Original Article

The subtyping of primary aldosteronism by adrenal vein sampling: sequential blood sampling causes factitious lateralization

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Background: The pulsatile secretion of adrenocortical hormones and a stress reaction occurring when starting adrenal vein sampling (AVS) can affect the selectivity and also the assessment of lateralization when sequential blood sampling is used. We therefore tested the hypothesis that a simulated sequential blood sampling could decrease the diagnostic accuracy of lateralization index for identification of aldosterone-producing adenoma (APA), as compared with bilaterally simultaneous AVS.

Methods and results: In 138 consecutive patients who underwent subtyping of primary aldosteronism, we compared the results obtained simultaneously bilaterally when starting AVS (*t*-15) and 15 min after (*t*0), with those gained with a simulated sequential right-to-left AVS technique (R ⇒ L) created by combining hormonal values obtained at *t*-15 and at *t*0. The concordance between simultaneously obtained values at *t*-15 and *t*0, and between simultaneously obtained values and values gained with a sequential R ⇒ L technique, was also assessed. We found a marked interindividual variability of lateralization index values in the patients with bilaterally selective AVS at both time point. However, overall the lateralization index simultaneously determined at *t*0 provided a more accurate identification of APA than the simulated sequential lateralization index$_{R \Rightarrow L}$ ($P = 0.001$). Moreover, regardless of which side was sampled first, the sequential AVS technique induced a sequence-dependent overestimation of lateralization index. While in APA patients the concordance between simultaneous AVS at *t*0 and *t*-15 and between simultaneous *t*0 and sequential technique was moderate-to-good ($K = 0.55$ and 0.66, respectively), in non-APA patients, it was poor ($K = 0.12$ and 0.13, respectively).

Conclusion: Sequential AVS generates factitious between-sides gradients, which lower its diagnostic accuracy, likely because of the stress reaction arising upon starting AVS.

Keywords: adrenal vein sampling, aldosterone, hypertension, lateralization, sequential, simultaneous

Abbreviations: 95% CI, 95% confidence interval; ACTH, adrenocorticotropic hormone; APA, aldosterone-producing adenoma; AUC, area under the curve; AVS, adrenal vein sampling; BP, blood pressure; CT, computed tomography; IVC, inferior vena cava; *K*, Cohen's kappa; L ⇒ R, sequential 'first left, second right' technique; LI, lateralization index; PA, primary aldosteronism; PAC, plasma aldosterone concentration; PCC, plasma cortisol concentration; PRA, plasma renin activity; R ⇒ L, sequential 'first right, second left' technique; RASI, relative aldosterone secretion index; ROC, receiving operator characteristics; SI, selectivity index

INTRODUCTION

In patients with primary aldosteronism, a common curable cause of arterial hypertension [1], the choice between surgical and medical treatment depends on adrenal vein sampling (AVS) results. AVS is recommended by the current Endocrine Society Practice Guidelines [1], because imaging is inadequate [2,3], as recently re-emphasized [4], and functional imaging with ^{11}C-metamidate PET–computed tomography (CT) [5] is not widely available. However, a recent randomized study underlined the need for improvements of currently uses to AVS procedures by showing that either a CT-based or a strategy based on synthetic adrenocorticotropic hormone (ACTH) 1–24 (cosyntropin)-stimulated AVS furnished equally bad results [6,7], likely because of the confounding effect of cosyntropin.

The key role of AVS for primary aldosteronism subtyping is underscored by the observation that, when systematically used, it identifies about two-thirds of all primary aldosteronism cases as being surgically curable [8]. However, AVS

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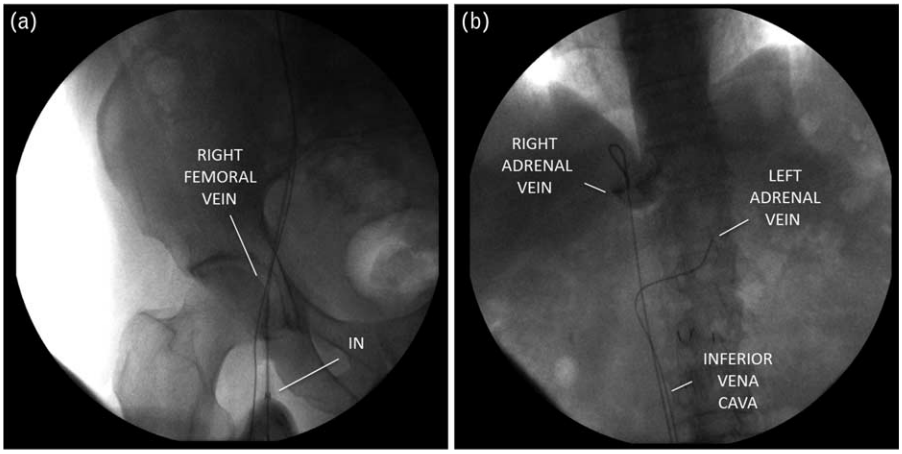

FIGURE 1 Simultaneous bilateral adrenal vein sampling. Panel (a) insertion of the introducer in the right femoral vein; the second introducer (IN) is inserted slightly more cranially and medially of the first introducer, under fluoroscopy guide. Panel (b) two differently shaped catheters are shown in the place for simultaneous sampling of the right and left adrenal vein (Simmons 1 and Simmons 2/3, respectively).

is technically demanding; moreover, unresolved issues concerning its performance and interpretation exist, as shown by the Adrenal Vein Sampling International Study [9] and highlighted in an expert consensus statement [10]. The blood sampling protocol is one such issues: major referral centers worldwide are split between use of the bilaterally simultaneous technique and the sequential technique [9]. The latter commonly implies stimulation with cosyntropin and catheterization of one side, usually the right, followed by that of the left, which is held to be technically simpler [1]. Cosyntropin increases steroids secretion, and thus the selectivity; furthermore, it minimizes the adverse impact of pulsatile secretion and time delay [10]. However, it did not increase the relative rate of adrenal production of aldosterone in a study that measured multiple steroids with liquid chromatography and tandem mass spectrometry [11]. Moreover, the expression of the ACTH receptors (melanocortin 2 receptor, MC2R) in the gland with aldosterone-producing adenoma (APA) is lower than contralaterally in a substantial proportion of cases, particularly those with the less florid clinical phenotype, which explains why cosyntropin confounds the assessment of lateralization in up to 30% of the cases because of the blunted aldosterone response of that gland [12–17].

The majority of the patients submitted to AVS experience a stress reaction, which raises cortisol secretion, and thus the selectivity index bilaterally [18]. Although swift, this reaction might affect the assessment of lateralization when the sequential AVS technique is adopted, a hypothesis that has never been examined so far. We therefore set out to evaluate if the timing of AVS affects the diagnostic accuracy of unstimulated AVS, and if the sequential and the bilaterally simultaneous technique performs equally or not.

METHODS

An extended version is available as online Supplemental material, http://links.lww.com/HJH/A851.

Study population

We prospectively recruited consecutive primary aldosteronism patients referred to our Specialized Centre for Hypertension for suspected primary aldosteronism from 2007 to 2015, who met the current guidelines indications for AVS [19]. Criteria for primary aldosteronism diagnosis and patient preparation have already been reported [8,20]. Diagnosis of APA was made according to the 'modified four-corners' criteria' upon confirmation of cure/improvement of hypertension and correction of the biochemical picture of primary aldosteronism after adrenalectomy, as described [8,20].

Exclusion criteria for the main analysis entailed refusal/lack of adrenalectomy; missing follow-up data at 1 and 6 months postsurgery; non-bilaterally selective AVS at *t*-15 or *t*0; need for potentially confounding drugs at AVS, because of allergy to the contrast medium, and/or drug-resistant hypertension without evidence of renin suppression [1].

All procedures followed the principles of the Declaration of Helsinki and the institutional guidelines; written consent was obtained from all participants.

Adrenal vein sampling procedure

AVS was performed between 0800 and 1200 h using bilaterally simultaneous blood collection with two catheters, shaped for each adrenal vein (Fig. 1 and Supplemental material, http://links.lww.com/HJH/A851) [21]. Briefly, we use two sheaths under US guidance: one 5F for the right adrenal vein catheter and the other 6F for the left adrenal vein. Due to its larger caliber, the latter also allows for blood sampling from the femoral vein [equivalent to the inferior vena cava (IVC)] simultaneous to the AVS. Blood samples for the measurements of plasma aldosterone concentration and cortisol concentration (PCC) were obtained simultaneously after visual confirmation of adequate bilateral cannulation (*t*-15) from the adrenal veins and the infrarenal IVC, and again 15 min later (*t*0). To avoid thrombosis, the right catheter was withdrawn from the adrenal vein after the *t*-15 blood sampling and was repositioned just before *t*0.

Indexes definitions and simulation of the sequential technique

The selectivity index, lateralization index, and relative aldosterone secretion index (RASI) were calculated as described [12,20] (refer to Supplemental Table 1, http://links.lww.com/HJH/A851). For diagnosis, the values derived at *t*0 were used. For the purpose of data analysis, the side with the higher RASI value at each time point was identified as 'dominant'. Following a consensus of experts [10] and guidelines [1,19], a cutoff of 2.00 was used for the selectivity index and the lateralization index to define selectivity and lateralization, and therefore the indication to adrenalectomy.

As cannulation usually is more difficult on the right than on the left side, the sequence 'first Right second Left' AVS technique (R $\Rightarrow$ L) is usually exploited in clinical practice to minimize the time lag between blood collections when the sequential technique is used. To simulate a sequential R $\Rightarrow$ L AVS technique, we combined the hormonal values of the right side at *t*-15 with those of the left side at *t*0, corrected for those simultaneously collected in the IVC. A 'first Left, then Right' approach (L $\Rightarrow$ R), as a sensitivity analysis, was also simulated. These sequential techniques were only simulated at the desk based on already available data and therefore did not involve any procedures/interventions beyond those already used for routine diagnostic AVS studies and approved by our NRC (Nucleo di Ricerca Clinica).

Statistical analysis

Results are expressed as mean ± SD, or median and interquartile range, as appropriate. Continuous variables were tested for normal distribution with Kolmogorov–Smirnov test. Parametric and nonparametric statistics were used for log-transformed data and variables with a skewed distribution, respectively. Within-patient comparison of paired *t* samples test was used in the whole cohort and in the APA and non-APA subcohorts; Pearson's χ^2 test was used for categorical variables.

The concordance between log-transformed indexes derived with compared strategies (simultaneous sampling at *t*-15 vs. *t*0; simulated sequential sampling vs. simultaneous at *t*0) was examined by calculating the concordance correlation coefficient ($\rho_c = \rho\ C_b$), an estimate of the degree to which pairs of observations fall on the identity line. ρ is the Pearson correlation coefficient, measuring precision, and C_b a bias correction factor that measures how far the best-fit line deviates from the identity line, and thus estimates accuracy. A Bland–Altman plot was used to detect systematic biases. The value of Cohen's kappa (*K*) was used to evaluate the diagnostic agreement between strategies. The latter was graded as poor ($K < 0.20$), fair (*K* between 0.21 and 0.40), moderate (*K* between 0.41 and 0.60), good (*K* between 0.61 and 0.80), and very good (*K* between 0.81 and 1.00).

The area under the curve (AUC) the receiver-operating characteristic (ROC) was used to estimate accuracy of the lateralization index with each sampling technique, using the diagnosis of APA as reference. Significance was set at *P* less than 0.05. MedCalc (MedCalc Software, version 15.8; Ostend, Belgium) and SPSS (version 24 for Mac; SPSS, Bologna, Italy) were used for data analysis.

RESULTS

Safety of bilaterally simultaneous adrenal vein sampling

The current analysis involved 138 primary aldosteronism patients (mean age 51.7 years, 38% women) who underwent bilateral simultaneous AVS. No major complications were seen. Moreover, with adequate hemostatic compression, no complications at the site of venipuncture were observed after removal of the two sheaths needed for catheterization.

Simultaneous adrenal vein sampling: impact of timing on selectivity index, relative aldosterone secretion index, and lateralization index

PCC was higher in both adrenal glands blood when starting AVS (*t*-15) than at *t*0 (not shown); thus, the selectivity index value fell from *t*-15 to *t*0 on both sides ($P < 0.001$; Suppl. Fig. 1, http://links.lww.com/HJH/A851). Even though the rate of bilaterally selective AVS did not change significantly, a trend toward a decrease was noted on the left side (Supplemental Table 2, http://links.lww.com/HJH/A851).

As bilateral selectivity is a prerequisite for the assessment of lateralization [10], the downstream analysis performed to evaluate the impact of timing of blood sampling on aldosterone secretion and lateralization considered only the 66 patients with bilaterally selective results at both

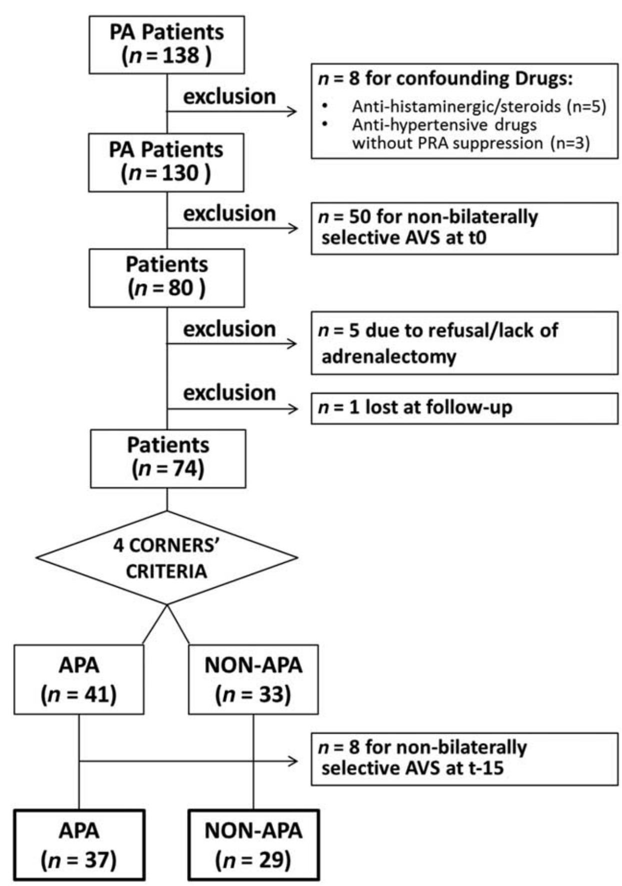

FIGURE 2 Flowchart of the study.

TABLE 1. Demographic and clinical features of the patients and diagnostic indexes

	Whole cohort	APA, $n=37$		Non-APA, $n=29$
Variable	Baseline	Baseline	Postadrenalectomy	Baseline
Age (years)	50 ± 11	49 ± 11	–	51 ± 11
Sex (F%)	32%	37%	–	24%
BMI (kg/m^2)	27.2 (24.3–30.6)	26.9 (24.2–31.0)	26.0 (24.4–29.1)	27.8 (24.3–30.6)
SBP (mmHg)	157 ± 21	159 ± 16	$126 \pm 09^{***}$	154 ± 28
DBP (mmHg)	97 ± 12	98 ± 12	$80 \pm 7^{***}$	96 ± 14
Treatment score (*n* of drugs)	2.5 ± 1.5	2.6 ± 1.7	$0.7 \pm 1.1^{***}$	2.3 ± 1.2
Serum K$^+$ (mmol/l)	3.6 ± 0.4	3.6 ± 0.4	$4.3 \pm 0.4^{***}$	3.7 ± 0.4
U-Na$^+$ excretion (mEq/24 h)	177 ± 78	166 ± 81	162 ± 52	192 ± 74
PRA (ng/ml/h)	0.32 (0.14–0.64)	0.21 (0.10–0.50)	1.00 (0.49–1.40)***	0.50 (0.34–0.80)*
PAC (ng/dl)	19.5 (15.2–27.8)	21.5 (15.4–28.2)	8.2 (4.3–10.2)***	17.7 (13.3–22.3)**
ARR (ng/dl)/(ng/ml/h)	68.7 (34.8–88.8)	70.6 (50.4–103.9)	8.4 (4.3–17.8)***	35.8 (22.1–77.6)**
APA diameter (mm)	–	12 ± 6	–	–
Hypertension cure (%)	–	58%	–	–

Indexes	APA *t*-15′	APA *t*0	Non-APA *t*-15′	Non-APA *t*0
RASI				
Dominant side	3.68 (1.56–6.76)	3.56 (1.33–8.15)	2.14 (0.98–3.67)	2.00 (1.34–4.52)
Nondominant side	0.46 (0.20–1.00)	0.46 (0.21–0.84)	1.05 (0.59–1.96)	1.65 (0.85–3.47)****
LI	8.04 (2.55–18.47)	6.54 (2.85–13.67)	1.63 (1.12–3.15)	1.28 (1.12–1.75)****

For ARR calculation, the minimum value of PRA was set at 0.20. Data presented as mean ± SD or median (IQR), as appropriate. APA, aldosterone-producing adenoma; ARR, aldosterone–renin ratio; AVS, adrenal vein sampling; LI, lateralization index; NA, not applicable; PAC, plasma aldosterone concentration; PRA, plasma renin activity; RASI, relative aldosterone secretion index; Serum K$^+$, kalemia at AVS.
$^{*}P<0.05$ vs. APA baseline.
$^{**}P<0.01$ vs. APA baseline.
$^{***}P<0.005$ vs. APA baseline.
$^{****}P<0.005$ vs. *t*-15′.

t-15 and *t*0 (Fig. 2). Of these, 37 patients were conclusively diagnosed as APA by the 'modified 4 corners criteria': after adrenalectomy, primary aldosteronism was biochemically cured in all of them; the high blood pressure (BP) was cured in 58% and showed a substantial decrease of BP and/or need for antihypertensive drugs to achieve normal BP in the remaining 42% of the patients. Table 1 shows the clinical and biochemical features of the patients included in the main analysis, their RASI values for the dominant and nondominant side, and their lateralization index values derived at *t*-15 and *t*0.

In the APA patients, the RASI showed no significant differences between *t*-15 and *t*0; accordingly, the lateralization index did not change significantly between *t*-15 (lateralization index$_{t\text{-}15}$) and *t*0 regardless of the side (lateralization index$_{t0}$; Table 1). However, there were highly variable individual responses, which resulted in a low concordance between *t*-15 (lateralization index$_{t\text{-}15}$) and *t*0 [correlation coefficient, ρ_c (95% confidence interval (95% CI)) $= 0.55$ (0.29–0.74); $\rho = 0.56$; $C_b = 0.98$; Fig. 3a].

In the non-APA patients, the RASI did not change significantly between *t*-15 and *t*0 in the dominant side. However, in the nondominant side, it was lower at *t*-15 than at *t*0 (Table 1; Fig. 3b), likely because the stress-induced stimulation of cortisol secretion exceeded that aldosterone at the beginning of AVS. Therefore, in these patients, the concordance between *t*-15 and *t*0 was lower than in the APA patients; moreover, there was an overestimation of lateralization index at *t*-15 compared with *t*0 [$\rho_c = 0.10$ (-0.14 to 0.33); $\rho = 0.160$; $C_b = 0.63$; Fig. 3b]. This systematic bias was by no means due to repositioning of the catheter in the right adrenal vein, as it was unrelated to the selectivity index value measured at any time or side (Supplemental Fig. 2, http://links.lww.com/HJH/A851). The Bland–Altman plot (Fig. 4) unveiled that it mainly affected the lateralization index$_{t0}$ values between 1.00 and 2.00, which are common in non-APA patients.

Impact of timing on diagnostic accuracy

Simultaneous samplings at *t*-15 and *t*0 showed a moderate diagnostic agreement in APA patients [K (95% CI) $= 0.55$ (0.35–0.76)]. However, no misdiagnosis of lateralization occurred using the lateralization index$_{t\text{-}15}$. In non-APA patients, the diagnostic agreement was poor [$K = 0.12$ (-0.21 to 0.46)] and the lateralization index$_{t\text{-}15}$ was overestimated compared with lateralization index$_{t0}$, which led to an excess of factitious diagnoses of lateralization (Fig. 3b). Accordingly, the diagnostic accuracy of lateralization index$_{t\text{-}15}$ for identification of APA was lower than of lateralization index$_{t0}$, albeit borderline significantly so ($P = 0.062$ for comparison of ROC curve AUC; Fig. 5, panel a).

Sequential adrenal vein sampling

In APA patients, lateralization index derived with an $R \Rightarrow L$ sequence (lateralization index$_{R \Rightarrow L}$) did not differ significantly from those derived simultaneously at *t*0 (lateralization index$_{t0}$). The concordance correlation coefficient showed no systematic bias [$\rho_c = 0.67$ (0.46–0.81); $\rho = 0.69$; $C_b = 0.98$; Supplemental Fig. 3, http://links.lww.com/HJH/A851]. In non-APA patients, the lateralization index$_{R \Rightarrow L}$ was systematically higher than the simultaneously determined lateralization index$_{t0}$ [$\rho_c = 0.12$ (-0.06 to 0.29); $\rho = 0.25$; $C_b = 0.48$; Supplemental Fig. 3, http://links.lww.com/HJH/A851], resulting in a relevant number of patients factitiously diagnosed as lateralized.

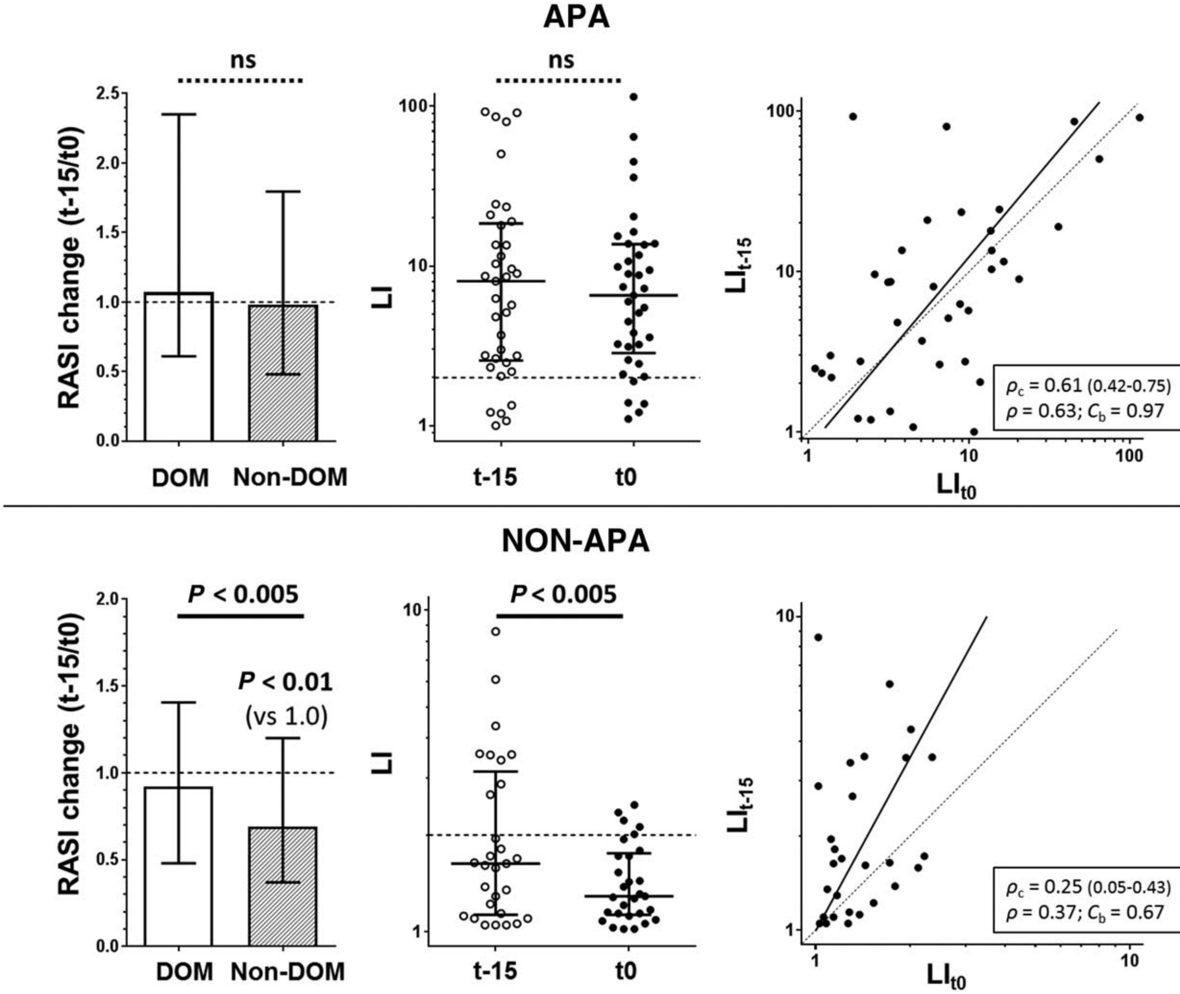

FIGURE 3 Impact of time delay on relative aldosterone secretion index (RASI) and lateralization index (LI) and concordance correlation coefficient between LI simultaneous samplings (left, mid, and right panels). In APA patients (upper panels), relative aldosterone secretion index values of both dominant and nondominant side (left panel), and therefore the lateralization index (mid panel) did not change between *t*-15 and *t*0, but a high variability, resulting in moderate concordance correlation coefficient (ρ_c), was observed, likely as a result of a stress reaction to starting adrenal vein sampling. C_b value points to no systematic bias (right panel). In non-APA patients (lower panels), relative aldosterone secretion index was lower at *t*-15 than at *t*0 in the nondominant side (left panel); therefore, the lateralization index fell from *t*-15 to *t*0 (mid panel), because of a systematic overestimation at *t*-15 (right panel). In the mid panels the: dashed line = lateralization $index_{cutoff}$ for lateralization; in the left panels the: solid lines = regression line, dotted lines = identity line.

Overall the diagnostic concordance was only moderate [68%; $K = 0.52$ (0.35–0.69)]; in fact, it was good (78%) for APA patients [$K = 0.66$ (0.46–0.86), Fig. 6, panel a], but poor (55%) for non-APA patients [$K = 0.13$ (−0.11 to 0.37); Fig. 6, panel b]. Of note, there was an excess of lateralization diagnoses to the left side with the R ⇒ L compared with the simultaneous technique at *t*0 (Fig. 6, panel b).

To gather further insight on this finding, we simulated a 'first Left, second Right' (L ⇒ R) sequence, which resulted into an excess of lateralization diagnoses to the right side (χ^2 test: $P < 0.05$; Fig. 6, panel d) and a poor (57%) diagnostic concordance [$K = 0.16$ (−0.07 to 0.38)]. This finding indicates occurrence of a side-dependent bias pointing to lateralization in the last sampled side. This bias added up to the aforementioned overestimation bias observed at *t*-15 with simultaneous sampling (slope of fitting line). Noteworthy, the worse accuracy ($P = 0.001$) for APA identification of the lateralization $index_{R \Rightarrow L}$ and lateralization $index_{L \Rightarrow R}$, (*not shown*) compared with lateralization $index_{t0}$ was confirmed with ROC curve analysis (Fig. 5, panel b).

DISCUSSION

Considering the key role of AVS for allocating patients with primary aldosteronism to treatment, alongside the poor performance of cosyntropin-stimulated AVS [6], efforts for optimizing the diagnostic accuracy of AVS are worth and urgent. We herein provide evidence that the timing of blood sampling has a clinically relevant impact on both the selectivity index and the lateralization index value. Likely because of a stress reaction, the former is higher when starting the procedure than 15 min afterward, and this occurs bilaterally (Supplemental Fig. 1, http://links.lww.com/HJH/A851), thus confirming results obtained in a smaller proof-of-principle study [18]. Apart from being pulsatile, because of this stress response, aldosterone secretion was higher at starting AVS. Furthermore, in non-APA patients, the increased aldosterone release occurred differently in the two adrenals, and was much less than that of cortisol, particularly in the nondominant side, leading to a decrease of the RASI values of this side. Notwithstanding a

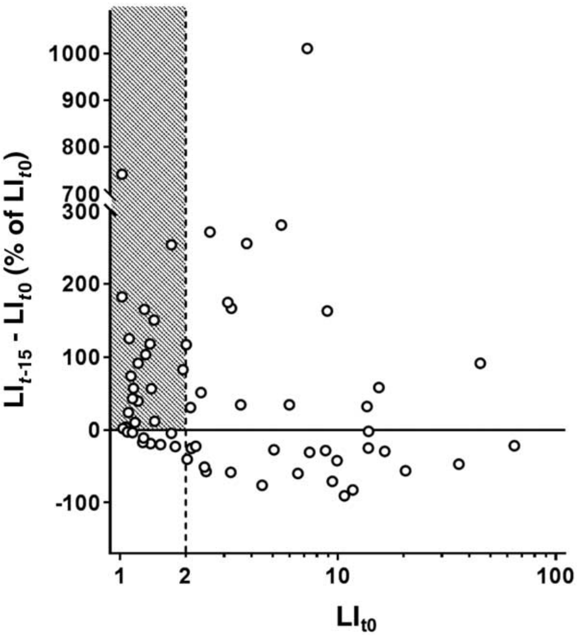

FIGURE 4 The Bland–Altman plot for lateralization index with simultaneous samplings at *t*15 and *t*0, calculated as difference of lateralization index$_{t\text{-}15}$ and lateralization index$_{t0}$, which is expressed as percent of LI$_{t0}$, plotted against lateralization index$_{t0}$. A systematic overestimation, which is clinically relevant in affecting final diagnosis, is evident for patients with lower lateralization index$_{t0}$ values and particularly for those with lateralization index$_{t0}$ below the cutoff for lateralization (2.00, vertical dashed line), which are those typically seen in non-APA. Solid line = identity.

large interindividual variability of hormones response to stress and the known molecular heterogeneity of APA [13,17], this phenomenon marginally affected the lateralization index in the APA patients with a florid unilateral aldosterone over-secretion, who usually exhibit contralateral suppression. However, it deeply affects lateralization in non-APA patients, as shown by the factitious increase of the lateralization index$_{t\text{-}15}$ seen in these patients: the overestimation of lateralization index at *t*-15 caused diagnostic accuracy to drop (Fig. 5). Thus, the timing of blood sampling critically affects the ascertainment of lateralized aldosterone excess, for example the accuracy of AVS.

Because of this time effect, we compared the performance of AVS between the bilaterally simultaneous and a sequential technique by simulating a real-life practice procedure. Unsurprisingly, we found that bilaterally simultaneous AVS outperformed sequential AVS for identification of APA, regardless of which approach was used, for example, 'right first, left second' or *vice versa*. In fact, the simulated sequential approach led to an excess of lateralization diagnoses to the last sampled side, regardless of it being the right or the left.

Investigation of the optimal lateralization index cutoff value for the identification of APA with bilaterally simultaneous and sequential AVS also showed some novel important information: with the former technique the optimal lateralization index cutoff was about two-fold lower than with the latter (2.48 vs. 5.11); at both cutoffs, the bilaterally simultaneous technique surpassed the sequential technique (sensitivity 78 vs. 62%; specificity 100 vs. 93%) (Fig. 5). This means that about 16% of the patients identified as APA with the bilaterally simultaneous AVS would be denied curative adrenalectomy if the sequential technique were used at its optimal cutoff. We would like to contend that the effect of timing on AVS detected in this study likely explains the wide variation of lateralization index cutoff values reported thus far [9,22].

To date, only two studies compared bilaterally simultaneous and sequential AVS and failed to detect differences between the techniques [23,24]. However, one was not powered to provide conclusive evidence [23], whereas in the other a sequential technique was simulated based on only a 5-min delay between sides [24]. This time lag is more alike bilaterally simultaneous sampling than what done in

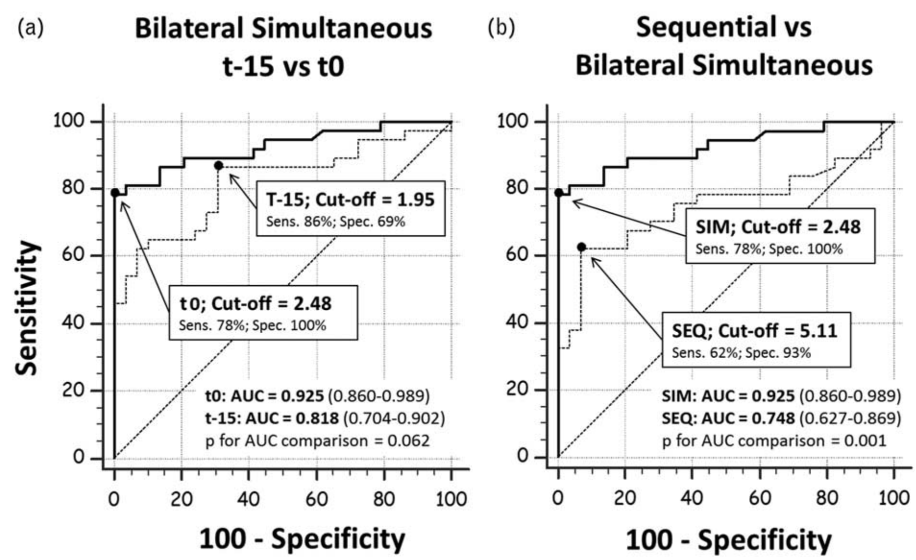

FIGURE 5 The receiver operator curve characteristics curves compare the bilateral simultaneous *t*-15 with *t*0 lateralization index (panel a) and sequential lateralization index$_{R \Rightarrow L}$ with simultaneous lateralization index$_{t0}$ (panel b) diagnostic performance, using a conclusive diagnosis of aldosterone-producing adenoma as reference. Lateralization index$_{t0}$ (solid line) provided a more accurate identification of aldosterone-producing adenoma compared with both lateralization index$_{t\text{-}15}$ and lateralization index$_{R \Rightarrow L}$ L [dotted line, panels (a) and (b), respectively]. Optimal cutoff values corresponding to the Youden index are denoted by closed circles.

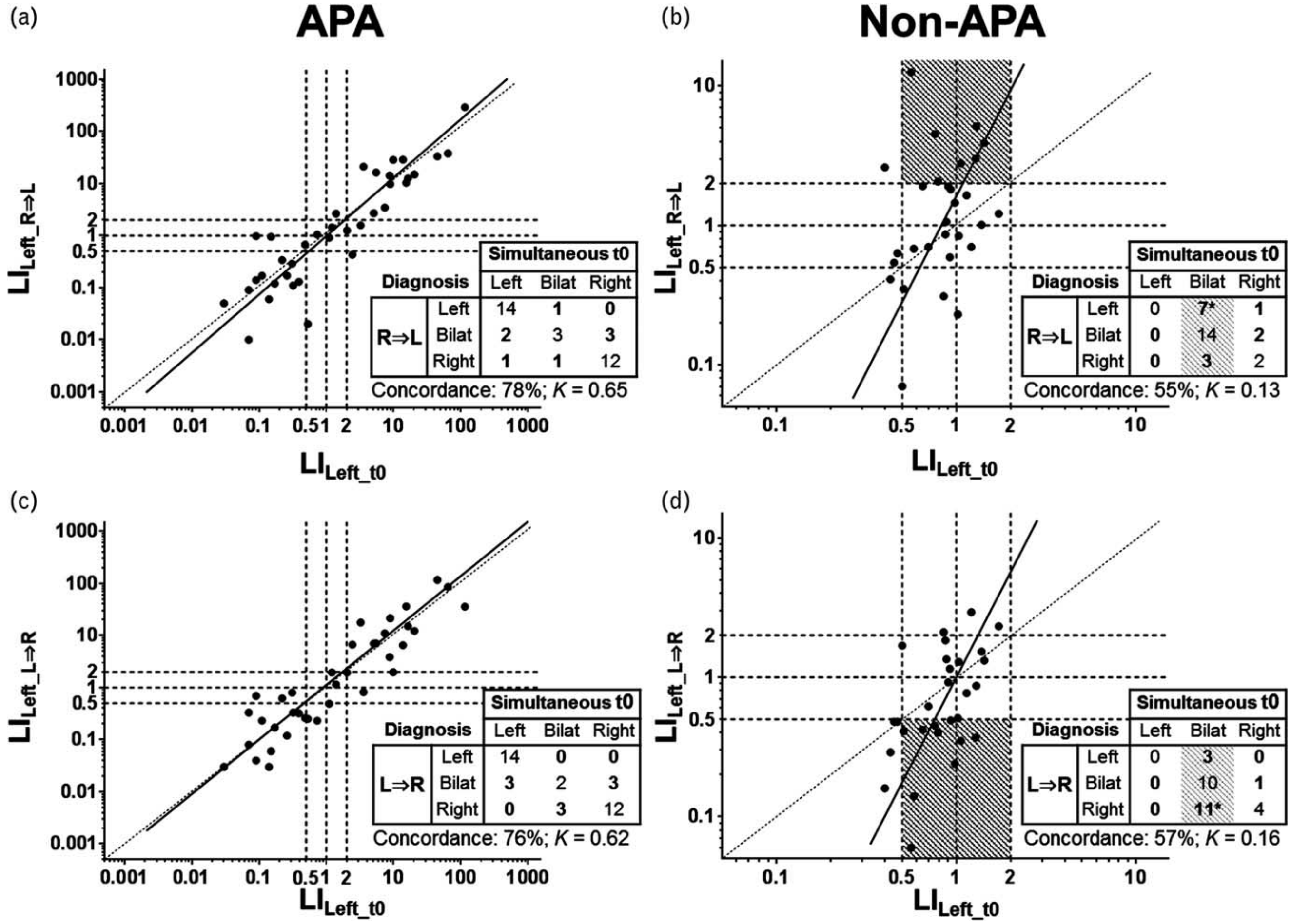

FIGURE 6 Diagnostic biases associated with the sequential techniques 'first right, second left' [R ⇒ L, panels (a) and (b)] and 'first left, second right' [L ⇒ R, panels (c) and (d)] compared with bilateral simultaneous adrenal vein sampling at *t*0. A good concordance was observed for both R ⇒ L and L ⇒ R techniques compared with *t*0 in APA patients [panels (a) and (c)]. At variance concordance was poor with either sequential technique in non-APA patients, as evidenced by deviation of the best-fit line from the identity line and a shift of lateralization diagnosis toward the last sampled side (shaded areas; *in tables; χ^2 test: $P < 0.05$). Vertical and horizontal dotted lines = lateralization index cutoff for lateralization diagnosis. Lateralization $index_{left}$ = relative aldosterone secretion $index_{left}$/relative aldosterone secretion $index_{right}$. Tables: allocation of cases to diagnosis, according to simultaneous *t*0 or R ⇒ L/L ⇒ R sequential technique; discordant diagnoses in bold.

clinical practice, in which blood collection requires longer, because it is exploited without aspiration to avoid adrenal vein collapse, for example, drop-by-drop. If everything goes smoothly, interventional radiologists can sample the last (left) side in less than 15 min once successful right adrenal vein catheterization has been achieved, but the rate of success in achieving this goal is variable and unpredictable: There are marked differences of training and expertise among radiologists [9] and even in experienced hands the time delay between sides sampling was reported to be '10-to-15 minutes' [22]. Moreover, the presence of anatomic adrenal vein variants, which can be seen in 15–20% [21], can prolong much the time for catheterization, particularly if super selective catheterization is performed [25]. In our hands, blood collection takes on average 2 min on the right side, thus leaving only 1 min to achieve cannulation of the left side and to sample the IVC. In less experienced hands, if differently shaped catheters (Fig. 1, panel b) are not used, and/or if there are anatomical variants, catheterization can take much longer, thus exposing to the effects of stress reaction resolution.

From the clinical standpoint, our results should discourage systematic use of the sequential blood sampling technique, whenever the time delay between the first and second AVS cannot be consistently kept within much less than 15 min. Moreover, they support the adoption of strategies to minimize stress [26], such as local anesthesia, benzodiazepines administration, and quiet rest for some minutes after the femoral vein access and before blood sampling [10]. On the contrary, these measures have not attained a wide acceptance thus far.

Limitations and strengths

Some limitations of this study need to be mentioned: first, even though we examined a relatively ample cohort, when our analysis was restricted to those with bilaterally selective AVS based on a selectivity index cutoff of 2.0 and who had outcome data the sample size was smaller. Second, we did not aim at comparing safety and costs of sequential and simultaneous AVS; thirds, our experimental design did not allow to break down the exact time when stress reaction resolves to any precise value less than 15 min. In the hands of skilled interventional radiologists, bilaterally simultaneous AVS was generally well tolerated with a complication rate similar to that reported for the sequential technique [10,28], and our experience supports this conclusion. Moreover, bilaterally simultaneous AVS was by no means more time consuming than the sequential technique, as blood collection takes place simultaneously; in addition, it was not more difficult or expensive to perform, as for optimal results two differently shaped catheters are needed to accommodate the different anatomy of the adrenal veins (refer to Fig. 1 and Supplemental extended methods, http://links.lww.com/HJH/A851). It might be argued that catheter's repositioning in the right adrenal vein might have introduced a bias; however, the observation that the diagnostic biases occurred on both sides, in a fashion that was totally independently on the selectivity index values, or on the sides-sequence chosen for sampling, speaks against this possibility. Finally, the factitious increase of the lateralization index value was seen in non-APA cases, the majority of which had no postadrenalectomy confirmation of the diagnosis. Hence, we cannot be totally sure that some had a less florid form of APA and/or might eventually develop a lateralizing phenotype with time. This contention seems, however, unlikely, and no proof for such natural history currently exists.

The following strengths need to be mentioned: a prospective within-patient study design, which minimized several potential confounders as, interindividual variability; the relatively large sample size; the state-of-the art criteria used to unambiguously diagnose APA, as confirmed at follow-up postadrenalectomy, as reference for determining accuracy [8,27].

In conclusion, nowadays the pathologies underlying surgically curable primary aldosteronism, for example, APA and micronodular unilateral hyperplasia [22,24], are detected when tiny, which means that they often escape detection with imaging with systematic screening of patients [1]. Hence, AVS is the cornerstone for deciding which patients should undergo unilateral adrenalectomy, as indicated by the guidelines. On the contrary, AVS performance is still suboptimal even at major referral centers, as recently shown [6].

We herein provide compelling evidence that a stress reaction occurring when starting AVS can confound interpretation of its results, and that a 15-min time delay between sampling each adrenal vein inflates the rate of non-APA patients falsely diagnosed as lateralized. Accordingly, a sequential approach was less accurate than a bilaterally simultaneous technique.

Overall, these results support generalized use of the bilaterally simultaneous technique under unstimulated conditions, and the implementation of measures aimed at preventing and/or minimizing the stress reaction triggered by AVS, particularly when the bilaterally simultaneous AVS is unfeasible. We propose that the latter should become the standard for current practice and for planning future prospective studies on use of AVS with biomarkers of selectivity more accurate than those currently used, that is, cortisol and aldosterone, as for example metanephrines [29], and other steroids that provide a better assessment of selectivity [11,30] as androstenedione [31].

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Conflicts of interest

There are no conflicts of interest.

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Reviewers' Summary Evaluations

Reviewer 1

Strenghts of the study:

The study is a proposal for a standardization of the adrenal vein sampling technique in primary aldosteronism since large differences among various approaches exist, provides an interesting comparison between simultaneous and sequential catheterization of adrenal veins with blood sampling without ACTH stimulation is presented. A further strength is the large group of subjects involved.

Weakness:

The blood sampling was performed at a relatively wide time interval, and this may affect plasma cortisol levels and hence interpretation of the data. Potential local side effects should be given more attention, because of simultaneous catheterization with two cathethers from one site.

Reviewer 2

The advantages of the present study are: i) a prospective within patient study design, which minimized several potential confounders as, for example, inter-individual variability; ii) the relatively large sample size; iii) the state-of-the art criteria used to unambiguously diagnose aldosterone-producing adenoma, as confirmed at follow-up postadrenalectomy a prospective within-patient study design, which minimized several potential confounders as, for example, inter-individual variability and finally diagnose aldosterone-producing adenoma, as confirmed at follow-up postadrenalectomy.