RESEARCH

Validation of three novel clinical prediction tools for primary aldosteronism subtyping

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Abstract

The 20-point clinical prediction SPACE score, the aldosterone-to-lowest potassium ratio (APR), aldosterone concentration (AC) and the AC relative reduction rate after saline infusion test (SIT) have recently been proposed for primary aldosteronism (PA) subtyping prior to adrenal vein sampling (AVS). To validate those claims, we performed a retrospective cross-sectional study that included all patients at our center who had positive SIT to confirm PA and were diagnosed with either bilateral disease (BPA) according to AVS or with lateralized disease (LPA) if biochemically cured after adrenalectomy from November 2004 to the end of 2019. Final diagnoses were used to evaluate the diagnostic performance of proposed clinical prediction tools. Our cohort included 144 patients (40 females), aged 32–72 years (mean 54 years); 59 with LPA and 85 with BPA. The originally suggested SPACE score ≤8 and SPACE score >16 rules yielded about 80% positive predictive value (PPV) for BPA and LPA, respectively. Multivariate analyses with the predictors constituting the SPACE score highlighted post-SIT AC as the most important predictor of PA subtype for our cohort. APR-based tool of <5 for BPA and >15 for LPA yielded about 75% PPV for LPA and BPA. The proposed post-SIT AC <8.79 ng/dL criterion yielded 41% sensitivity and 90% specificity, while the relative post-SIT AC reduction rate of >33.8% criterion yielded 80% sensitivity and 51% specificity for BPA prediction. The application of any of the validated clinical prediction tools to our cohort did not predict the PA subtype with the high diagnostic performance originally reported.

Key Words

- adrenal vein sampling
- endocrine hypertension
- primary hyperaldosteronism
- subtype prediction

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Introduction

Primary aldosteronism (PA) is the most common curable secondary cause of high blood pressure, which generates more damaging cardiovascular, renal and metabolic outcomes than equally severe essential hypertension (1, 2). When lateralized PA (LPA) is reliably proven, the best treatment option is unilateral laparoscopic adrenalectomy, which represents the only way to completely remove the harmful aldosterone excess (3). Therefore, subtyping to differentiate LPA from bilateral PA (BPA) is of major clinical importance for all patients with PA who are eligible for surgery (4).

Adrenal imaging alone can often lead to PA subtype misclassification and poorer adrenalectomy cure rates (3, 5), so adrenal vein sampling (AVS) is recommended to precede adrenalectomy in most surgical candidates (4, 6, 7). Unfortunately, AVS is an invasive, costly and technically difficult procedure, which is not widely available (8). As the real prevalence of PA might be around 20% among hypertensive population (9), much needed alternative approaches for subtyping are constantly being sought (10, 11, 12); however, they are currently restricted to few centers or they are insufficiently consistent to replace AVS.

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Alternatively, clinical prediction tools based on patient clinical and biochemical characteristics, which are obtained during the routine diagnostic work-up, might be employed to better select patients for AVS (13). These aids mainly rely on the well-known observation that LPA is more likely associated with a more severe phenotype and a unilateral adrenal nodule on CT than BPA (14). There have been several attempts to predict LPA (15, 16). Lately, criterions to identify patients with BPA were developed to choose patients that could avoid AVS and immediately commence with medical treatment (17, 18). Regrettably, subsequent external validation of the suggested approaches in independent cohorts of PA patients disputed their originally reported robust predictive utility (19, 20), but also stimulated ongoing search for the ideal clinical prediction tool.

Burrello *et al.* have recently proposed several prediction models including a comprehensive SPACE score, a 20-point score on six clinical and biochemical variables to discriminate patients with LPA and BPA, which performed better than several previous scores when internally and externally validated (20). Contrary to this rather complex approach, Puar *et al.* addressed the same problem using only the plasma aldosterone concentration (AC) to lowest plasma potassium ratio (21). Additionally, Nagano *et al.* have found the criteria of the plasma aldosterone relative reduction rate and of plasma AC after saline infusion test (SIT) useful to determine which subset of patients should proceed to AVS (22).

Primarily, we aimed to validate the diagnostic performance of the SPACE score and the accompanying predictive models for PA subtyping in our sizeable cohort of PA patients. Secondary objectives were to test the other two recent, more straightforward approaches: the aldosterone-to-potassium ratio and aldosterone responses post-SIT.

Materials and methods

Study design

A retrospective cross-sectional study was conducted from November 2004 to the end of 2019 at the national tertiary endocrine referral center. The study was conducted in accordance with the Declaration of Helsinki and approved by the National Medical Ethics Committee, ID 0120-216/2020/3. Informed consent was waived for the present study, but it was obtained in written form from all the included patients at the time of the AVS when they were registered into the AVS database.

Patients

Patients with confirmed PA who underwent successful AVS at our center during the study period were candidates for enrollment. PA was diagnosed according to the existing Endocrine Society guidelines (4, 23) and the expert opinions (24, 25). Briefly, AC and plasma renin activity (PRA) were measured in the morning in ambulatory patients after they had been seated for at least 15 min. Patients were instructed to have unrestricted dietary salt intake before blood withdrawal and they were potassium-replete, as requested by the guidelines (4, 23). AC to PRA ratio (ARR) values above 36 ng/dL/ng/mL/h together with AC >10.8 ng/dL were considered a positive screening test. The minimum PRA value for statistical analysis was set at 0.2 ng/mL/h to avoid overinflating ARR (26). The diagnosis of PA was confirmed by recumbent SIT until the end of 2018 with post-SIT AC >5 ng/dL as the diagnostic threshold (4). SIT was performed in a seated position afterwards, and post-SIT AC >6 ng/dL was deemed positive (27). Confirmatory testing was omitted if the diagnosis of PA was straightforward due to spontaneous hypokalemia, undetectable PRA values and consistently increased AC (>20 ng/dL) (4).

Adrenal imaging and AVS

All patients with confirmed PA underwent a thin-slice CT scan (Siemens). For the purpose of this study, CT scan results were interpreted according to the published criteria by Burrello *et al.*, as follows: (i) nodule: defined as an adrenal mass ≥8 mm in diameter; (ii) unilaterally abnormal: in the presence of a thickening >4 mm and/or the presence of a nodule (as previously defined) on one side; (iii) bilaterally abnormal: in the presence of any combination of nodule or thickening >4 mm on both sides (i.e. nodule on one side + contralateral thickening; bilateral nodules; bilateral thickening); (iv) bilaterally normal: absence of any lesion (a thickening up to 4 mm was considered as normal) (20).

As already reported, AVS was performed sequentially during continuous adrenocorticotrophic hormone (ACTH) stimulation in all patients and it was regarded successful if the selectivity index (SI), determined as the ratio of concentrations of cortisol from an adrenal vein and the infra-adrenal inferior vena cava (IVC), was at least 5 on both sides (28, 29). Lateralization index (LI) was calculated as the gradient of the higher over the lower cortisol-corrected aldosterone ratio (6, 28).

LPA was diagnosed in patients who had successful AVS with LI of more than 4 (6, 28, 29), if they were

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biochemically cured according to the Primary Aldosteronism Surgical Outcome (PASO) criteria 6–12 months after unilateral adrenalectomy (30). Further, five patients with equivocal LI values between 3 and 4 (6, 28) who were operated on and biochemically cured were also classified as LPA.

BPA was diagnosed in patients with bilaterally successful adrenal vein cannulation and LI below 3 (6, 28). To reinforce the diagnosis of BPA, the AV/IVC index was also calculated as the gradient of the cortisol-corrected aldosterone ratio between the non-dominant adrenal vein and inferior vena cava to double-check for potentially missed lateralization. As suggested, the non-dominant AV/IVC index values of ≤0.5 were considered indicative of probable contralateral LPA (31). Figure 1 shows a flowchart of the patient selection for our study.

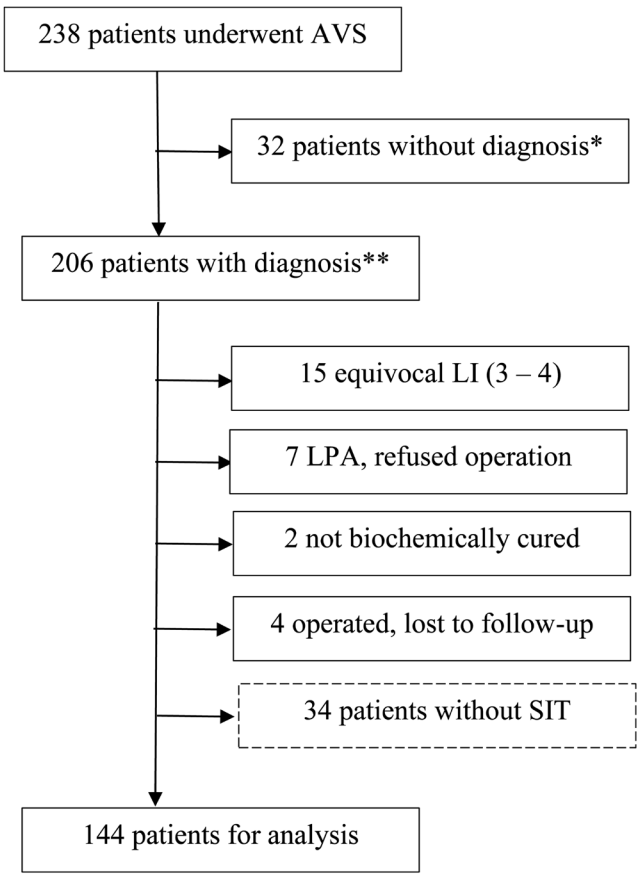

Figure 1
Flowchart demonstrating the patient selection for our validation analysis. *Without diagnosis – inconclusive outcome after unsuccessful AVS; **With diagnosis – conclusive outcome after successful AVS or after unilateral adrenalectomy with biochemical cure. AVS, adrenal vein sampling; LI, lateralization index; LPA, lateralized primary aldosteronism; SIT, saline infusion test.

Assays

Serum aldosterone was measured with the Active® Aldosterone RIA (Beckman Coulter, Immunotech, Czech Republic). Serum cortisol was measured with an automated chemiluminescent immunoassay on the Immulite® 2000 XPi (Siemens Healthcare). PRA was measured by the Angiotensin I RIA KIT (Beckman Coulter, Immunotech). The respective within- and between-assay coefficients of variation were below 4.5 and 9.8% for aldosterone, below 6.8 and 9.4% for cortisol and below 11.3 and 20.9% for PRA.

Validation of the clinical prediction tools

SPACE score and the associated prediction models

Original six clinical and biochemical parameters found to be associated with a diagnosis of LPA (20) were used for the validation analysis: AC at screening and after SIT, presence/absence of nodules, largest nodule diameter, and CT descriptive findings. The data were obtained from the AVS database and from electronic hospital records. The lowest ever documented serum potassium value when off supplements and diuretics was used for the analysis. Special care was taken to avoid any other confounding factors, such as intercurrent illnesses (e.g. gastroenteritis) (14, 21). All patients who were apparently misclassified according to the SPACE score (i.e. missed LPA due to SPACE score ≤8 or BPA with SPACE score >16 wrongly assigned to the LPA category) were carefully authenticated.

Aldosterone-to-potassium ratio and aldosterone responses after SIT

Aldosterone-to-potassium ratio (APR) was calculated by applying the baseline AC used for the ARR at screening as the numerator and the lowest ever recorded serum potassium with the above-mentioned precautions as the denominator, as suggested by Puar *et al.* (21). Performance of the APR was tested against final diagnoses in our cohort by using the originally reported thresholds of less than 5 for BPA and of more than 15 for LPA (21). The criteria of AC <8.79 ng/dL and the AC relative reduction rate >33.8% pos-SIT that were proposed by Nagano *et al.* (22) were tested in the same way.

Statistical analysis

Numerical variables were described as mean, median and inter-quartile ranges; categorical variables were presented as frequencies and proportions. The differences in the characteristics between patients with LPA and BPA were

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tested using *t*-test and exact Mann–Whitney test for numerical variables, and Fisher's exact test for categorical variables. The ability of the six selected parameters to distinguish patients with lateralized from those with bilateral disease was studied with univariate logistic regression, multiple logistic regression (without and with Firth's bias correction (32, 33)), linear discriminant analysis (LDA) and three tree-based classification methods: CHAID (34), CART (34) and C5.0 (35). In multiple logistic regression and LDA, all the predictors were entered at once into the model. The tree-based methods were applied using default settings and equal miss-classification cost for the two possible outcomes. We therefore attempted to replicate and augment the approach of Burrello *et al.* (20). The accuracy of other subtyping criteria was assessed in terms of sensitivity, specificity, positive predictive value (PPV) and diagnostic accuracy (where applicable). Statistical significance level was set at $P \leq 0.05$. Statistical analyses were conducted using IBM SPSS Statistics 25 (IBM Corp.).

Results

Patient characteristics

A total of 238 patients had AVS during the study period (Fig. 1). Thirty-four subjects with straightforward PA did not undergo SIT (4) and they had to be excluded. Finally, the inclusion criteria were met in 144 patients, 40 females and 104 males, aged 54 years on average (range 32–72 years); 59 with LPA (41%; 29 right; 30 left) and 85 with BPA (59%). More than half of them had a unilateral abnormality (76 patients (53%); 37% left; 16% right) on CT scan, which was in nine cases smaller than 8 mm. Bilateral adrenal abnormalities were present in 15 cases (10%). The average abnormality size was 15.9 mm (SD 8.8 mm). Both adrenals were interpreted as normal in 37% of the cases. Patients were referred due to hypertension with hypokalemia (57%), due to resistant hypertension (37%) or as hypertensive patients with an adrenal incidentaloma (6%). Unsurprisingly, patients with LPA had more severe disease with significantly lower potassium values and smaller relative reduction of AC post-SIT. On the other hand, their AC and ARR values were higher, not only in basal conditions but also post-SIT. A nodule at CT scanning was detected in 80% of patients with LPA and in only 40% of patients with BPA. Lateralized patients were more likely to harbor a unilateral abnormality on CT than bilateral patients (76% vs 37%); at the same time, they were less likely to have bilaterally normal adrenals than their bilateral counterparts (15% vs 52%). As expected, LI at AVS was significantly higher in LPA than in BPA patients. More detailed clinical characteristics and laboratory parameters of the study group are presented by disease subtype in Supplementary Table 1 (see section on supplementary materials given at the end of this article).

The total number of patients on less interfering antihypertensive medications (ACE inhibitors or angiotensin receptor blockers) potentially affecting the renin–angiotensin–aldosterone system was 101 (70%; 44 (74% of) LPA and 57 (67% of) BPA patients). Thirty-seven of these patients (14 LPA and 23 BPA patients) were also receiving a beta blocker.

Validation of the clinical prediction tools

SPACE score and the associated prediction models

We first tried the simple criterion of predicting LPA if the SPACE score exceeds 12 (20). In this way, 41 of the 71 patients predicted to have LPA and 55 of the 73 patients predicted to have BPA were classified correctly, respectively, which yielded 70% specificity and 65% sensitivity with a moderate overall diagnostic accuracy of 67%.

When the two-threshold criterion of the SPACE score was applied (BPA if ≤8, LPA if >16, 'gray zone' otherwise) (20), 46 patients out of 56 were predicted to have BPA and 25 out of 32 were predicted to have LPA were classified correctly, thus yielding 78% (95% CI 61–89%) and 82% (95% CI 70–90%) PPV for BPA and LPA, respectively. The remaining 56 (39%) patients were classified as 'gray zone'; 32 (57%) of them had BPA and 24 (43%) had LPA. The proportion of actual PA subtypes by two-point intervals of the SPACE score is presented in Fig. 2.

Six out of ten missed LPA patients were ultimately diagnosed with unilateral adrenal hyperplasia, while the remaining four harbored adrenal microadenomas. All BPA patients with SPACE score values in the LPA range (>16) had a unilateral adrenal nodule, either on the ipsilateral (five cases) or on the contralateral side (two cases) regarding the LI values. Their non-dominant AV/IVC indices were not indicative of unilateral disease in six out of seven cases. The remaining patient with a large ipsilateral nodule had lower aldosterone/cortisol ratios in both adrenal veins than in the inferior vena cava, which was suggestive of apparent bilateral aldosterone suppression (36), thus making the final diagnosis of BPA questionable. The same phenomenon was found in additional three patients in the BPA group (2.8% overall); one was classified as such and the other two as 'gray zone'. Complete CT, AVS and/or

histopathology features of the apparently misclassified patients are presented in Supplementary Table 2.

The results of logistic regression models are summarized in Table 1. In the univariate models, all the six studied predictors were statistically significant. In the standard and Firth's bias-corrected multiple logistic regression model, only AC post-SIT proved to be a statistically significant predictor of LPA.

In order to further validate the results of Burrello *et al.* (20), we conducted LDA with the same set of predictors. The discriminant function (there could be only one because the dependent variable had two categories) was statistically significant (Wilks' lambda: $P < 0.001$); it explained 28% of variance of the dependent variable. The model correctly classified 75% of the patients in the sample (a priori classification accuracy without a model was 59%, which was the proportion of the more frequent category, i.e. BPA patients) and 73% of the patients using leave-one-out cross-validation. The standardized canonical discriminant function coefficients as well as the structure matrix indicated AC post-SIT to be the relatively most important predictor (details omitted).

The final step in our attempt to validate the findings of Burrello *et al.* (20) was to conduct a classification-tree analysis. Unlike Burrello *et al.* (20), who used an assembly ('black-box') classifier (random forest), we used single-tree models to make the results easier to interpret. The trees resulting from the CHAID, CART and C5.0 algorithm are displayed in Figs 3, 4 and 5, respectively. All three methods used the largest nodule diameter and AC post-SIT for the splits and identified them among the three most important predictors (Table 2). Low values of largest nodule diameter (8 mm and below) were predictive of BPA, while higher values were predictive of LPA but required further confirmation from AC post-SIT (Figs 3, 4 and 5). Low values of AC post-SIT (below 10 or 6.5 ng/dL) were predictive of BPA, while high values (above 20 or 23 ng/dL) were predictive of LPA. The third key predictor was AC at screening according to CART, and CT scanning findings according to C5.0 (Figs 4 and 5). In general, classification accuracy was comparable to the LDA model; it was better for the training data, but the cross-validation estimate was worse (Table 2).

Aldosterone-to-potassium ratio and aldosterone responses after SIT

According to the two-threshold criterion of Puar *et al.* (BPA if APR <5, LPA if APR >15, 'gray zone' otherwise) (21), 27 patients out of 37 were predicted to have BPA and 17 out of 22 were predicted to have LPA were classified correctly, thus yielding 77% (95% CI 57–90%) and 73% (95% CI 57–90%) PPV for BPA and LPA, respectively. The remaining 85 (59%) patients were classified as 'gray zone'; 53 (62%) of them had BPA and 32 (43%) had LPA. Therefore, the APR was able to reliably exclude LPA in 18% and confirm it in 12% of our cohort, respectively. Three out of four patients with apparent bilateral aldosterone suppression were classified as 'gray zone' and one as LPA.

The AC <8.79 ng/dL post-SIT criterion (22) classified correctly 53 of the 103 patients (51%) predicted to have LPA and 35 of the 41 patients predicted to have BPA (85% PPV; 95% CI 72–93%), respectively, which yielded 90% specificity and 41% sensitivity for predicting BPA. The AC relative reduction rate post-SIT >33.8% criterion (22) classified correctly 30 of the 47 patients (63%) predicted to have LPA and 68 of the 97 (70% PPV; 95% CI 60–78%) patients predicted to have BPA, respectively, which yielded 51% specificity and 80% sensitivity for predicting BPA.

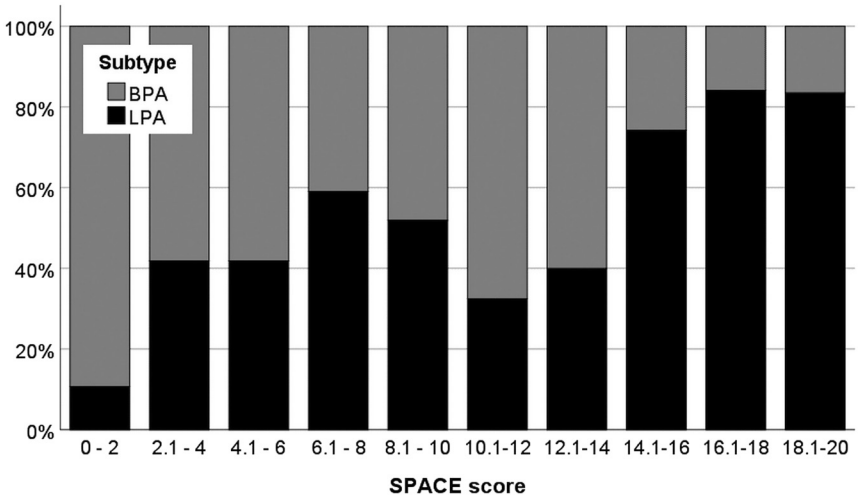

Figure 2
Stacked-bar chart showing the proportion of primary aldosteronism subtypes depending on the SPACE score (20). BPA, bilateral primary aldosteronism; LPA, lateralized primary aldosteronism.

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Table 1 Summary of logistic regression analyses for predicting LPA subtype.

Predictor	Univariate analysis				Multivariate analysis			
					Standard[a]		Firth bias correction[b]	
	H-L	R^2_N	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Aldosterone at screening (ng/dL)	0.513	0.094	1.03 (1.01–1.05)	0.003	1.00 (0.97–1.03)	0.830	1.00 (0.97–1.03)	0.849
Lowest potassium (mmol/L)	0.798	0.215	0.14 (0.06–0.34)	<0.001	0.44 (0.13–1.47)	0.183	0.47 (0.14–1.47)	0.196
Aldosterone post-SIT (ng/dL)	0.619	0.295	1.10 (1.06–1.15)	<0.001	1.07 (1.02–1.12)	0.007	1.06 (1.02–1.12)	0.005
Nodule at CT scanning (presence vs none)	NA	0.202	5.88 (2.73–12.67)	<0.001	2.50 (0.61–10.29)	0.206	2.37 (0.61–9.45)	0.213
Largest nodule (diameter, mm)	0.003	0.134	1.07 (1.04–1.11)	<0.001	1.00 (0.94–1.06)	0.940	1.00 (0.94–1.06)	0.963
CT scanning findings (unilateral vs bilateral abnormality or none)	NA	0.152	4.30 (2.10–8.80)	<0.001	1.65 (0.58–4.70)	0.347	1.62 (0.59–4.43)	0.348

H-L – *P*-value from Hosmer–Lemeshow test (<0.05 indicates inadequate model fit); R^2_N – Nagelkerke pseudo-R^2 (ranges from 0 to 1, higher values indicate better explanatory potential of the model); OR (95% CI) – estimated odds ratio per unit change of the predictor (with 95% CI); [a]$P < 0.001$ from likelihood-ratio test of the model, $P = 0.642$ from Hosmer–Lemeshow test, R^2_N=0.388; [b]$P < 0.001$ from likelihood-ratio test of the model. NA, not applicable; SIT, saline infusion test.

Discussion

Validation of the SPACE score in our cohort of patients with PA resulted in only moderate overall diagnostic accuracy of 67% when using 12 as a cut-off, which was lower than the originally reported accuracy of 89.3% at training, 81.5% at internal validation and 78.8% at external validation, respectively (20). Application of the two-threshold criterion of the SPACE score (BPA if ≤8, LPA if >16, 'gray zone' otherwise) resulted in better PPV of around 80% for both subtypes in our cohort. Still, the diagnostic performance was clearly inferior to sensitivities and specificities that were around 95% in the original training and the internal validation cohorts (20).

We did not calculate the diagnostic accuracy for the two-threshold criterion of the SPACE score, because it might be potentially misleading for clinical prediction tools with a substantial 'gray zone', which encompassed approximately 40% of our patients. Ten out of our 59 patients with LPA (17%) (Supplementary Table 2) would have been denied a potentially curative operation if the SPACE score alone had been used for subtyping. Three of these patients had a histopathologically proven adrenal microadenoma that was not detected by CT. If the opposite were true, these three patients would not have been entirely missed, but classified as 'gray zone'. Additional five misclassified patients who were surgically cured and were finally diagnosed with unilateral adrenal hyperplasia had also bilaterally normal CT results. Therefore, imaging-related parameters were crucial for subtype diagnosis according to the SPACE score, which is not surprising as only 3.8% of the original LPA patients displayed a bilaterally normal CT scanning, whereas 85.7% had a defined nodule. Moreover, CT descriptive findings with any adrenal thickening of >4 mm considered abnormal were also among the six main predictors in the model (20). All this points to meticulous radiologic assessment in the original cohort, which has been already reported (37). Imaging is often performed routinely outside the center where AVS is ultimately done, so it might be therefore difficult to impose rigorous standards that are obvious in research institutions (38). Accordingly, subtle radiologic findings, such as adrenal microadenomas with less than 10 mm in diameter that are sometimes defined as the CT-undetectable feature could be missed (4). Of note, 15% of our LPA patients had bilaterally normal CT scanning, which is comparable to a reported prevalence of 13–30% of CT-undetectable microadenomas in lateralized patients with PA (28, 38, 39, 40, 41). Previous prediction models that included CT results considered a cutoff of 10 mm (42, 43, 44) or at least 8 mm (15, 17, 18) for adrenal abnormality to preserve clinical applicability. Interestingly, all our single-tree models pointed to the largest adrenal nodule diameter of at least 8 mm as one of the strongest predictors of LPA in our cohort, while CT descriptive findings were less important.

Seven out of our 85 patients with BPA (8%) (Supplementary Table 2) were classified as LPA and would have been put at risk of inappropriate adrenalectomy if the SPACE score alone would direct the clinical decision making. However, uniformly accepted criteria to conclusively diagnose BPA are lacking and some of our patients diagnosed with BPA might in fact have had LPA.

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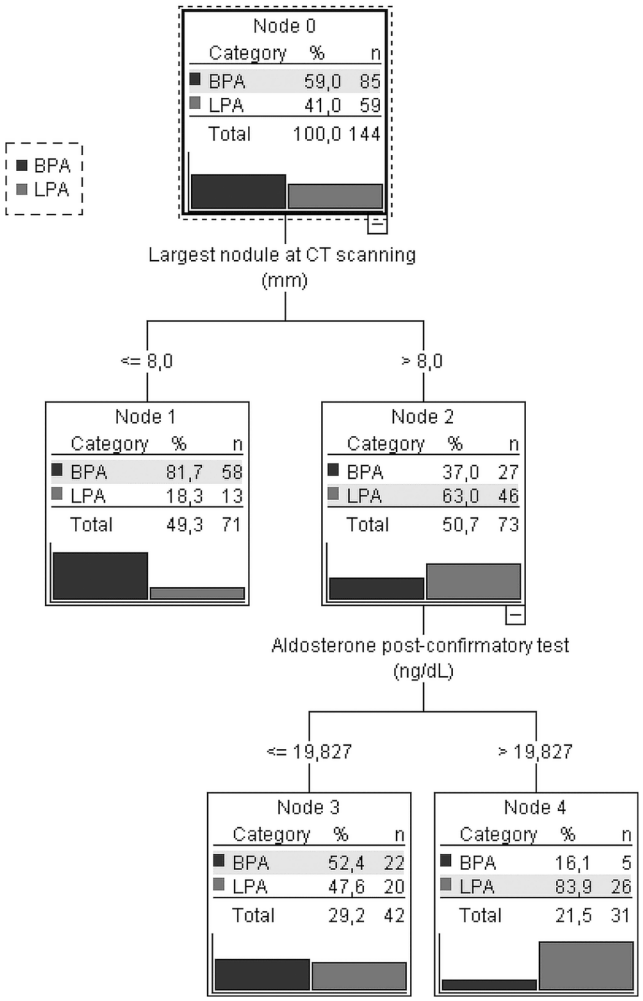

Figure 3
Classification tree for predicting primary aldosteronism subtype obtained using the CHAID algorithm. In each node, the predicted category is shaded in gray. BPA, bilateral primary aldosteronism; LPA, lateralized primary aldosteronism.

To partially overcome this problem, we also examined the contralateral AV/IVC index of ≤0.5 as indicative of missed lateralization in the BPA subgroup (31). Notably, with this approach, which was successfully validated in some (45), but not in all studies (46), no clearly missed LPA cases in our cohort were detected. However, we found four patients (including one of the BPA cases predicted as LPA) with apparent bilateral aldosterone suppression that might have been uninterpretable with respect to lateralization of excess aldosterone production (36). Such cases could remain unrecognized (47), so we decided not to exclude them from our analysis to better represent the real-world practice. The contralateral AV/IVC indices were not reported in any of the studies that proposed the presently validated clinical prediction tools (20, 21, 22).

Univariate estimates of the effects of the studied predictors from logistic regression were essentially like those of Burrello *et al.* (20). However, the multivariate model revealed AC post-confirmatory test to be the only reliable independent predictor of the PA subtype (Table 1). Our linear discriminant analysis model correctly classified 73% of the validation cohort, which is inferior to the originally reported accuracy of 81.4%. This method also identified AC post-confirmatory test as the key predictor, and likewise, it was one of the two most salient predictors in the tree-based models. This is in contrast with Burrello *et al.* (20), who identified the lowest potassium and nodule presence as the strongest predictors in their linear discriminant analysis and the lowest potassium and nodule diameter in their random forest approach. Comparably, as mentioned previously, our single-tree models also determined nodule diameter as one of the two strongest predictors that was able when combined only with AC post-SIT to achieve at least moderate discriminatory accuracy for both subtypes (Figs 3 and 4).

Nagano *et al.* have recently found AC post-SIT and also the relative AC reduction rate value to determine which subset of patients with PA should undergo AVS (22). We obtained high specificity but low sensitivity for the absolute AC value post-SIT and the converse for the post-SIT AC relative reduction rate criterion when predicting BPA. This was similar to the original authors (22) and to some earlier reports (48, 49). Post-SIT AC apparently indicates the severity of PA (49), which has been already employed in several clinical prediction tools (17, 50). Actually, Burrello *et al.* (20) also found AC post-SIT or post-captopril challenge test to be among the six most important predictors in their model. However, confirmatory tests could be spared in patients with florid PA (4, 25). Accordingly, SIT was omitted in 34 out of 178 (19%) of our PA patients with final diagnosis in the present study. Furthermore, it has been recently recommended to skip confirmatory tests completely due to cost, unreliability and misleading results (51). In fact, confirmatory testing was performed in only one-third of 435 recently operated patients with PA within a worldwide cohort (52).

More streamlined approaches might be better suited for most clinicians, such as the two-threshold APR-based criterion, which contains only plasma AC and the lowest plasma potassium (21). However, this tool yielded only moderate positive predictive values for both PA subtypes in our cohort, which was like the originally reported predictive ability for BPA, but worse than that for LPA. The difference might have ensued from using serum instead of plasma to measure both AC (53) and potassium (54) in our clinical

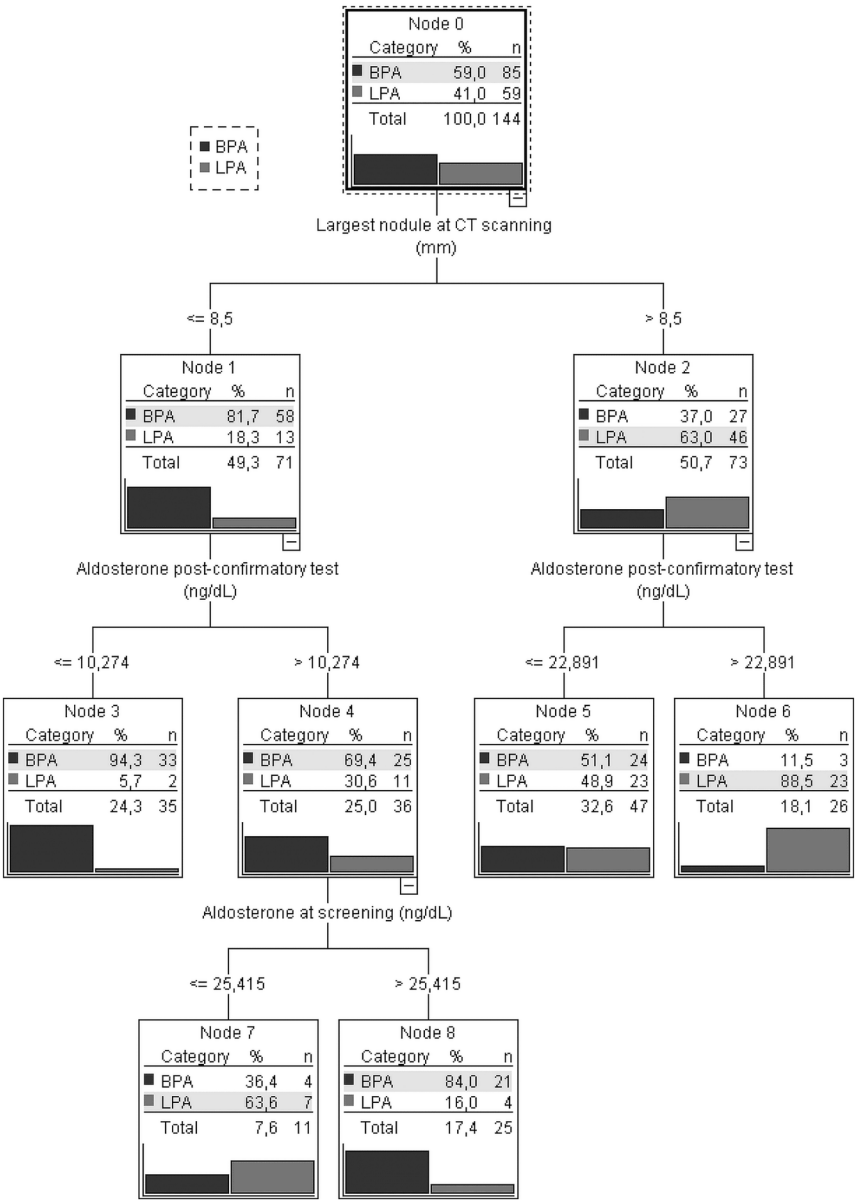

Figure 4
Classification tree for predicting primary aldosteronism subtype obtained using the CART algorithm. In each node, the predicted category is shaded in gray. BPA, bilateral primary aldosteronism; LPA, lateralized primary aldosteronism.

practice. Furthermore, Puar *et al.* studied an even more florid disease than us (21), which might have influenced the outcome. Importantly, BPA was defined differently with LI values less than 2 taken as a cut-off, but LI values between 2 and 4 were also allowed with additional clinical adjudication (21). Ultimately, if therapeutic decisions had been APR-guided, slightly less than one-third of our patients predicted to have BPA who had LPA would have been denied AVS and potentially curative adrenalectomy. Similarly, one-fourth of our patients predicted as LPA who had BPA would have inappropriate surgery. In addition, according to the APR, almost 60% of our patients were classified as 'gray zone' and only 18% of the cohort was reliably diagnosed with BPA (APR <5) to immediately commence medical treatment. Thus, in terms of diminishing the number of unnecessary AVS procedures, the benefit of this clinical prediction tool might not be considerable.

Based on the present study and other reports (19, 20, 21, 43), clinical prediction tools might not be ideal for selecting the right PA patients for AVS and surgery. Hopefully, functional imaging with positron emission tomography (PET)/CT or PET/MR will present a better solution, when longer half-life radiotracers will become more widely available (55, 56). Alternatively, mass spectrometry-based steroid profiling combined with applications of artificial intelligence could address this problem (57).

The present study has some limitations. First, the design was retrospective. Ideally, a prospective validation

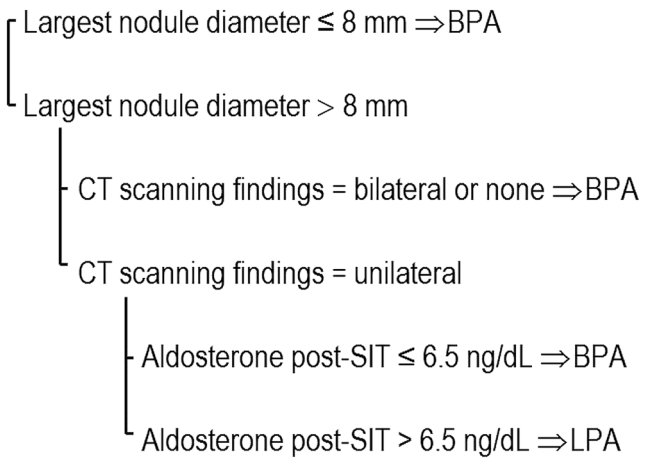

Figure 5
Classification tree for predicting primary aldosteronism subtype obtained using the C5.0 algorithm.. BPA, bilateral primary aldosteronism; LPA, lateralized primary aldosteronism.

of the suggested clinical prediction tools should have been done in a large, multicenter prospective cohort. However, the evaluation of our patients was consistent throughout the study and most of the important clinical and laboratory data have been prospectively logged in our AVS database almost without missing values. Secondly, less interfering antihypertensive drugs such as ACE inhibitors, angiotensin receptor blockers and beta blockers, which might have affected serum potassium and aldosterone values and therefore our results, were not withdrawn during the diagnostic workup as strictly as in the three original cohorts (20, 21, 22). Consequently, the clinical prediction tools might not have been validated in the exact same conditions that they were developed in. However, we merely followed the expert opinion that a complete medication washout is not needed, if the patient had a suppressed PRA value, which allows correct interpretation of the ARR, confirmatory tests, and even AVS results (25). Still, only very few patients with eGFR <60 mL/min/1.73 m^2 might have experienced significantly spuriously higher potassium values and they were well balanced between the LPA and BPA groups. In addition, the offending drugs only moderately decrease aldosterone values (4), such that experts believe that their influence is not sufficient to interfere with diagnostic decision making (50). Concordantly, Solar *et al.* showed that extensive medication switching is not needed in all patients before confirmatory testing, because more severely excessive aldosterone overproduction might not be effectively suppressed by less interfering antihypertensive medication (58). Thirdly, the relatively large within- and between-assay coefficients of variation, especially for PRA, could have had potential effects on the diagnosis of PA in our cohort. To make our diagnostics more robust, the minimum PRA value was set to avoid overinflating ARR (26). In addition, one should consider that comparable coefficients of variation were also reported elsewhere, probably due to the absence of a standardized approach for PRA measurement (59). Another possible source of error might have been unrecognized autonomous cortisol cosecretion in some cases because dexamethasone suppression testing was initially performed only in selected patients according to the guidelines at the time (23). Fortunately, low-grade cortisol cosecretion in PA seems to have only a limited effect on ACTH-stimulated AVS parameters without alterations in patient management (60). Additionally, the use of ACTH stimulation before and/or during AVS is sometimes considered controversial because it might potentially stimulate the contralateral gland and camouflage the lateralization of aldosterone production, thus making some patients with LPA seemingly inappropriate for surgical treatment (7). However, AVS was done at least partly by ACTH stimulation also in all three original cohorts (20, 21, 22), which reflects established clinical practice (8).

The main strength of our study is that our results are based on a relatively large and well-defined national cohort

Table 2 Relative predictor importance and estimated classification accuracy for the tree-based models.

Algorithm	CHAID	CART	C5.0
Most important predictors (in decreasing order)	Largest nodule diameter Aldosterone post-SIT	Aldosterone post-SIT (100) Largest nodule diameter (74) Aldosterone at screening (61)	Largest nodule diameter (100) CT scanning findings (51) Aldosterone post-SIT (42)
Classification accuracy			
In-sample	74%	75%	78%
Ten-fold cross-validation	67%	69%	71%

CHAID only provides a ranked list of the most important predictors; CART and C5.0 provide a normalized measure of predictor importance on a 0–100 scale, which is reported in parentheses.
SIT, saline infusion test.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0532

of patients with PA who were managed in a standardized way and according to the Endocrine Society clinical guidelines when feasible (4, 23). Throughout the study period, we strictly followed the same AVS protocol with almost 90% of patients overall having a successful sampling (29). Most stringent selectivity (SI >5) and lateralization criteria (LI >4) were used to optimize the diagnostic reproducibility of AVS (13). Importantly, the PASO criteria for post-adrenalectomy biochemical cure were used as the golden standard for the diagnosis of LPA (30), while all subjects with equivocal LI between 3 and 4 who were not surgically cured were excluded from the analysis to ensure better approximation for the diagnosis of BPA (6).

Conclusions

Application of any of the validated clinical tools to our cohort did not predict the PA subtype with the high diagnostic performance originally reported. Such tools provide useful clinical information and scientific insight but are not perfect.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/EC-21-0532.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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