was not significantly
218 different from the machine learning models (accuracy at validation of 79.1% and 87% for the LDA
219 and the RF model, respectively). Confusion matrix for training, internal validation, and combined
220 developmental cohort are reported in Figure 3D. The difference between the accuracy of the
221 prediction score in the training cohort compared with the internal validation cohort, revealed a
222 modest bias due to an expected overfitting effect (7.8%), which did not affect the reliability of the
223 model. A cut-off of greater than 8 or of greater than 16 optimized sensitivity or specificity,
224 respectively (Table S4) [24]. With a cut-off of 8, sensitivity was increased to 97.8% and 95%,
225 correctly classifying 91 of 93, and 38 of 40 patients with LPA, in the training cohort and in the
226 validation cohorts, respectively. With a cut-off of 16, specificity was increased to 98.2% and 92%,
227 correctly classifying 56 of 57, and 23 of 25 patients with BPA, in the training cohort and in the
228 validation cohort, respectively.

229 To evaluate further the diagnostic performance of the SPACE score, 7 previously published scores
230 [17-23] were tested on our combined cohort (Table S5) [24]. The accuracy of our prediction score
231 (89.3% and 81.5% at training and internal validation analysis, respectively) was superior to all
232 available scores (accuracy ranging from 58.2% to 86.3% at training and from 67.3% to 78% at
233 validation). Of note, the RF classification algorithm outperformed all other models with an accuracy
234 of 87% at validation, higher than all score evaluated at training.

235 ***External validation***

236 LDA, RF model and the SPACE score were validated on an external independent cohort from
237 Munich of 118 patients, 57 with LPA and 61 with BPA (Table S6) [24]. Compared with the
238 developmental cohort, the prevalence of LPA was significantly lower in the external validation
239 cohort (48.3% vs. 61.9%; $P = 0.017$) and mean BP (153/94 mmHg vs. 164/99 mmHg), DDD (2.5
240 [1.0; 4.0] vs. 3.3 [2.0; 5.0]), potassium levels (3.1 ± 0.5 mEq/L vs. 3.4 ± 0.7 mEq/L) were also
241 significantly lower. PRA at screening (0.29 vs 0.25 ng/mL/h) and after confirmatory testing (0.21
242 vs 0.15 ng/mL/h) was significantly higher and aldosterone levels at screening (17.9 vs 33.4 ng/dL)
243 and after confirmatory testing (11.2 and 16.4 ng/dL) were significantly lower ($P < 0.05$ for all
244 comparisons) in the validation compared with the developmental cohort. The reliability of the
245 diagnostic performance of our prediction models was confirmed at external validation. The accuracy
246 was 78.8%, 80.5%, and 78.8%, respectively for LDA, RF, and the score system (Figure S1) [24],
247 with a minimum overfitting bias compared with the internal validation on the developmental cohort
248 (range between 0.3% and 6.5%).

249 ***Management of PA patient***

250 The SPACE score was directly correlated with the proportion of patients with a diagnosis of LPA
251 (Table S7) [24] and with the lateralization index (LI) at the AVS (Table S8) [24]. Figure 4A clearly
252 illustrates the stratification of patients with a diagnosis of LPA vs. BPA for the prediction score and
253 graphically confirmed the cut-offs of 8, 12 and 16, which maximize sensitivity, accuracy, and

254 specificity, as defined by ROC curve analysis. In addition, all patients with a score greater than 18
255 had LPA, whereas all patients with a score lower or equal than 2 had BPA.

256 Finally, our score was integrated in a flow chart for PA management (Figure 4B). Patients with a
257 score less than or equal to 8 were classified as “probable BPA” and treated with MRA (N = 32),
258 thus resulting in 28 patients with true bilateral disease correctly managed, and 4 patients with a LPA
259 (1.9%) which missed the possibility to undergo adrenalectomy. Patients with a score greater than
260 16 were classified as “probable LPA”, with indication to unilateral adrenalectomy (N = 62).
261 Accordingly, 3 patients with bilateral disease would undergo inappropriate surgery (1.4%), and 1
262 patient with LPA would have resection of the wrong adrenal (0.5%). All remaining patients (N =
263 121), with a score comprised between 8.5 and 16 would undergo AVS with management according
264 to the result of the procedure. Sensitivity, specificity, positive, and negative predictive values are
265 reported in the confusion matrix (Figure 4C).

266 We combined patients from the developmental and external validation cohorts (N=333) and
267 stratified these patients into 3 groups according to the points of the SPACE score (score \leq 8 vs.
268 8.5-16 vs. $>$ 16): the median LI displayed a gradual increase in the 3 groups of patients (Table S8)
269 [24]. Moreover, clinical and biochemical outcomes in patients with LPA misclassified as BPA were
270 worse than patients with a correct prediction of LPA (83.3% vs. 48.8% partial + absent clinical
271 success, and 5.6% vs. 0.6% partial + absent biochemical success; Table S9) [24].

272 After stratification for the confirmatory test performed during the diagnostic work-up (Table S10)
273 [24], the SPACE score confirmed its applicability both for patients diagnosed by saline infusion
274 testing (accuracy 84%) or captopril challenge testing (accuracy 84.6%).

275 The application of the prediction score in our clinical context would result in the correct
276 management of 207 of 215 patients (96.3%) with a reduction of 43.7% (94 of 215) of AVS
277 procedures in the developmental combined cohorts. Notably, the accuracy of the flow chart for
278 patient management at external validation remained high (94.9%), with a reduction of 66.1% (78 of
279 118) of AVS procedures (Figure S1) [24].

280

281 **DISCUSSION**

282 In our study, we developed and validated two different prediction models based on supervised
283 machine learning algorithms and a clinical score for the subtype diagnosis of PA. An online tool
284 was developed to allow the application of the RF algorithm to clinical practice. Moreover, we
285 proposed a flow-chart for patient management which integrates our score-system in a second user-
286 friendly downloadable tool.

287 Küpers et al. proposed for the first time a clinical score to diagnose lateralized PA; the major
288 advantages were easy applicability and a very high positive predictive value, resulting in the correct
289 classification of all patients predicted as LPA [17]. However, this score displays very low
290 sensitivity, misclassifying 43% of LPA patients, who would miss the chance of potentially curative
291 adrenalectomy. In addition, validation on independent cohorts did not confirm its diagnostic
292 performance with a low accuracy, between 56.0% and 72.7% [26-29]. Six other score-systems were
293 proposed (Table S11) [24]. Two of them [18;23] used only biochemical or demographic features,
294 thus applicable before imaging. However, these scores were useful only to detect patients that could
295 avoid AVS due to BPA. The other scores [19;20] combined biochemical parameters with
296 radiological findings and displayed a high negative predictive value (82.2-100%), with the
297 identification of patients with BPA to be allocated to medical treatment. Limitations of these studies
298 were the low number of enrolled patients, the absence of an internal or external validation, and the
299 applicability only to patients undergoing captopril challenge [18;20] or intravenous saline loading
300 [19;23] for confirmatory testing. The score proposed by Kamemura et. al was developed in more
301 than 200 patients but was applicable only to patients without evidence of an adrenal mass at CT
302 scanning, which represent a minority of patients with PA [21]. Finally, Kobayashi et al. proposed
303 and validated a score on more than 1,000 patients, reporting a negative predictive value of 92.5%
304 [22] but with insufficient accuracy. The application of this score in our patients resulted in an
305 accuracy of 67.4-72.7%.

306 In our diagnostic models, the highest performance was reached by the RF algorithm, which
307 identified 132 of 133 patients with LPA and correctly classified 68 of 69 patients with BPA,
308 resulting in a sensitivity of 99.2% and a negative predictive value of 98.5%. The model accuracy
309 was 93.0% and 87.0% at training and internal validation, respectively. Our SPACE score displayed
310 an equally high performance with an overall accuracy of 89.3% in the training cohort and 81.5% in
311 the internal validation cohort (using 12 as cut-off), outperforming all previously proposed clinical
312 scores. A cut-off of 8 maximized sensitivity and negative predictive value (97% and 87.5%,
313 respectively, in the combined cohort), correctly identifying 28 of 32 patients with BPA, whereas a
314 cut-off of 16 maximized specificity and positive predictive value (96.3% and 95.2%, respectively,
315 in the combined cohort), correctly identifying 59 of 62 patients with LPA.

316 All previously proposed score-systems were applied in our cohort, to assess their generalizability.
317 The accuracy at validation was not suitable for clinical use, ranging between 67.3% and 78% and
318 suggesting a moderate overfitting bias (up to 19%). Conversely, the overfitting effect was low in
319 our models (from 2.3% to 7.8%) with a high accuracy at validation (from 81.5% to 93%).

320 To exclude selection bias and further assess the generalizability of our diagnostic models, we
321 performed an external validation on an independent cohort of patients. LDA, RF model, and the
322 SPACE score confirmed a high diagnostic performance (accuracy range 78.8-94.4%), with a
323 minimum overfitting bias.

324 We combined 3 biochemical variables with 3 imaging-related parameters associated with subtype
325 diagnosis. These parameters were selected considering the results of univariate and multivariate
326 regression analysis, and then used for the LDA, the RF model, and the score-system. Potassium
327 levels, aldosterone levels at screening and after confirmatory test are clinical criteria associated with
328 a high probability of LPA and reflect the severity of disease [30]. Imaging-related parameters
329 resulted to be crucial for subtype diagnosis; in our cohort only 5 of 133 LPA patients (3.8%)
330 displayed a bilaterally normal CT scanning, whereas 85.7% had a defined nodule.

331 The SPACE score was integrated in a flow chart for the management of patients with PA, resulting
332 in the correct classification of 96.3% of patients, potentially reducing almost half of the AVS. The
333 lower cut-off identifies patients with BPA to address to MRA treatment: 28/32 patients with BPA
334 were correctly classified, whereas 4 patients with LPA would be diagnosed as BPA and treated with
335 MRA, therefore, missing the chance of treatment by adrenalectomy. These 4 patients displayed
336 bilaterally normal adrenals at CT scanning and are thus at high risk of partial/absent clinical success
337 after surgery according with the recently proposed prognostic PASO score [7;25]. The higher cut-
338 off identifies patients with LPA, who could undergo unilateral adrenalectomy in centers where AVS
339 is not available. With this strategy, 58 patients with LPA would be correctly adrenalectomized,
340 whereas 4 patients would receive inappropriate surgery (3 patients with BPA and 1 patient with
341 lateralization on the other side). The 3 BPA patients would also be misclassified by all other
342 previously published scores.

343 The external validation resulted in similar performance, with correct management of 94.9% of
344 patients and a potential reduction of 66.1% in the number of AVS, thus excluding a significant inter-
345 center variability. The assessment of clinical and biochemical outcomes of patients with a correct
346 prediction of LPA compared with those misclassified by the SPACE score, reinforced our findings.
347 Finally, unlike previous models, our score system was applicable both to patients with PA diagnosed
348 by saline infusion testing and by captopril challenge testing, with a similar accuracy (84.0% vs.
349 84.6%, respectively).

350 The present score is expected to be of interest to hypertension and endocrine centers and in particular
351 for those that perform systematic screening of patients with hypertension, therefore having a high
352 rate of diagnosis of BPA [31]. With our score a high proportion of BPA patients can avoid
353 unnecessary AVS with a significant reduction of costs and potential complications.

354 The failure to define with certainty the side of aldosterone hypersecretion represents the main limit
355 of our score and of all others previously proposed. A second limit is the retrospective inclusion of
356 the patients with PA: a prospective validation in a large number of patients is warranted to confirm

357 and further validate our prediction models. Moreover, our score cannot be applied to patients with
358 PA diagnosed by the furosemide upright posture test or the oral saline loading test. Finally, it should
359 be noted that dichotomization into LPA and BPA reflects the need to address patients to surgical vs.
360 medical treatment and does not represent the complexity of the disease. Many patients with
361 unilateral disease are cases with bilateral but asymmetrical aldosterone production displaying a high
362 LI at AVS. These cases should benefit from adrenalectomy and are therefore considered as patients
363 with unilateral or lateralized PA [32]. The strengths of our study include the reliable accuracy of
364 our diagnostic models after internal and external validation, using both machine learning algorithms,
365 or a simple scoring system, with a potential impact on clinical practice for centers where AVS is
366 not available. In addition, we proposed two user-friendly downloadable tools which integrate the
367 RF model and the flow-chart based on the SPACE score, allowing their application for the
368 management of PA patients.

369

370 **CONCLUSIONS**

371 We here developed and validated two prediction model and an easy applicable scoring system for
372 the subtype diagnosis of PA. Our findings support the integration of clinical, biochemical, and
373 imaging parameters by advanced computational approaches, to define PA subtype diagnosis,
374 potentially reducing the number of AVS for patients with confirmed PA and guiding surgical
375 decision in centers where AVS is not available.

376

377 **Source(s) of Funding:** This research did not receive any specific grant. CA and MR are supported
378 by the Else Kröner-Fresenius Stiftung (2019_A104) and by the Deutsche Forschungsgemeinschaft
379 within the CRC/Transregio 205/1 “The Adrenal: Central Relay in Health and Disease”.

380 **Conflict(s) of Interest/Disclosure(s):** the authors have nothing to disclose.

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500 **FIGURE LEGENDS**

501

502 *Legend to Figure 1 – Diagnostic Modelling: Linear Discriminant Analysis.* The LDA model
503 included the 6 variables with the highest classification power for subtype diagnosis in the combined
504 cohort (N = 215). **Panel A**, canonical plot representing diagnostic performance of LDA; each patient
505 is indicated by a point and subtype diagnosis are reported by color (LPA, lateralized PA, black;
506 BPA, bilateral PA, grey). The axes (canonical component 1 and 2) are calculated by weighted linear
507 combination of the 6 variables included in the model to maximize the separation between groups.
508 The crosses indicate the means of (canonical 1; canonical 2) for patients with LPA or BPA, the
509 ellipse included patients with a linear combination coefficient that falls within the mean \pm SD. **Panel**
510 **B**, confusion matrix reporting real and predicted diagnosis, accuracy, sensitivity, specificity, and
511 10K-cross validation. **Panel C**, histogram representing normalized LDA coefficients for each
512 variable included in the model. CT, Computed Tomography.

513

514 *Legend to Figure 2 – Diagnostic Modelling: Random Forest.* The RF algorithm included the 6
515 variables with the highest classification power for subtype diagnosis in the combined cohort (N =
516 215). **Panel A**, the first classification tree of the forest is shown for the prediction of LPA (lateralized
517 PA) vs. BPA (bilateral PA). **Panel B**, confusion matrix reporting real and predicted diagnosis,
518 accuracy, sensitivity, specificity, and 10K-cross validation. **Panel C**, histogram representing
519 normalized predictive coefficients for each variable included in the model. CT, Computed
520 Tomography.

521

522 *Legend to Figure 3 – Score development and validation.* Univariate/multivariate regression
523 analyses and coefficients from the LDA and RF models were used to assign points to each variable
524 according to stratification level. The score was developed in the training cohort (N = 150) and tested
525 on the validation cohort (N = 65). **Panel A**, table showing included variables and final point system

526 used for the score. **Panel B**, receiver operating characteristics (ROC) curve to assess AUC (area
527 under the curve) and the best cut-off for the score in the combined cohort (N = 215). **Panel C**,
528 representation of cut-offs and assigned points for each variable after categorization: subtype
529 diagnosis is represented by colors (LPA, lateralized PA, black; BPA, bilateral PA, grey); the bars
530 indicate median and interquartile range for each group. **Panel D**, confusion matrix representing real
531 and predicted subtype diagnosis, accuracy sensitivity, specificity for the training cohort (N = 150),
532 the validation cohort (N = 65), and the combined cohort (N = 215). CT, Computed Tomography;
533 CI, Confidence Interval.

534

535 *Legend to Figure 4 – Score performance and management of PA patients.* Flow chart for PA
536 patient management using our prediction score. **Panel A**, histogram showing the proportion of
537 patients (y-axis, %) for each subtype diagnosis (LPA, lateralized PA, black; BPA, bilateral PA,
538 grey), stratified by score points (x-axis) on the combined cohort (N = 215). The total number of
539 patients (N) for each AVS score level and their proportion (%) are reported in Table S7 [24]. **Panel**
540 **B**, PA patient management using our score; the number of patients is indicated in bold; cut-offs and
541 misclassified patients are indicated in grey. **Panel C**, confusion matrix representing real and
542 predicted subtype diagnosis, sensitivity, specificity, positive predictive value (PPV), and negative
543 predictive value (NPV). AVS, Adrenal Venous Sampling; MRA, Mineral Receptor Antagonist.

544 **Table 1. Patient Characteristics of Study Cohort**

Variable	LPA (N = 133)	BPA (N = 82)	P-value
Female sex, n (%)	56 (42.1)	19 (23.2)	0.005
Age at diagnosis (years)	49 ± 10.5	50 ± 7.7	0.248
Duration of HTN (months)	74 [27; 168]	63 [22; 123]	0.284
Systolic BP (mmHg)	165 ± 25.0	163 ± 20.5	0.613
Diastolic BP (mmHg)	99 ± 14.5	99 ± 11.7	0.873
Antihypertensive medication (DDD)	3.8 [2.2; 5.7]	3.0 [1.3; 4.7]	0.027
eGFR (mL/min)	96 [81; 109]	94 [80; 102]	0.146
Lowest Potassium (mEq/L)	3.1 ± 0.6	3.8 ± 0.4	< 0.001
PRA at screening (ng/mL/h)	0.30 [0.20; 0.40]	0.20 [0.10; 0.40]	0.554
Aldosterone at screening (ng/dL)	38.0 [25.7; 49.7]	28.7 [19.8; 37.9]	< 0.001
Confirmatory testing			
Saline infusion test, n (%)	102 (76.7)	63 (76.8)	0.982
Captopril Challenge test, n (%)	31 (23.3)	19 (23.2)	
PRA post-confirmatory test (ng/mL/h)	0.15 [0.10; 0.20]	0.15 [0.10; 0.21]	0.850
Aldosterone post-confirmatory test (ng/dL)	20.5 [13.3; 32.9]	11.5 [8.2; 17.7]	< 0.001
Microalbuminuria, n (%)	42 (31.5)	24 (29.4)	0.800
LVH at Echo, n (%)	81 (60.7)	48 (59.1)	0.831
CV events, n (%)	17 (12.6)	15 (18.1)	0.320
Presence of nodule at CT scanning, n (%)	114 (85.7)	34 (41.5)	< 0.001
Largest nodule at CT scanning (diameter, mm)	14 [10; 20]	12 [10; 19]	0.315
CT scanning findings			
Bilaterally Normal	5 (3.8)	20 (24.4)	< 0.001
Bilaterally Abnormal	19 (14.2)	18 (22.0)	
Unilateral Abnormality	109 (82.0)	44 (53.7)	
AVS protocol			
Basal, n (%)	43 (32.3)	37 (45.1)	0.051
ACTH continuous infusion, n (%)	51 (38.4)	32 (39.0)	
Both (Basal + ACTH), n (%)	39 (29.3)	13 (15.9)	
Lateralization Index at AVS	12.0 [6.9; 21.3]	1.8 [1.3; 2.6]	< 0.001
Clinical outcome: Complete, n (%)	72 (54.1)	N.A.	N.A.
[only for LPA] Partial, n (%)	55 (41.4)		
Absent, n (%)	6 (4.5)		
Biochemical outcome: Complete, n (%)	131 (98.5)	N.A.	N.A.
[only for LPA] Partial, n (%)	2 (1.5)		
Absent, n (%)	0 (0.0)		

545

546 Clinical characteristics of patients included in the analysis stratified for diagnosis: patients with
547 lateralized PA (LPA; N = 133) vs. bilateral PA (BPA; N = 82). The DDD is the assumed average
548 maintenance dose per day for a drug used for its main indication in adults. AVS, Adrenal Venous
549 Sampling; HTN, Hypertension; BP, Blood Pressure; DDD, Defined Daily Dose; eGFR, estimated

550 Glomerular Filtration Rate; PRA, Plasma Renin Activity; LVH, Left Ventricular Hypertrophy;
 551 Echo, Echocardiography; CV, Cardiovascular; CT, Computed Tomography. Normally and non-
 552 normally distributed variables were reported as mean \pm standard deviation or median [interquartile
 553 range], respectively. Categorical variables were reported as absolute number (n) and proportion (%).
 554

555 **Table 2. Selected discriminant variables for a diagnosis of lateralized PA**

Variable (ref. LPA)	Univariate analysis		Multivariate Analysis	
	OR (CI 95%)	P-value	OR (CI 95%)	P-value
Aldosterone at screening (ng/dL)	1.04 (1.02 – 1.07)	< 0.001	1.05 (1.01 – 1.10)	0.017
Lowest Potassium (mEq/L)	0.10 (0.05 – 0.21)	< 0.001	0.09 (0.03 – 0.30)	< 0.001
Aldosterone post-confirmatory test (ng/dL)	1.09 (1.05 – 1.12)	< 0.001	1.09 (1.02 – 1.16)	0.012
Nodule at CT scanning (ref. presence)	8.33 (4.35 – 16.67)	< 0.001	12.50 (2.94 – 47.62)	0.001
Largest nodule at CT scanning (diameter, mm)	1.12 (1.07 – 1.16)	< 0.001	1.11 (1.06 – 1.16)	0.013
CT scanning findings (ref. unilateral abnormality)	9.91 (3.50 – 28.05)	< 0.001	4.44 (1.30 – 13.21)	0.016

556
 557 Logistic regression analysis was performed to assess the odds ratio (OR) and the 95% confidence
 558 interval (CI) for each variable. Univariate and multivariate analysis are shown as indicated. An OR
 559 greater than 1 indicates an increased likelihood of lateralized PA (LPA), and an OR less than 1 a
 560 decreased likelihood. Aldosterone at screening, lowest potassium, aldosterone post-confirmatory
 561 test, and largest nodule at CT were treated as continuous variables. An OR increase of 0.01
 562 represents a 1% increased likelihood of a diagnosis of LPA for each unit of the reference variable.
 563 Presence/absence of nodule at CT scanning, and CT scanning findings were treated as categorical
 564 variables. CT, Computed Tomography.