

Quantitative Value of Aldosterone-Renin Ratio for Detection of Aldosterone-Producing Adenoma: The Aldosterone-Renin Ratio for Primary Aldosteronism (AQUARR) Study

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Background—Current guidelines recommend use of the aldosterone-renin ratio (ARR) for the case detection of primary aldosteronism followed by confirmatory tests to exclude false-positive results from further diagnostic workup. We investigated the hypothesis that this could be unnecessary in patients with a high ARR value if the quantitative information carried by the ARR is taken into due consideration.

Methods and Results—We interrogated 2 large data sets of prospectively collected patients studied with the same predefined protocol, which included the captopril challenge test. We used an unambiguous diagnosis of aldosterone-producing adenoma as reference index. We also assessed whether the post-captopril ARR and plasma aldosterone concentration fall furnished a diagnostic gain over baseline ARR values. We found that the false-positive rate fell exponentially, and, conversely, the specificity increased with rising ARR values. At receiver operating characteristics curves and diagnostic odds ratio analysis, the high baseline ARR values implied very high positive likelihood ratio and diagnostic odds ratio values. The baseline and post-captopril ARR showed similar diagnostic accuracy (area under the receiver operating characteristics curve) in both the exploratory and validation cohorts, indicating lack of diagnostic gain with this confirmatory test (between-area under the curve difference, 0.005; 95% CI, -0.031 to 0.040 ; $P=0.7$ for comparison, and 0.05 ; 95% CI, -0.061 to 0.064 ; $P=0.051$ for comparison, respectively).

Conclusions—These results indicate that the ARR conveys key quantitative information that, if properly used, can simplify the diagnostic workup, resulting in saving of money and resources. This can offer the chance of diagnosis and ensuing adrenalectomy to a larger number of hypertensive patients, ultimately resulting in better control of blood pressure. (*J Am Heart Assoc.* 2017;6:e005574. DOI: 10.1161/JAHA.117.005574.)

Key Words: accuracy • aldosterone • aldosterone-producing adenoma • aldosterone-renin ratio • diagnostic method • high blood pressure • hypertension • primary aldosteronism • specificity

The recent Lancet Commission on Arterial Hypertension called attention to the disastrous status of blood pressure (BP) control worldwide and included the search for

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Accompanying Data S1, Tables S1 through S4, and Figures S1 through S6 are available at <http://jaha.ahajournals.org/content/6/5/e005574/DC1/embed/inline-supplementary-material-1.pdf>

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secondary hypertension among the 9 key steps to combat high BP. Primary aldosteronism (PA) is the most prevalent endocrine cause of arterial hypertension. According to all guidelines, it should be screened with the aldosterone-renin ratio (ARR),¹⁻³ although the guidelines do not specify which ARR cut-off value should be used to define a positive result. However, in clinical practice the test is considered positive when its value exceeds a given cutoff, commonly placed between 20 and 40 (in [ng/dL]/[ng/mL per hour] for plasma aldosterone concentration [PAC] and plasma renin activity [PRA]).^{1,4,5}

Such liberal cutoffs maximize sensitivity, but generate many false positives. For example, in the Primary Aldosteronism Prevalence in hYpertension (PAPY) Study, by using a cutoff of 30, the false positive (FP) rate was 18%.⁴ The latter must be excluded from the further invasive and expensive subtyping, which requires adrenal vein sampling (AVS), a costly and minimally invasive procedure available only in

highly specialized referral centers.^{1,3,5} Hence, to the end of selecting the patients for AVS, both the Endocrine Society and the Japanese guidelines concur in supporting the use of confirmatory tests.

Algorithms entailing confirmatory tests may carry some limitations in that they have theoretical drawbacks and furthermore are not evidence based.⁶ For example, the basic assumption that aldosterone is autonomous (from angiotensin II) secretion in all aldosterone-producing adenoma (APA) patients is not proven. In the seminal study that introduced the saline infusion test, only 5 patients had an APA by currently accepted criteria, and, moreover, 2 of them showed an increase of PAC with standing up indicating responsiveness to angiotensin II.⁷ The existence of angiotensin II-responsive APA in up to 70% of the cases has been thereafter documented by multiple independent studies.^{8–11} Furthermore, the 2 most popular confirmatory tests, the captopril challenge and the saline infusion, when assessed prospectively in a large cohort of consecutive patients, showed such an overlap of PAC responses between patients with and without APA that abated their value for individual patients discrimination¹¹ and disproved the basic assumption of angiotensin II independence.

The systematic use of confirmatory tests in clinical practice increases times, complexity, and costs of the diagnostic workup, thus contributing to the underdiagnosis of PA. A simplification of the diagnostic algorithm would therefore be a crucial step for improving the detection rate of PA, which can ultimately result in more patients referred for adrenalectomy or for specific therapies, which are highly efficacious^{12,13} and cost-effective.¹⁴

The surgically curable APAs usually feature a more florid biochemical phenotype than the medically treatable bilateral adrenal hyperplasia (BAH).⁴ Hence, high ARR values point to an APA subtype, more than to BAH.^{4,15} We therefore hypothesized that: (1) the ARR carries quantitative information, which wanes when its results are dichotomized as positive or negative, and (2) high ARR values could be associated with a high likelihood of APA. If verified, these hypotheses would indicate no need for further confirmation and the possibility for the patients with high ARR values to proceed directly to AVS. In line with this proposition, recognizing the burden of the systematic undertaking of confirmatory tests, the recently released guidelines suggest to proceed directly to AVS in patients with a PAC value >20 ng/dL (550 pmol/L), plasma renin below detection levels, and concurrent hypokalemia,¹ albeit as recommendation of class 2 with very-low-quality evidence.

Hence, we set out to determine whether increasing ARR values allows unambiguous identification of an APA with a high diagnostic specificity. We also sought for investigating

the diagnostic gain of the captopril challenge test over baseline ARR as a function of different ARR cutoffs.

Materials and Methods

The protocol of the Aldosterone-Renin Ratio for Primary Aldosteronism (AQUARR) study was already published¹⁶; hence, it will be only briefly recalled here. The study entailed 2 large cohorts of referred prospectively recruited hypertensive patients, all of which underwent measurement of the ARR at baseline and after the captopril challenge test. All patients provided an informed written consent and the protocol was approved by the ethics committee of the University of Padua (Padua, Italy).

Exploratory Analysis

The exploratory analysis was performed in the PAPY Study data set, which was carried out between 2000 and 2005⁴ and prospectively recruited 1125 consecutive patients newly diagnosed with hypertension, who were referred to specialized centers for diagnosis and treatment of hypertension. Details on exclusion criteria, screening procedure, pharmacological treatment, and diagnosis are given in Data S1.

By protocol PA was diagnosed if patients had an ARR ≥ 40 at baseline and/or an ARR ≥ 30 post-captopril (method 1) and/or a logistic discriminant function score ≥ 0.50 .^{17,18} To avoid a factitious inflation of the ARR value when PRA values were <0.20 ng/mL per hour, they were fixed at 0.20 ng/mL per hour for the ARR calculation.

The study followed the STARD Statement for Reporting Diagnostic Accuracy Studies, according to which diagnostic approaches/tests should be evaluated against a clinical reference standard or a gold standard.¹⁹ Thus, recognizing that discrimination of BAH from low-renin primary (essential) hypertension is arbitrary,²⁰ and that a diagnosis of PA can be made unambiguously only for APA, it was decided beforehand to use the diagnosis of APA as the gold standard. Accordingly, all the following “4 corners criteria” had to be fulfilled for diagnosing an APA: (1) a biochemical finding of PA; (2) lateralization of aldosterone secretion either at AVS or at ¹³¹I-norcholesterol dexamethasone-suppressed adrenocortical scintigraphy; (3) adenoma demonstration at pathology assessment; and (4) evidence of normokalemia and cure or improvement of hypertension at follow-up at least 120 days after adrenalectomy. Cure was defined as a BP <140/90 mm Hg, for systolic and diastolic, respectively, without antihypertensive medications; improvement entailed systolic and diastolic BP <140/90 mm Hg on the same or a decreased number and/or of defined daily doses of medications.²⁰ Patients with biochemical PA without lateralized

aldosterone excess were held to have BAH. Upon completion of the diagnostic workup and follow-up, an adjudication committee established the final diagnosis.

Validation Phase

Consecutive white patients referred to the ESH Excellence Hypertension Center of the University of Padua between January 2012 and February 2015 were prospectively recruited in the validation cohort. They were recently diagnosed with hypertension and, after publication of the PAPY Study results,⁴ all were systematically screened for secondary hypertension.¹ All underwent the same workup described above for the exploratory data set under carefully standardized conditions, the only differences being a centralized measurement of all biochemical indexes, a longer (4 weeks) minimum wash-out period (for diuretics, beta-blockers, angiotensin-converting enzyme inhibitors, and angiotensin II receptor type 1 antagonists), no consideration to the postcaptopril PRA and PAC value for the diagnosis, and systematic use of AVS when indicated.¹ Exclusion criteria entailed a previous diagnosis of secondary hypertension, including the Familial Hyper-aldosteronism-1, as identified by genetic testing.²¹

Of the 1266 patients initially recruited, 190 had to be excluded to prevent any confounding effect on the analysis of diagnostic accuracy: 153 because of unfeasible washout due to very high BP levels and/or target organ damage, and 37 with PA owing to unfulfilled 4 corners criteria because of either lack of subtyping data (n=31) or refused adrenalectomy (n=6) in spite of lateralized aldosterone excess at AVS. Thus, 1076 patients were available for the final analysis (Figure 1).

PRA, PAC, Na⁺, and K⁺ serum levels and 24-hour urinary excretion were centralized and measured in a certified (International Organization for Standardization [ISO] 9001) laboratory. Normal values and within- and interassay coefficient of variation have been reported.^{4,22} PA and APA were diagnosed by the aforementioned criteria by an adjudication committee (G.M., G.P.R.), with the only relevant difference that PA diagnosis was made based on the unstimulated ARR level ≥ 40 regardless the post-captopril data. For the purpose of this study, the patients with lateralized aldosterone excess at AVS, who had a biochemical correction of PA and showed an improvement/cure of hypertension after adrenalectomy, but at pathology were found to have unilateral multinodular hyperplasia, were included in the APA group.

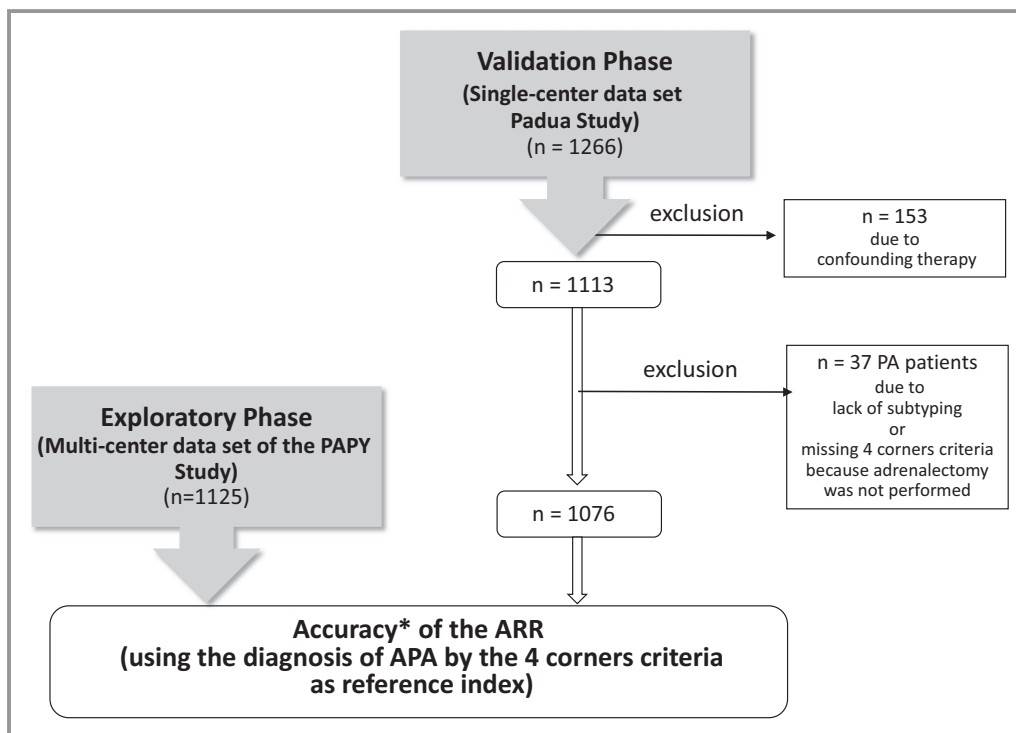


Figure 1. Flow chart of the AQUARR (Aldosterone-Renin Ratio for Primary Aldosteronism) study. *The accuracy of the aldosterone-renin ratio (ARR) was determined by analysis of the area under the receiver operating characteristics curve, positive and negative likelihood ratio, diagnostic odds ratio, and error rate. The optimal cut-off values were established by Youden index analysis. APA indicates aldosterone-producing adenoma; PA, primary aldosteronism; PAPY, Primary Aldosteronism Prevalence in hYpertension.

Statistical Analysis

Transformation (log or square root) of skewed quantitative variables was exploited to achieve a normal distribution, as appropriate, before statistical analysis. One-way ANOVA followed by Bonferroni's post-hoc test, or *t* test, were used to compare quantitative variables among/between groups. The distribution of categorical variables was compared by chi-square analysis.

The operative features of the ARR (sensitivity, specificity, positive and negative likelihood ratios, and diagnostic odds ratio [DOR]) and identification of optimal cut-off values were calculated using the conclusive diagnosis of APA, as defined above, as reference.¹⁹

The positive and negative likelihood ratio, error rate, and DOR were calculated to estimate the test accuracy for identifying patients with APA. The positive likelihood ratio (eg, the ratio of probability of a positive test result in patients with and without disease) was calculated as sensitivity/(1–specificity); the negative likelihood ratio (eg, the ratio of probability of a negative test result in patients with and without disease) was estimated as (1–sensitivity)/specificity. The error rate was calculated as follows:

$$P(T-|D+) \times P(D+) + P(T+|D-) \times P(D-)$$

where $P(T-|D+)$ stands for the false negative (FN) rate, $P(D+)$ is the rate of diseased patients, $P(T+|D-)$ stands for the FP rate, and $P(D-)$ is the rate of nondiseased patients.

The DOR, a prevalence-independent indicator of test performance, was computed as the odds of positivity among diseased patients divided by the odds of positivity among nondiseased patients. It can also be defined as the ratio of the odds of disease in test positives relative to the odds of disease in test negatives. Its values range from zero to infinity, with a value of 1 indicating that the test does not discriminate between patients with and without disease and higher values indicating progressively better discriminatory performance of the test.^{23,24}

The area under the receiver operating characteristics (ROC) curve was used to estimate the accuracy of the test for identification of APA. The Youden Index (*J*), which defines the maximum potential effectiveness of a biomarker, can be formally defined as $J = \max(c) \{ \text{Sensitivity}(c) + \text{Specificity}(c) - 1 \}$. The cut-point that achieves this maximum is referred to as the optimal cut-point (c^*) because it is the cut-off that optimizes the biomarker's differentiating ability when equal weight is given to sensitivity and specificity. Hence, C^* was used to identify the baseline and post-captopril ARR cut-off values corresponding to the best combination of sensitivity and specificity.

We first plotted specificity and FP rate for the diagnosis of APA as a function of the ARR values in both data sets, as a

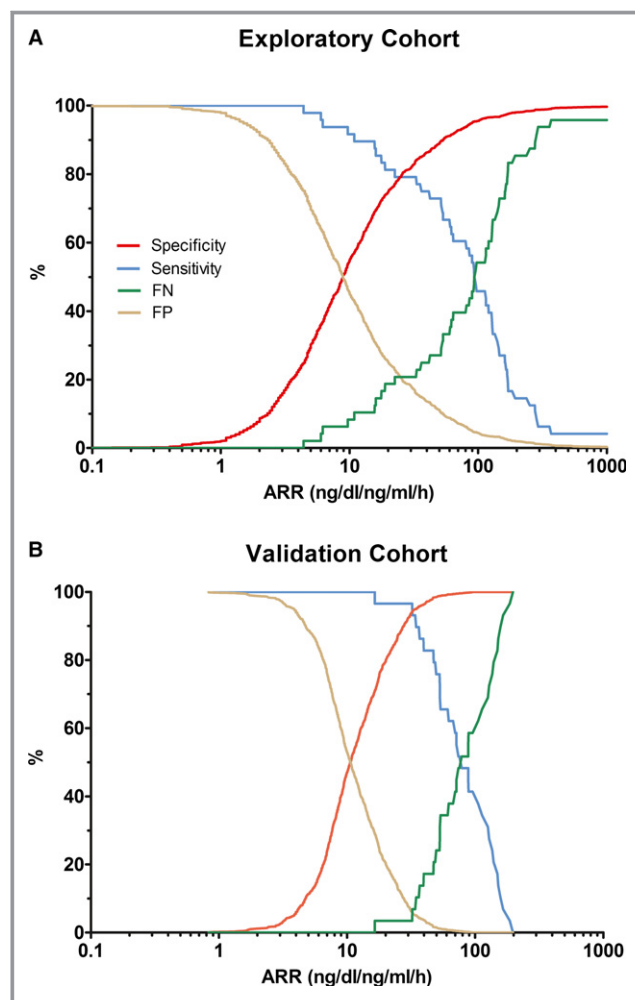


Figure 2. Diagnostic yield of the aldosterone-renin ratio (ARR) values. The plot shows that increasing ARR values are associated with an exponential increase of specificity and an exponential decrease of false negative (FN) rate for identification of aldosterone-producing adenoma patients in the exploratory (A) and validation (B) cohort. FP indicates false positive.

preliminary inspection of the quantitative value of the baseline ARR (Figure 2). Next we examined the ARR cut-off values corresponding to a 5% FP rate and to 1% step-wise decreases. To further verify the robustness of these findings, acknowledging that some assays have lower limits of detection of PRA, a sensitivity analysis was carried out using a minimum cutoff for PRA (0.10 ng/mL per hour).

To determine the diagnostic gain yielded by the captopril challenge test, we compared the ROC areas under the curve (AUCs) of baseline and post-captopril ARR (method 1) and of baseline and post-captopril PAC decrease (method 2).¹

Significance was set at $P < 0.05$. For the statistical analysis, we used the SPSS (version 23 for Mac; IBM Italy SpA, Rome, Italy), GraphPad Prism (version 6.00 for Mac; GraphPad Software, La Jolla, CA), and MedCalc (version 8.1.1.0; MedCalc Software, Mariakerke, Belgium) software.

Results

Baseline Characteristics of the Patients

Table S1 shows the baseline characteristics of the 1125 PAPY Study patients divided by diagnosis. As described, 84% in the BAH group and more than 50% in the APA group did not have hypokalemia.⁴ However, APA patients showed a more-florid PA phenotype than BAH patients, as indicated by higher PAC and ARR and lower serum K⁺. Compared to PH patients, APA patients were older, had higher systolic BP, and lower serum K⁺; they also showed the expected higher PAC, and lower PRA, which translated into higher ARR values.

Rate of PA was slightly lower in the validation (6.9%) than in the exploratory cohort (11.2%), even though the overall baseline characteristics of the patients in the validation cohort (Table 1) closely mimicked those of the exploratory data set. This expected finding is readily explained by the fact that after the publication of the PAPY Study in 2006, a systematic screening was implemented at our center. Comparison of the features of the 3 diagnosis groups between the exploratory and the validation data set showed only slight differences of age, estimated glomerular filtration rate, baseline PRA, PAC, and ARR (Figures S1 through S5) and overall less spread values reflecting the single-center nature of this cohort. Baseline and post-adrenalectomy APA patients' features are provided in Table S2.

Diagnostic Yield of the ARR at Different Cut-Off Values

Progressively increasing ARR values were associated with an exponential increase of specificity for identification of APA

patients and, conversely, with an exponential decay of the FP rate, both in the exploratory and in the validation data set, albeit with better results in the latter (Figure 2). Table S3 and Table 2 show the specificity, FP rate, positive and negative likelihood ratio, and DOR in the exploratory and validation data sets at prespecified ARR cutoffs.

The ROC AUC, an overall measure of the ARR accuracy for the diagnosis of APA (Figure 3), was higher ($P<0.001$) than the identity line AUC (0.500) in both data sets, with a trend to lower values in the exploratory (0.878; 95% CI, 0.821–0.923) than in the validation data set (0.980; 95% CI, 0.960–1.000; $P<NS$ [not significant] for comparison). However, the ARR value that maximized sensitivity and specificity, as identified by the Youden index, showed almost identical values in the exploratory and the validation data sets (33.3 versus 32.2, respectively). The corresponding ARR values based on direct renin concentration (mUI/L) and PAC (ng/dL) assay, calculated with the available App,²⁵ were 1.75 and 1.69, respectively. These values are slightly lower than those found in a recent prospective study,²⁶ because, in the present cohort, the minimum PRA values were arbitrarily fixed at 0.20 ng/mL per hour and therefore the derived direct renin concentration led to a decrease of the estimated ARR.

We analyzed the exploratory data set at 12 ARR cutoffs progressively increasing stepwise from 10 to 120 and found that the positive and negative likelihood ratio and the DOR increase progressively alongside the ARR cut-off value (Table S3).

The results were practically identical in the validation data set: at high ARR values, the FP rate was negligible and the specificity approached 100% (Table 2); the positive likelihood ratio increased exponentially alongside the DOR and tended

Table 1. Demographic Characteristics of the Patients Enrolled in the Validation Cohort

Variable	PH (n=1036)	P (PH vs APA)	APA (n=29)	P (APA vs BAH)	BAH (n=11)	P (BAH vs PH)
Age, y	47±13	ns	48±13	ns	54±12	ns
Sex (M/F), %	49/51	ns	62/38	ns	73/27	ns
BMI, kg/m	27±7	ns	25±8	ns	30±1	ns
Systolic BP, mm Hg	148±24	ns	153±18	ns	155±13	ns
Diastolic BP, mm Hg	91±14	ns	92±13	ns	89±11	ns
Serum K ⁺ , mEq/L	4.0±0.4	<0.001	3.3±0.6	0.031	3.7±0.4	ns
Na ⁺ _u V, mEq/day	158 (113–209)	ns	141 (120–169)	ns	177 (143–226)	ns
GFR, mL/min	97 (82–112)	ns	103 (90–122)	ns	81 (56–108)	ns
PRA, ng/mL per hour	0.90 (0.53–1.41)	0.004	0.35 (0.2–0.55)	ns	0.40 (0.20–0.91)	ns
PAC, ng/dL	10.0 (7.5–12.7)	<0.001	28.0 (22.4–37.1)	ns	18.8 (17.5–37.5)	<0.001
ARR, (ng/dL)/(ng/mL per hour)	10.5 (7.1–17.5)	<0.001	78.8 (51.4–137.7)	<0.001	61.3 (41.2–88.4)	<0.001

Data are expressed as mean value±SD or median and 25th to 75th percentile in parentheses for variables not normally distributed. APA indicates aldosterone-producing adenoma; ARR, aldosterone-renin ratio; BAH, bilateral adrenal hyperplasia; GFR, glomerular filtration rate; K⁺, potassium; Na⁺_uV, sodium urinary excretion; ns, not significant; PAC, plasma aldosterone concentration; PH, primary (essential) hypertension; PRA, plasma renin activity.

Table 2. Diagnostic Yield of the ARR at Specified Cutoffs in the Validation Data Set

ARR	FP n (%)	TN n (%)	FN n (%)	TP n (%)	Sens. (%)	Spec. (%)	PLR	NLR	DOR	ER
10	555 (51)	492 (46)	0 (0)	29 (3)	100	47 (41–53)	1.9	NA	NA	NA
20	213 (20)	834 (77)	1 (0)	28 (3)	97 (83–100)	80 (75–85)	4.8	0.04	110	0.20
30	81 (7)	966 (90)	1 (0)	28 (3)	97 (83–100)	92 (89–95)	12.5	0.04	334	0.08
40	39 (4)	1008 (93)	5 (1)	24 (2)	83 (55–100)	96 (94–98)	22.2	0.18	124	0.04
50	16 (1)	1031 (96)	7 (1)	22 (2)	76 (34–100)	98 (96–100)	49.6	0.25	202	0.02
60	10 (1)	1037 (96)	10 (1)	19 (2)	66 (31–100)	99 (98–100)	68.6	0.35	197	0.02
70	6 (1)	1041 (97)	12 (1)	17 (1)	59 (22–96)	99 (98–100)	102.3	0.42	246	0.02
80	4 (0)	1043 (97)	15 (1)	14 (1)	48 (11–85)	100 (99–100)	126.4	0.52	243	0.02
90	2 (0)	1045 (97)	17 (2)	12 (1)	41 (6–78)	100 (99–100)	216.6	0.59	369	0.02
100	0 (0)	1047 (97)	17 (2)	12 (1)	41 (6–78)	100	NA	NA	NA	NA

ARR indicates aldosterone-renin ratio expressed in (ng/dL)/(ng/mL per hour); DOR, diagnostic odds ratio; ER, error rate; FN, false negative; FP, false positive; NA, not available; NLR, negative likelihood ratio; PLR, positive likelihood ratio; sens, sensitivity; spec, specificity; TN, true negative; TP, true positive.

to infinity with ARR above 100, attributed to the fact that the denominator entailing the odds of disease in test negatives was 0, because all patients with an ARR above this cutoff had an APA (Figures 2 and 4; Table 2). A sensitivity analysis carried out using a minimum cutoff for PRA of 0.10 ng/mL per hour (Figure S6) led to practically identical conclusions.

Diagnostic Gain of the Captopril Challenge Test

To determine the diagnostic gain of the captopril challenge test, the ROC AUCs were compared by 2 methods: between

the baseline and the post-captopril ARR (method 1) and between the baseline ARR and the post-captopril PAC fall (method 2), in both cases using the APA diagnosis as reference. This showed a negligible between-AUC difference (0.005; 95% CI, -0.031 to 0.040 ; $P=0.7$ for comparison, and 0.009; 95% CI, -0.051 to 0.069 ; $P=0.8$, with method 1 and method 2, respectively), indicating that the captopril challenge test provides no diagnostic gain over the baseline ARR (Figure 3A).

Results were similar in the validation data set (between-AUC difference, 0.05; 95% CI, -0.061 to 0.064 ; $P=0.51$ for

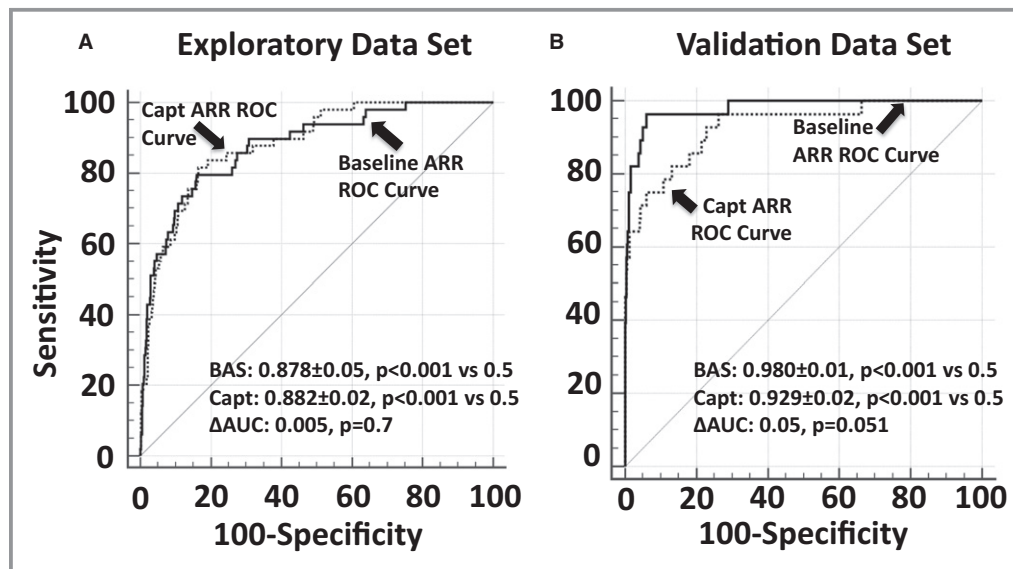


Figure 3. Receiver operating characteristics (ROC) curve of baseline (BAS) and post-captopril (Capt) aldosterone-renin ratio (ARR) for diagnosing aldosterone-producing adenoma (APA). In both the exploratory (A) and the validation (B) cohort, the area under the curve (AUC) of the baseline and captopril ARR was higher than the 0.500 (eg, the AUC under the identity line). However, the difference between the AUC of the baseline and the post-captopril ARR was negligible and not statistically significant in both the exploratory (A) and the validation (B) cohort.

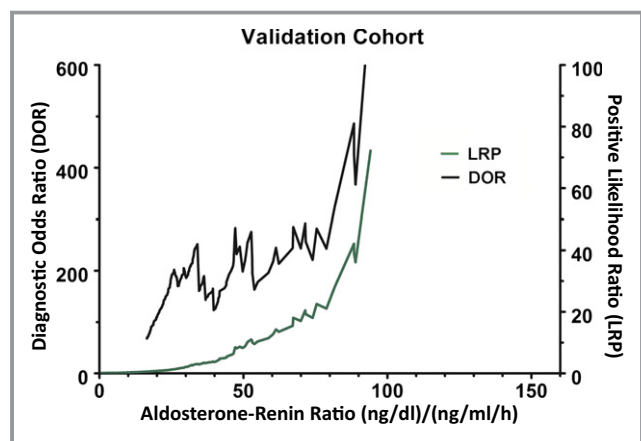


Figure 4. The plot of positive likelihood ratio (LRP) and diagnostic odds ratio (DOR) as a function of increasing aldosterone-renin ratio (ARR) in the validation data set. Please note that raising ARR values are associated with an exponential increase of both LRP and DOR.

comparison with method 1; 0.02; 95% CI, -0.056 to 0.095 ; $P=0.6$ with method 2 (Figure 3B). Of note, in the high specificity range of values corresponding to high ARR values, the curves were superimposed in all comparisons (Table 2, Data S1, and Table S4).

Discussion

This study included 2 large data sets of patients that were studied with a similar predefined protocol: the multicenter PAPHY study and a single-center validation cohort. In both cohorts, the overall prevalence of PA was high (11.2% and 6.9%, respectively), albeit with a slightly lower difference in the validation cohort, which reflected multiple factors. The latter included the systematic screening of newly diagnosed hypertensive patients implemented at our center after the publication of the PAPHY Study,⁴ which led to screen a less-selected cohort, and the tighter diagnostic criteria used.

With the strength provided by a painstaking investigation of the patients, this study demonstrates that the baseline (unchallenged) ARR carries essential quantitative information: Progressively increasing ARR values implied an exponential increase of specificity and, conversely, an exponential decay of FP rate in both the exploratory and (even more so) in the validation data sets. In the exploratory data set, both the positive likelihood ratio and the DOR—a disease prevalence-independent measure of diagnostic accuracy—were high (6.35 and 17.7, respectively) for ARR values above 50. Corresponding values were even higher in the validation data set (Figure 4). Our main goal, however, was not to support use of a given cutoff instead of another, but rather to enable physicians to make their own choice of the ARR cutoff, and

thus of specificity, based on patient's past probability of APA. Hence, in Table 2, we provided the specificity, FP rate, positive and negative likelihood ratio, and DOR values of the validation data set as a function of the ARR values.

Noteworthy, in this study, the aforementioned Endocrine Society guidelines' strategy for bypassing confirmatory tests (in patients with hypokalemia, markedly suppressed renin, and with PAC >20 ng/dL) was feasible in only a tiny proportion (2.5% in the exploratory cohort) of our patients. This finding is by no means surprising inasmuch as the existence of normoaldosteronemic APA has been well documented^{27,28} and hypokalemia is no longer regarded as a *conditio sine qua non* of PA.^{4,29}

The second major result of this study entails the demonstration that the systematic use of the captopril test provided no diagnostic gain over baseline ARR, even in a set of patients studied in a carefully standardized way. Comparison of the accuracy (ROC AUC) of the baseline and post-captopril ARR (method 1, Figure 3) or the post-captopril PAC fall (method 2) showed no differences, not even in the portion of the ROC curves corresponding to a high sensitivity where FP results must be pinpointed.

Thus, overall, these findings furnished compelling evidence that: (1) Detection of increasing ARR values are associated with an exponential increase of the likelihood of an APA and (2) high ARR values renders the undertaking of any further confirmatory tests useless, if not counterproductive. This is likely explained by the aforementioned fallacy of the premise that all APAs are autonomous from angiotensin II.^{11,30,31}

With the strength of a prospective design, a STARD-based methodology funded on robust criteria to diagnose APA,¹⁹ a large sample size, and a rigorous formal analysis, this study therefore furnishes evidence-based support to the contention that confirmatory tests can be skipped when the biochemical picture of PA is florid.^{1,32}

It might be argued that the general applicability of these results is limited by use of the specific assays used at our center and also by adoption of the PRA instead of the direct concentration of (active) renin. To overcome this possible limitation, based on the finding of a tight linear relationship between the PRA and the direct renin concentration assay in a large, prospective, head-to-head comparison of the 2 assays,²⁶ the Working Group on Endocrine Hypertension of the European Society of Hypertension has developed the ARR-App²⁵ that allows a swift conversion of PRA into direct renin concentration, and vice versa, and a straightforward calculation of the ARR based on either assay. It could also be argued that the reproducibility of the ARR is limited. However, when performed under standardized conditions, even in a multicenter study, the ARR was found to be within-patients reproducible³³; moreover, its reproducibility was found even higher when an automated (hands-off) chemiluminescent assay was used.²⁶

Some limitations of this study, however, deserve to be mentioned before its conclusions can be generalized: our cohorts were recruited at third-level referral centers and therefore results need to be replicated in different settings and to conclusively demonstrated in a randomized diagnostic trial. Additionally, even though the cohorts comprised similar patients, the accuracy of the ARR was slightly higher in the validation (Figure 4) than in the exploratory data set. The multicenter nature of the exploratory study with no centralized biochemical assays and the single center with a centralized measurement of all biochemical variables of the validation study explain this finding, given that less-spread PAC, PRA, and ARR values were found in the validation data set than in the exploratory cohort (Figures S1 through S5). Moreover, our study examined only whites and only the captopril challenge test (ie, 1 of the 5 confirmatory tests for PA). Hence, we would like to underline that our conclusions pertain to this ethnicity and this test, which, however, is widely used and provides results comparable to the saline infusion test if patients are not on a low-sodium intake.¹¹ Notwithstanding these potential limitations, the robustness and generalizability of the following conclusions are strongly supported by the replication of our findings in both our large cohorts.

Conclusion

The ARR is the most used test exploited to diagnose primary aldosteronism, the most frequent cause of curable hypertension, but is categorized as positive or negative, which means disregarding the quantitative information that it bears. In 2 large data sets of prospectively recruited hypertensive patients undergoing screening tests for primary aldosteronism, we found that high ARR values identified patients with an exponentially increasing probability of carrying an APA and with a likelihood of FP result approaching zero. Hence, the ARR carries important quantitative information, which should not be neglected by categorizing its results simply as positive or negative. We showed that a proper use of this quantitative information increases the diagnostic accuracy of the ARR for identification of the surgically curable subtype of primary aldosteronism (eg, APA). Increasing values of the ARR imply a high positive likelihood ratio and a high DOR of APA. Above these levels, the captopril challenge confirmatory test furnished no diagnostic gain over the baseline ARR. Accordingly, we would like to suggest that the diagnostic algorithm can be simplified in an ample number of hypertensive patients screened for primary aldosteronism, thus engendering an improved case detection of APAs and, ultimately, a more cost-effective treatment of arterial hypertension. An App that allows calculation of the ARR²⁵ in the unit of measure used in this study starting from assays that measure direct active

renin concentration is a further argument supporting the application of these findings in clinical practice.²⁶

Appendix

Participating Centers and PAPY Study Investigators: Padova DMCS Internal Medicine 4: Gian Paolo Rossi, Andrea Semplicini, Chiara Ganzaroli, Achille Cesare Pessina; Padova, Endocrinology: Franco Mantero, Decio Armanini, Giuseppe Opocher, Mee-Yung Mattarello; Ancona, Endocrinology: Gilberta Giacchetti, Vanessa Ronconi, Marco Boscaro; Reggio Emilia, Internal Medicine: Ermanno Rossi; Pisa, Internal Medicine: Giampaolo Bernini, Angelica Moretti; L'Aquila, Department of Internal Medicine and Public Health: Claudio Ferri, Giovambattista Desideri; Palermo, Internal Medicine: Giuseppe Andronico; Brescia, Internal Medicine: Damiano Rizzoni, Enzo Porteri; Legnano, Internal Medicine: Gaetana Palumbo; Roma, Internal Medicine: Claudio Letizia, Chiara Caliumi; Trieste, Internal Medicine: Bruno Fabris; Firenze, Endocrinology: Massimo Mannelli, Gabriele Parenti; Torino, Endocrinology: Mauro Maccario, Ezio Ghigo; Reggio Calabria, Nephrology: Francesca Mallamaci, Carmine Zoccali; Bari, Internal Medicine: Anna Belfiore.

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Disclosures

None.

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Supplemental Material

Data S1.

Supplemental Methods

Exclusion criteria, screening procedure, pharmacological treatment, and diagnosis: The exclusion criteria comprised only patient's refusal to participate in the study and a prior diagnosis of any secondary form of HT. Patients underwent screening while off antihypertensive treatment or, if already treated, after switching to calcium channels blockers and/or doxazosin following guidelines. In patients on a mineralocorticoid receptor antagonist (spironolactone, canrenone, or potassium canrenoate), or on agents affecting the renin-angiotensin-aldosterone system (diuretics, beta-blockers, angiotensin-converting enzyme inhibitors, and angiotensin II type 1 receptor antagonists) at least six-weeks or two-weeks wash-out period was required, respectively. The screening test was performed after one-hour in sitting position (supine rest) and again 60 minutes after 50 mg oral captopril administration while the patient remained sitting (supine). It comprised measurement of PAC and PRA, Na⁺ and K⁺ in serum and in 24-hours urine were also assayed. The PAC (in ng/dl)/PRA (in ng/ml/h) ratio (ARR), at baseline and after captopril was calculated. Further work-up (comprising a saline infusion test, and a high-resolution computed tomography (CT) scan and/or magnetic resonance (MR) imaging) was performed in all patients with and ARR ≥ 40 baseline and/or ≥ 30 post-captopril, and in one every four consecutive patients not fulfilling such criteria. All those fulfilling the aforementioned biochemical criteria with a positive saline infusion test underwent subtyping. AVS was used, if bilaterally selective, for the PA subtyping wherever available. At centers where AVS was unfeasible, lateralized aldosterone excess production was ascertained by dexamethasone-suppressed adrenocortical ¹³¹I-norcholesterol scintigraphy.¹² PRA was measured by radioimmunoassay with commercial kits (Ren CTK, Sorin Biomedica Saluggia, Italy, in 10 centers; or Angiotensin I RIA CT, Radim, Pomezia, Italy, in the others); intra-assay and inter-assay coefficient of variation was within 8% and 10%, respectively. PAC was measured with a commercial kit (Aldosterone Mirya, Technogenetics, Cassina de Pecchi, Italy; normal range between 1.0 to 15.0 ng/dl supine and 3.0 to 32.0 ng/dl upright on a normal Na⁺ diet; intra-assay and inter-assay coefficients of variation <5.6%).

Results. Diagnostic yield of the ARR at different cut-off values: to examine the diagnostic accuracy conveyed by the quantitative value of the ARR the exploratory dataset was analyzed at five predefined ARR cut-offs, which yielded high specificity (95% to 99%) and very low false positive rates decreasing stepwise (by 1%) from 5% to 1% (Table S4). This analysis showed a progressive increase of the positive and negative likelihood ratio, and the diagnostic odds ratio alongside the ARR cut-off value (Table S4). Moreover, ARR values above the cut-off associated with a false positive rate of 2%, e.g. 115.4 [in (ng/dl)/(ng/ml/h)], provided no incremental positive likelihood ratio (Table S4).

Diagnostic gain of the captopril challenge test: the diagnostic gain of the captopril challenge test was also investigated in the exploratory dataset by selecting the 42 patients with an ARR ≥ 115.4 , e.g. positive at the screening test by such restrictive cut-off value. Of them, 48.6% of the 35 found to be positive by the PAPY Study criterion (e.g. post-captopril ARR > 30), were false positive (Method 1, Table S4).

Even less accurate was the result when a post-captopril decrease of PAC < 30% (e.g. the criterion designated as diagnostic for APA by the Endocrine Society guidelines) in that 52.4% of the 42 positive patients were

false positive (Method 2, Table S4). Selection of the patients with an ARR ≥ 115.4 in the exploratory or validation dataset also showed no diagnostic gain of the captopril challenge test over baseline ARR by using either Method 1 or Method 2). In these sub-cohorts both criteria for positivity performed disappointingly bad, as shown by high false positive rate (0.40 and 0.52, Method 1 and 2, respectively) (Table S4), low values of the positive (1.10 and 1.34, for Method 1 and 2, respectively) and the negative likelihood ratio (0.55 and 0.76, for Method 1 and 2, respectively). The error rate was also high for both criteria (0.48 and 0.43, Method 1 and 2, respectively) and the DOR was low (2.0 and 1.77, Method 1 and 2, respectively).

Variable	PH (n=1003)	P (PH vs APA)	APA (n=50)	P (APA vs BAH)	BAH (n=72)	P (BAH vs PH)
Age, yrs	46±12	0.017	51±13	ns	49±11	ns
Sex (M/F, %)	56/44	ns	54/46	ns	61/39	ns
BMI, (Kg/m ²)	27±5	ns	27±4	ns	27±5	ns
Systolic BP, (mmHg)	147±17	<0.001	158±23	ns	153±16	0.021
Diastolic BP, (mmHg)	95±10	ns	98±10	ns	99±11	0.005
Serum K ⁺ , (mEq/L)	4.0±0.4	<0.001	3.4±0.6	<0.001	3.8±0.4	<0.001
Na ⁺ _u V, (mEq/d)	144 (102-205)	ns	130 (88-166)	ns	130 (98-166)	ns
GFR, (mL/min)	86±21	ns	83±17	ns	85±18	ns
PRA, (ng/mL/h)	1.1 (0.6-18)	0.005	0.2 (0.2-0.6)	ns	0.3 (0.2-0.6)	<0.001
PAC, (ng/dL)	9.3 (5.4-14.8)	<0.001	26.2 (14.9-43.1)	0.001	21.9 (14.6-32.6)	<0.001
ARR, (ng/dl)/ (ng/ml/h)	8.3 (4.1-16.7)	<0.001	89.7 (33.8-145.5)	<0.001	66.0 (31.3-96.6)	<0.001

Table S1. Demographic Characteristics of the Patients Enrolled in the PAPY Study.

Data are expressed as mean value SD or median and 25-75 percentile in parentheses for variables not normally distributed.

APA, aldosterone producing adenoma; ARR, aldosterone-renin ratio; BMI, body mass index; BP, blood pressure; GFR, glomerular filtration rate; BAH, idiopathic hyperaldosteronism; K⁺, potassium; Na⁺_uV, sodium urinary excretion; ns, not significant; PAC, plasma aldosterone concentration; PH, primary (essential) hypertension; PRA, plasma renin activity.

Variable	Baseline	After adrenalectomy	P
Age, y	48±13	51±13	ns
Sex (M/F, %)	62/38	62/38	ns
BMI, Kg/m²	25±8	25±3	ns
Systolic BP, mmHg	153±18	133±30	= 0.036
Diastolic BP, mmHg	92±13	87±13	ns
Antihypertensive therapy, (number of drugs)	1.4±0.6	0.3±0.7	<0.001
Serum K⁺, mEq/L	3.3±0.6	4.3±0.5	<0.001
Na⁺_uV, mEq/day	141 (120–169)	163 (111–188)	ns
GFR, mL/min	103 (90-122)	91 (84-117)	ns
PRA, ng/mL/h	0.35 (0.20-0.55)	0.50 (0.26-1.00)	ns
PAC, ng/dL	28.0 (22.4-37.1)	5.9 (4.1–8.6)	<0.001
ARR,(ng/dl)/ (ng/ml/h)	78.8 (51.4-137.7)	13.6 (7.3–21.0)	<0.001

Table S2. Demographic Characteristics of the aldosterone producing adenoma patients (n=29) of the Validation Cohort at baseline and after adrenalectomy.

ARR, aldosterone-renin ratio; BMI, body mass index; BP, blood pressure; GFR, glomerular filtration rate; K⁺, potassium; Na⁺_uV, sodium urinary excretion; ns, not significant; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

ARR	FP n (%)	TN n (%)	FN n (%)	TP n (%)	Sens. (%)	Spec. (%)	PLR	NLR	DOR	ER
10	488 (43)	587 (52)	4 (1)	46 (4)	92 (77–100)	55 (49–61)	2.0	0.15	13.8	0.44
20	272 (24)	803 (71)	11 (1)	39 (4)	78 (55–100)	75 (70–80)	3.1	0.29	10.5	0.25
30	200 (18)	875 (78)	11 (1)	39 (4)	78 (55–100)	81 (76–86)	4.2	0.27	15.5	0.19
40	144 (13)	931 (83)	14 (1)	36 (3)	72 (47–97)	87 (83–91)	5.4	0.32	16.6	0.14
50	115 (10)	960 (85)	16 (2)	34 (3)	68 (42–94)	89 (85–93)	6.4	0.36	17.7	0.12
60	89 (8)	986 (87)	19 (2)	31 (3)	62 (35–89)	92 (89–95)	7.5	0.41	18.1	0.10
70	64 (6)	1011 (90)	22 (2)	28 (2)	56 (28–84)	94 (91–97)	9.4	0.47	20.1	0.08
80	53 (5)	1022 (91)	22 (2)	28 (2)	56 (28–84)	95 (92–98)	11.4	0.46	24.5	0.07
90	38 (4)	1037 (92)	25 (2)	25 (2)	50 (22–78)	96 (94–98)	14.1	0.52	27.3	0.06
100	30 (3)	1045 (93)	28 (2)	22 (2)	44 (16–72)	97 (95–99)	15.8	0.58	27.3	0.05
110	26 (2)	1049 (93)	29 (3)	21 (2)	42 (14–70)	98 (96–100)	17.4	0.59	29.2	0.05
120	20 (2)	1055 (93)	33 (3)	17 (2)	34 (7–61)	98 (96–100)	18.3	0.67	27.2	0.05

Table S3. Diagnostic yield of the ARR at specified cut-offs in the exploratory dataset.

ARR, aldosterone-renin ratio expressed in (ng/dl)/(ng/ml/h); FN, false negative; FP, false positive; TN, true negative; TP, true positive; DOR, diagnostic odds ratio; ER, error rate; NLR, negative likelihood ratio; PLR, positive likelihood ratio; NA, not available; Sens, sensitivity; Spec, specificity.

Method*	n	FP n (%)	TN N (%)	FN n (%)	TP n (%)
Exploratory dataset (n = 42) patients)					
1	42	17 (40)	5 (12)	2 (5)	18 (43)
2	42	22 (52)	0	0	20 (48)
Validation dataset (n = 10 patients)					
1	10	0	0	0	10 (100)
2	10	0	0	0	10 (100)

Table S4. Diagnostic yield of the captopril challenge test in the exploratory and the validation dataset in patients with ARR exceeding the predefined cut-off (115.4) corresponding to a 2% false positive rate in the exploratory dataset.

* Method 1: test positive if post-captopril ARR ≥ 30 ; Method 2: test positive if post captopril PAC decrease $\leq 30\%$ from baseline PAC value.

ARR, aldosterone-renin ratio; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

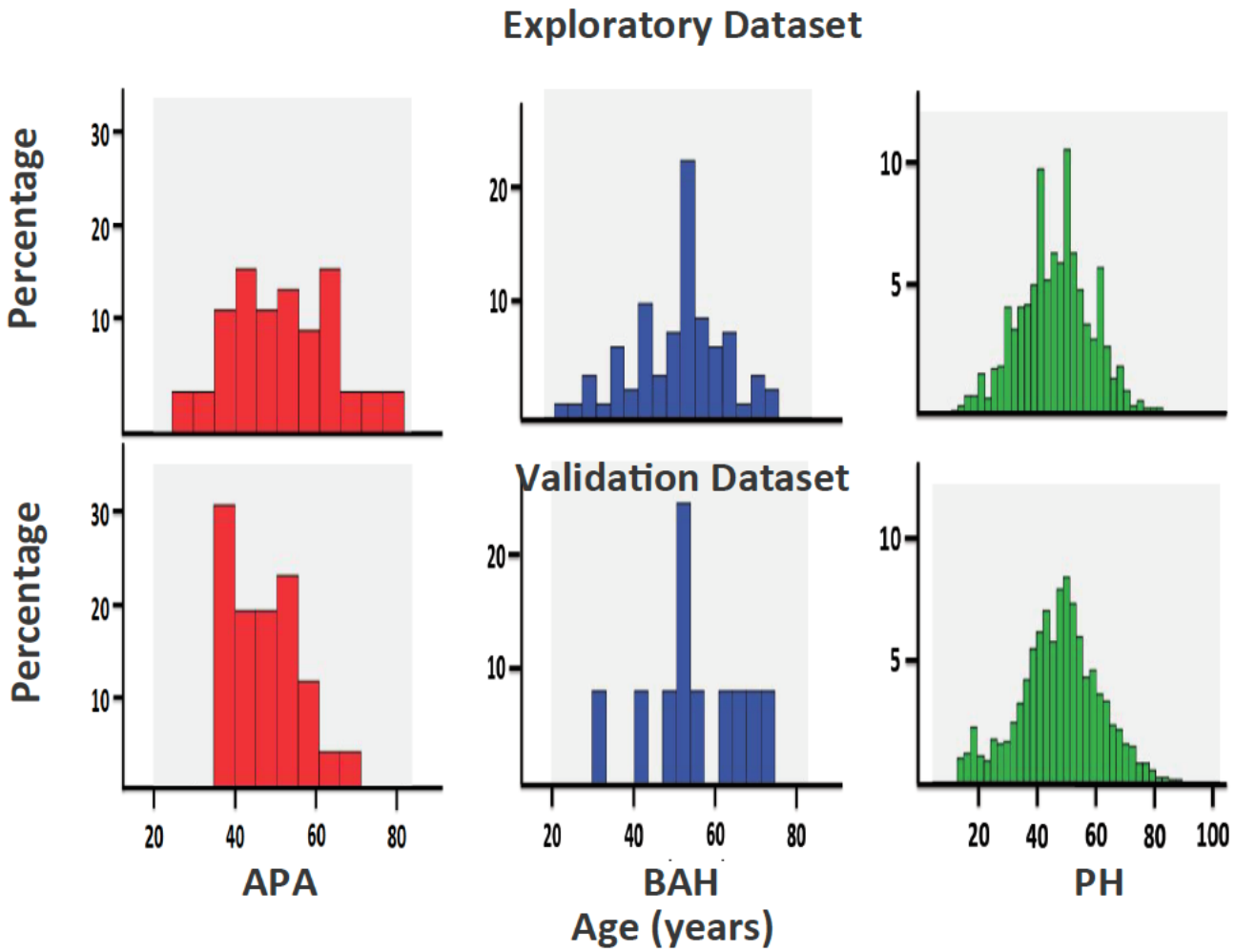


Figure S1. Age distribution in the aldosterone-producing adenoma (APA), idiopathic hyperaldosteronism (IHA), essential hypertension (EH) subgroups in the exploratory and validation datasets.

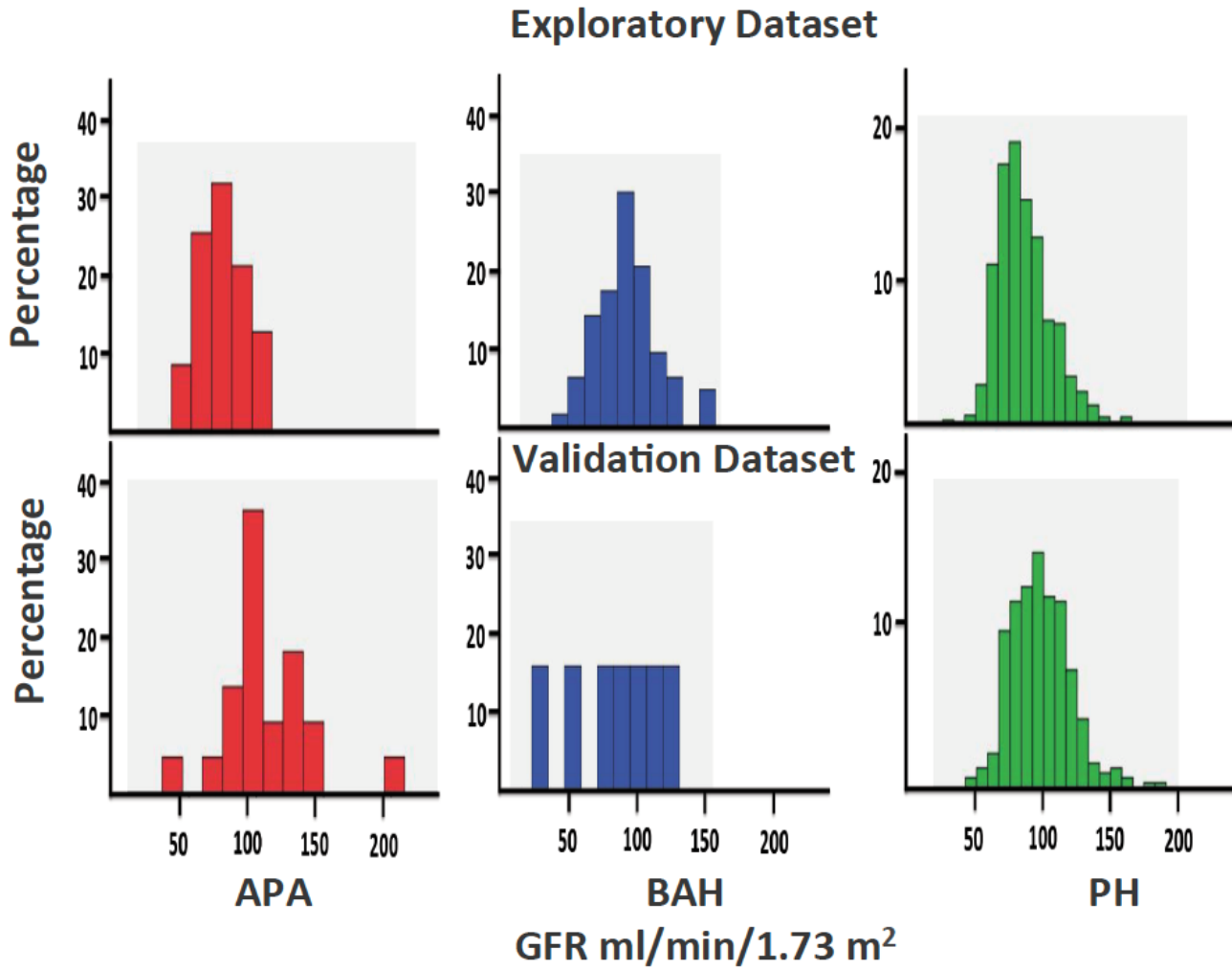


Figure S2. Glomerular filtration rate (GFR) distribution in the aldosterone-producing adenoma (APA), idiopathic hyperaldosteronism (IHA), essential hypertension (EH) subgroups in the exploratory and validation datasets.

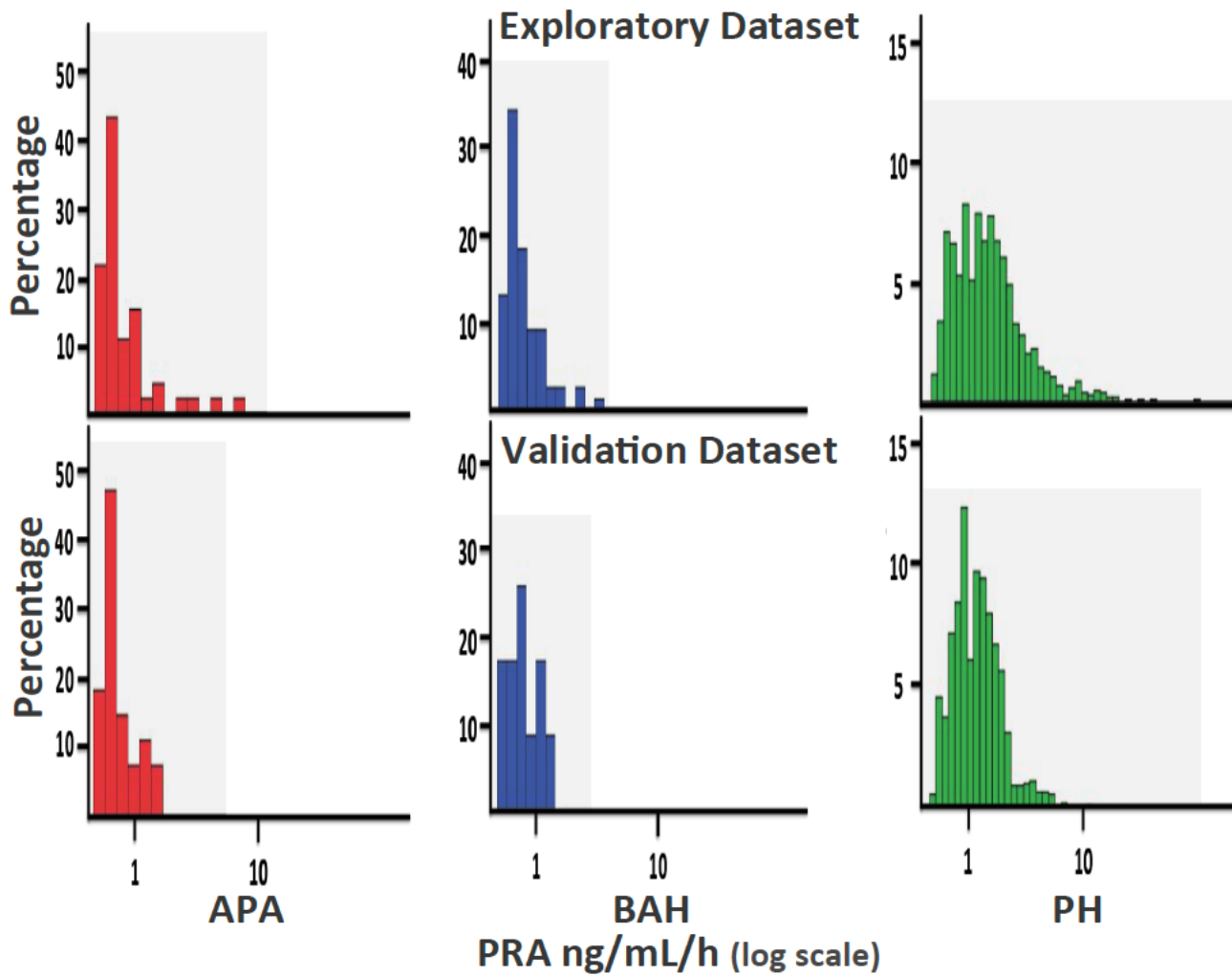


Figure S3. Plasma renin activity (PRA) distribution in the aldosterone-producing adenoma (APA), idiopathic hyperaldosteronism (IHA), essential hypertension (EH) subgroups in the exploratory and validation datasets.

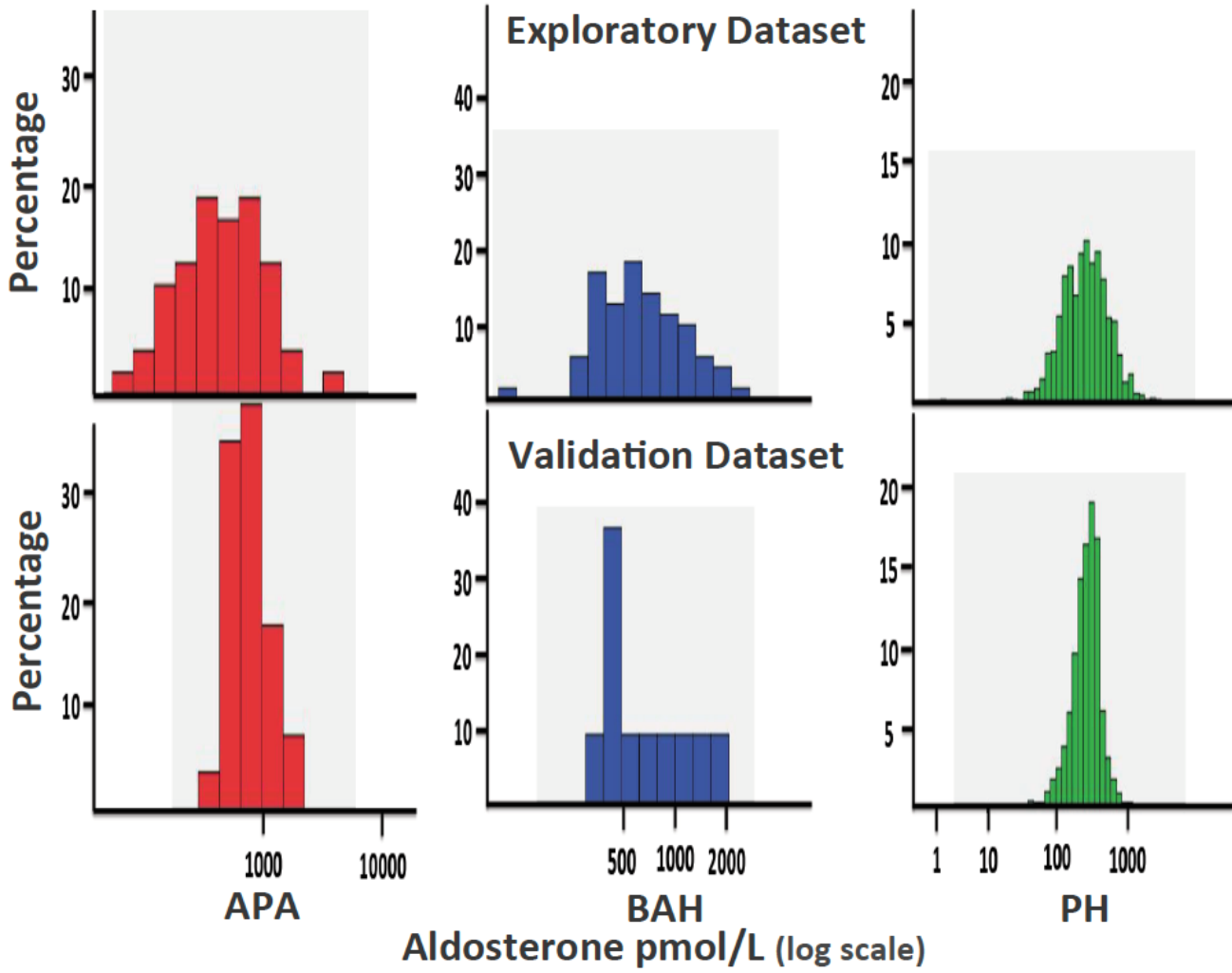


Figure S4. Plasma aldosterone distribution in the aldosterone-producing adenoma (APA), idiopathic hyperaldosteronism (IHA), essential hypertension (EH) subgroups in the exploratory and validation datasets.

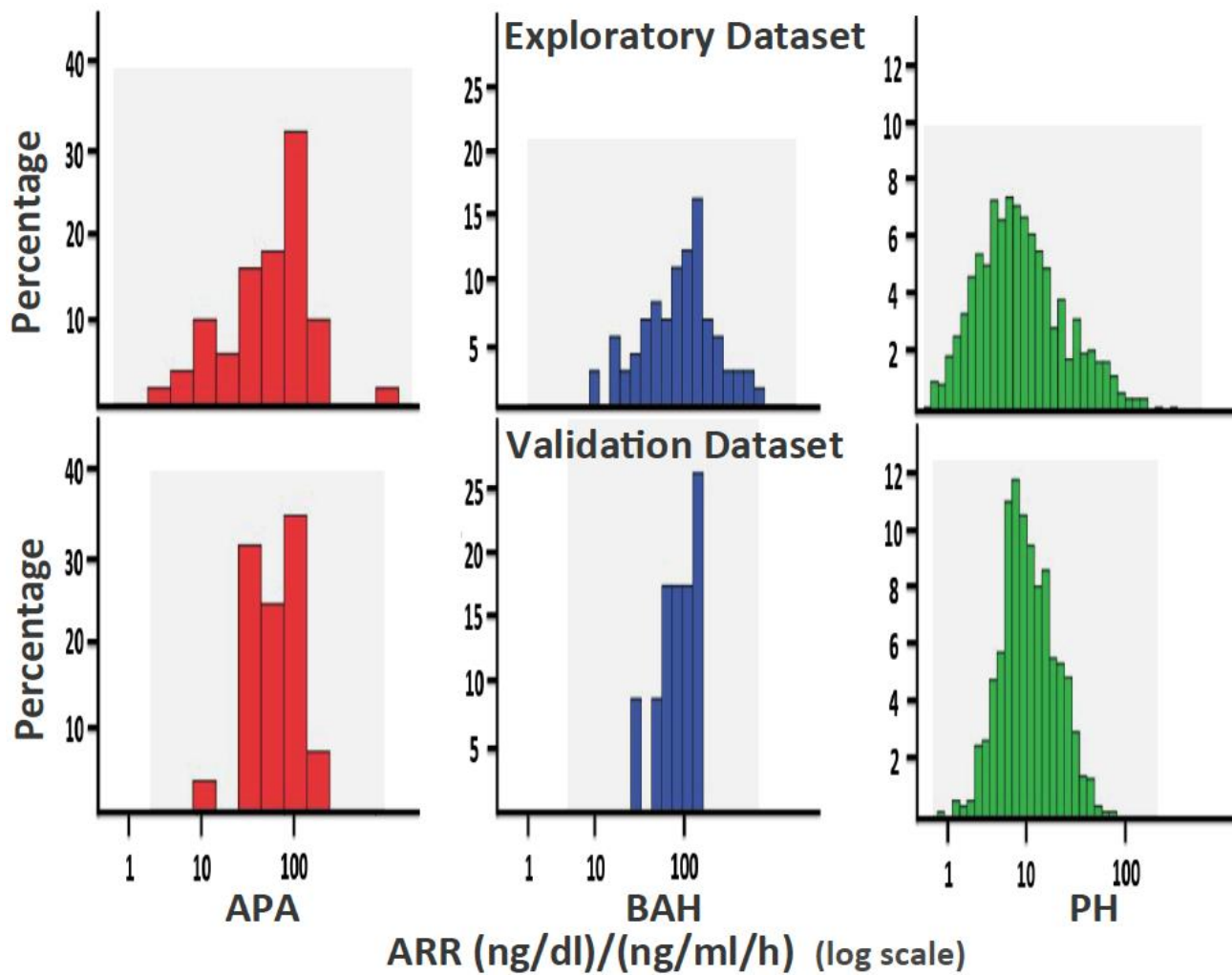


Figure S5. Aldosterone-renin ratio (ARR) distribution in the aldosterone-producing adenoma (APA), idiopathic hyperaldosteronism (IHA), essential hypertension (EH) subgroups in the exploratory and validation datasets.

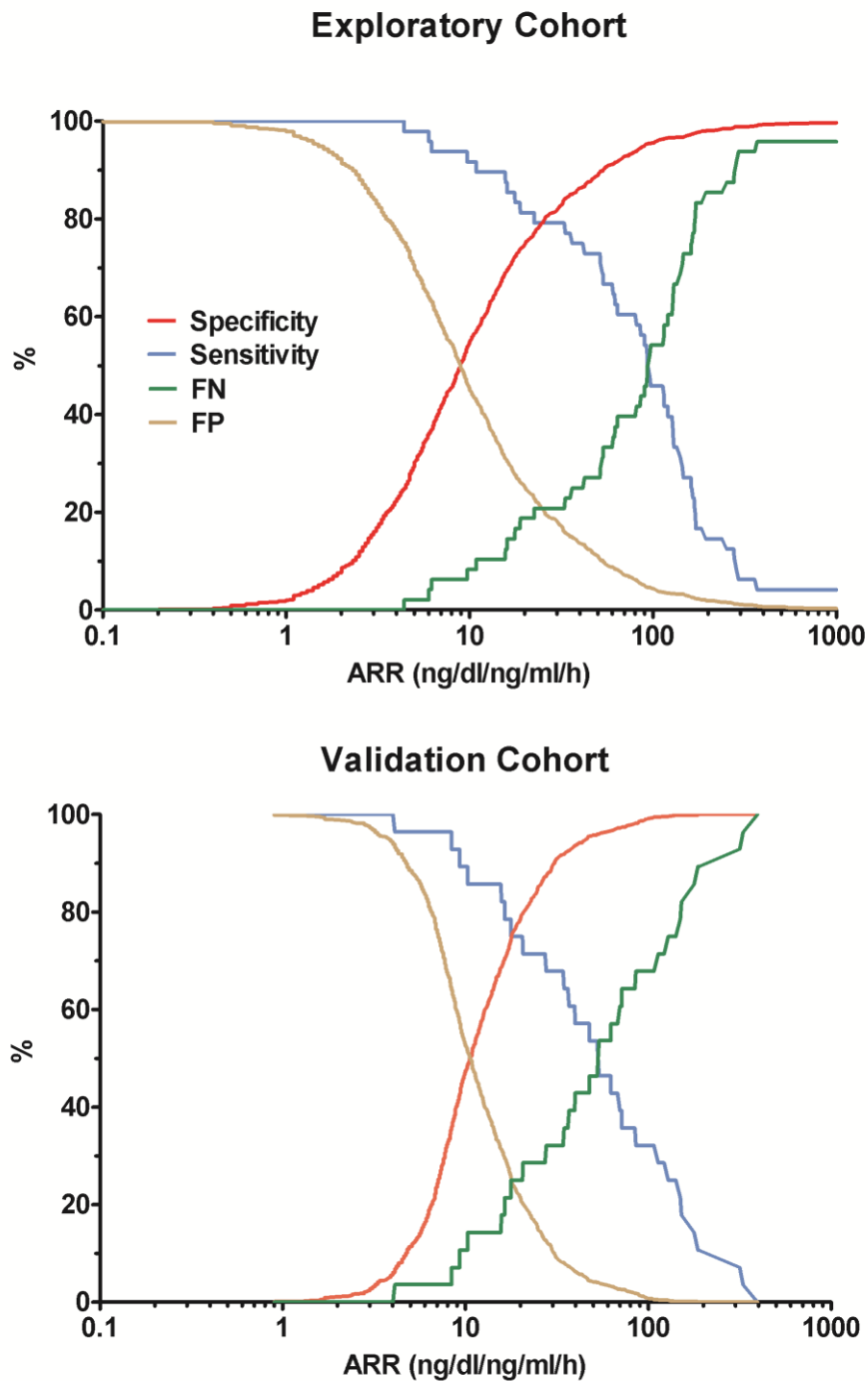


Figure S6. Diagnostic yield by increasing aldosterone-renin ratio (ARR) values. The plot shows the increase of specificity and false negative (FN) rate for identification of aldosterone-producing adenoma (APA) patients and the decrease of sensitivity and false positive (FP) rate with increasing and decreasing ARR values, respectively, in the exploratory (Upper Panel) and validation (Lower Panel) datasets. Please note that ARR was computed using the minimum cutoff for PRA of 0.10 ng/mL/hr.