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ABSTRACT

In patients with primary aldosteronism (PA), adrenal vein sampling (AVS) is considered the only reliable technique to distinguish between unilateral and bilateral autonomous production of aldosterone, but agreement is lacking on the best criteria indicating successful cannulation and lateralisation. The objective of this study was to assess the impact of differing criteria for successful cannulation and lateralization on reproducibility of subtype diagnosis. Sixty two patients with confirmed PA underwent AVS on two separate occasions, because the first was unsatisfactory. We compared the different diagnoses of PA subtype reached using AVS data assessed by permissive (type 1), intermediate (type 2) and strict (type 3) criteria. Although 91.1% of all (both first and second) AVS were "successful" by type 1 criteria (50.8% by type 2 and 33.9% by type 3), in only 32.2% of patients was the diagnosis concordant between first and second AVS. Type 1 criteria also led to a higher rate of diagnosis of unilateral PA (67.3% of "successful" procedures) than type 2 (36.5%) or type 3 (26.2%). There was considerable disparity in the diagnosis reached using the three different criteria, with concordance in only 32.2%. Using either type 1 or 2 criteria, the minimal adrenal/peripheral vein cortisol ratio necessary to obtain the same diagnosis in the first and second AVS procedures was ≥ 2.75. In conclusion, permissive criteria for successful cannulation and lateralisation on AVS achieve poor diagnostic reproducibility and should be avoided.

Key words: endocrine hypertension, primary aldosteronism, aldosterone, aldosterone-producing adenoma, bilateral adrenal hyperplasia.

Abbreviations: PA = plasma aldosteronism; PAC = plasma (or serum) aldosterone concentration; PRA = plasma rennin activity; ARR = aldosterone/renin ratio; APA = aldosterone-producing adenoma; BAH = bilateral adrenal hyperplasia; CT = computed tomography; AVS = adrenal vein sampling; PCC = plasma (or serum) cortisol concentration; PV = peripheral vein; IVC = inferior vena cava; A/C = aldosterone/cortisol ratio; ACTH = adrenocorticotropic hormone

INTRODUCTION

Primary aldosteronism (PA) is currently believed to be the most frequent form of secondary endocrine hypertension accounting for up to 5-10% of all hypertensive patients[1,2]. The rate of diagnosis of PA increased markedly after use of the aldosterone/renin ratio (ARR) as a screening test became widespread[3]. The diagnosis of PA should not be missed since it has been recently demonstrated that patients with PA exhibit a higher rate of cardiovascular complications, target organ damage and metabolic syndrome compared to matched essential hypertensives[4,5,6], and that this increased rate is reversed with specific surgical or medical treatment[7]. A definitive diagnosis is made after a positive confirmatory test (usually a suppression test) that is performed in patients with positive ARRs[8]. After confirming the diagnosis of PA, it is fundamental to distinguish between unilateral and bilateral subtypes because individuals with unilateral forms [mainly aldosterone-producing adenomas (APA)] can be cured or at least experience significant amelioration of the disease by unilateral laparoscopic adrenalectomy[1,3,9,10], whereas patients with bilateral adrenal hyperplasia (BAH) are usually treated and benefit from targeted pharmacotherapy with aldosterone antagonists[1,11]. CT scanning lacks sensitivity and specificity[10,12,13,14] and should therefore be followed by adrenal venous sampling (AVS), which is the only reliable means of differentiating unilateral from bilateral PA and lateralizing APAs pre-operatively.

During AVS, both plasma (or serum) aldosterone (PAC) and cortisol (PCC) concentration are determined in blood collected from the adrenal veins, and in simultaneously collected blood from a peripheral vein (PV) or the inferior vena cava (IVC). Comparison of adrenal venous and PV (or IVC) cortisol permits an assessment of the "adequacy" or "success" of cannulation of the adrenal veins. However, agreement is lacking on which criteria should be used for defining successful cannulation with some centers using more permissive criteria ($C_{adrenal\ vein}/C_{IVC}>1.1$)[15], and others more restrictive criteria ($C_{adrenal\ vein}/C_{PV}>3$ without ACTH stimulation[10] or $C_{adrenal\ vein}/C_{IVC}>4$ under low-dose continuous ACTH stimulation[16]). Cortisol levels are also used in the calculation of aldosterone/cortisol ratios (A/C) which serve to "correct" adrenal venous aldosterone levels for

differing degrees of dilution of adrenal with non-adrenal venous blood. However, criteria for defining "lateralization" of aldosterone secretion are again not uniform, with some centers using more permissive criteria [$(A/C)_{adrenal\ vein}/(A/C)_{contralateral\ adrenal\ vein}$ at least 2.0][15], and others more restrictive criteria [$(A/C)_{adrenal\ vein}/(A/C)_{PV}$ at least 2.0 AND $(A/C)_{contralateral\ adrenal\ vein}/(A/C)_{PV}$ 1.0 or less without ACTH stimulation;[10] or $(A/C)_{adrenal\ vein}/(A/C)_{Icontralateral\ adrenal\ vein} > 4.0$ under low-dose continuous ACTH stimulation[16]].

In the current study, we sought to assess the impact of differing criteria for defining cannulation success and for defining lateralization on (1) rates of subtype (unilateral versus bilateral PA) diagnosis and (2) reproducibility of subtype diagnosis in patients who had undergone two separate AVS procedures, in each case because the cannulation during the first attempt was deemed unsuccessful according to locally used criteria.

METHODS

The study was carried out in two referral centers: (1) the Endocrine Hypertension Research Centre, Greenslopes and Princess Alexandra Hospitals, Brisbane and (2) the Division of Internal Medicine and Hypertension Unit, University of Torino. Patients were enrolled after written informed consent and approval of the study protocol by the local ethics committees.

Brisbane

We selected all patients with PA who underwent two AVS studies in the period 2000-2006, because the first one did not meet our criteria for successful cannulation. This comprised 45 (8.5%) of 531 patients. Patients were studied as previously described[17,18]. Briefly, all hypertensive patients with an ARR >70 (plasma aldosterone expressed in pmol/L, plasma active renin concentration in mU/L) or >590 [renin as plasma renin activity (PRA) in ng/ml/h] on at least 2 occasions were subjected to a fludrocortisone suppression test (FST) to definitively confirm or exclude PA. Blood was collected in the sitting position between 0800 and 1100 h after at least 2 hours of upright posture for

measurement of ARR after hypokalemia had been corrected with potassium supplements and while patients were being encouraged to maintain a liberal dietary salt intake. Prior to testing, diuretics (including spironolactone and amiloride) were always ceased for at least 4 weeks and, wherever possible, beta-blockers, dihydropyridine-type calcium channel antagonists, angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists were ceased for at least 2 weeks and withheld throughout the subsequent diagnostic steps. In order to maintain hypertension control, treatment was instituted where necessary with verapamil slow-release, with or without added hydralazine and/or prazosin[18].The diagnosis of PAL was based on FST criteria[17] when PAC, measured at 1000 h in seated patients after at least 2 hours upright, failed to suppress to <166 pmol/L (6 ng/dL) at the conclusion of 4 days administration of a high sodium diet, slow-release sodium chloride (Slow Na, 30 mmol three times daily with meals) and fludrocortisone acetate (0.1 mg every 6 h), provided that on day 4: (i) upright renin was suppressed to <8.4 mU/L (for plasma active renin concentration) or 1 ng/ml/h (for PRA); (ii) plasma potassium was within the normal range; and (iii) plasma cortisol was lower at 1000 h than at 0800 h, excluding an acute increase in adrenocorticotrophic hormone which could prevent suppression of aldosterone. In all patients with PAL confirmed by FST, peripheral blood DNA was tested[19] for the hybrid *CYP11B1*/*CYP11B2* gene responsible for glucocorticoid-remediable PAL and adrenal CT performed with fine (2.5-3.0 mm) slices, pre and post intravenous contrast. All who tested negative for the hybrid gene, regardless of CT findings, underwent AVS[17,20] in order to differentiate unilateral from bilateral adrenal aldosterone overproduction. 'Gradients' of at least 3.0 between adrenal and peripheral venous cortisol concentrations were taken to indicate adequate sampling from adrenal veins. If the A/C ratio on one side was at least 2.0 times the simultaneous peripheral venous ratio, and on the other side was the same as or less than the peripheral ("contralateral suppression"), the study was considered to demonstrate lateralization of aldosterone production. Patients demonstrating adrenal venous A/C ratios higher than peripheral on both sides were considered to have bilateral autonomous aldosterone production. For patients in whom the repeat (second) AVS procedure was

successful by cannulation criteria, but in whom adrenal A/C was less than peripheral in one side and between 1.1 and 2 times the peripheral in the other side, those AVS results were considered "inconclusive". All AVS studies were performed between 8.00 and 11.00, to minimize the chance that "poor" adrenal/peripheral cortisol gradients could be due to a low cortisol secretory rates from the adrenals as might be expected in the afternoon.

Anti-hypertensive therapy was unaltered in the period between the two AVS studies. Hormonal assays were performed as described previously[13]. Diagnosis of APA was confirmed after surgery, pathology, blood pressure outcome and normal suppressibility of aldosterone after post-operative FST[13].

PAC was measured by solid-phase radioimmunoassay technique using Coat-A-Count assay (Diagnostic Product Corporation, Los Angeles, California, USA). The interassay coefficient of variation at 98, 631 and 1458 pmol/L was 15%, 6% and 6% respectively. The intra-assay coefficient of variation was 2.3-5.4% for values in the range 180-2256 pmol/L. PCC was determined by chemiluminescent immunoassay on the DxI 800 Immunoassay System (Beckman Coulter, Brea, California, USA). The interassay coefficient of variation at 140, 562 and 958 nmol/L is 6.5%, 5.0% and 4.9% respectively.

Torino

We selected all PA patients who underwent two AVS in the period 2002-2007 in our Unit at the University of Torino. This comprised 17 (18%) of 93 patients. PA patients were selected as previously described[14]. Briefly, patients were screened using the ARR: the cut-off level for a "positive" ARR was 1100 (aldosterone in pmol/L, PRA in $ng*ml^{-1}\ h^{-1}$) together with a PAC of >416 pmol/L. Blood samples were obtained in the sitting position between 8 and 10 a.m. All anti-hypertensive drugs were stopped at least 3 weeks before the aldosterone and PRA measurements (at least 6 weeks before for diuretics and at least 8 weeks before for spironolactone). Patients that, for clinical reasons, could not be left untreated were allowed to take an alpha-blocker (doxazosin)

and/or a calcium channel blocker (verapamil or amlodipine), and maintained the same therapy during and for the period between the screening and the final diagnosis. The confirmatory test was an intravenous saline load (2 L of 0.9% NaCl infused over 4 hours), that was considered positive if post-test PAC levels were > 139 pmol/L[21].

CT scanning with fine cuts (2.5 mm) of the adrenal with contrast was performed in all PA patients. Adrenal vein cannulation, performed in all patients with positive saline load test (unless found to have GRA by genetic testing[22]), was considered successful if the adrenal vein/IVC cortisol gradient was at least 2. The study was considered to show lateralisation when the A/C ratio from one adrenal was at least 4 times the ratio from the other adrenal gland or if it was at least 3 times contralateral together with an A/C in the contralateral vein lower than that in the peripheral vein.

All AVS studies were performed between 8.00 and 11.00, to minimize the chance that "poor" adrenal/peripheral cortisol gradients could be due to a low cortisol secretory rates from the adrenals as might be expected in the afternoon. Anti-hypertensive therapy was unaltered in the period between the two AVS studies. Diagnosis of APA was confirmed after surgery, pathology, blood pressure outcome and normal suppressibility of aldosterone after post-operative intravenous saline loading[14]. Hormonal assays were performed as described previously[14]. PAC was assessed by solid-phase radioimmunoassay ALDOCTK-2 (DiaSorin, Saluggia, Vercelli, Italy). Within-run precision tests yielded coefficient of variations of 5.3%, 3.8% and 2.7% on samples with a mean aldosterone values of 8.6 ng/dL, 24.9 and 49.6, respectively. Between-run coefficients of variations were 13.7%, 10.4% and 8.3% at concentrations of 9.1 ng/dL, 23 and 60.7, respectively. PCC were determined using chemiluminescent microparticle immunoassay technology (CMIA) automated on ARCHITECT® analyzer (Abbott Laboratories, Abbott Partk, IL, USA). Precision studies yielded within-run CVs of 8.1%, 5.6%, and 4.5% and between-run CVs of 9.9%, 7.3%, and 4.6% at cortisol concentrations of 34, 173, and 437 microg/L, respectively.

RESULTS

The study involved 62 patients (45 assessed at the Brisbane centre and 17 at the Torino centre) who underwent two AVS procedures (a total of 124 procedures). Clinical and biochemical parameters are summarized in Table 1. During the period of the study the success rate of cannulation was 83% in Torino and 81% in Brisbane, using the criteria specific to the corresponding institution. We analysed the AVS data according to a relatively permissive set of criteria currently in use elsewhere[23] (type 1), a relatively strict set of criteria (type 3, used in the Brisbane Centre) and an "intermediate" set of criteria (type 2, used in the Torino Unit) (Table 2).

Cannulation success rates

Of the first AVS studies (all deemed unsatisfactory in either Brisbane or Torino), 83.4% would have been considered "successful" using the type 1 criteria. As expected, 98.4% of the second, repeat AVS studies met the type 1 criteria (Table 3). In contrast, using the type 3 criteria, cannulation would have been judged successful in 0% with first AVS and 67.7% with second AVS (33.9% overall), and using the type 2 criteria, 19.4% with the first and 82.3% with the second (50.8% overall).

Rates of subtype diagnosis

Application of type 1 criteria led to a high rate of diagnosis of unilateral PA, with 67.3% of those deemed "successful" AVS by type 1 criteria lateralising (61.3% of the total 124). Using type 2 criteria, 36.5% of successful AVS procedures lateralised (18.5% of the total) and using type 3 criteria, only 26.1% of the successful AVS procedures lateralised (8.9% of the total).

With marked differences between the three criteria sets in terms of diagnostic conclusion reached for each AVS study, only 32.2% of them were concordant (Table 4). However, in over 75% of the AVS studies the same conclusion was reached using criteria type 2 or type 3. Results using criteria type 1 were concordant with results using criteria type 2 in only 46.7% and concordant with criteria

type 3 in only 33%. Importantly, in 21 of the 25 studies in which conclusions reached using criteria 2 and 3 were discordant, this was due to a lack of any satisfactory diagnosis being reached, because of failed cannulation when the stricter type 3 criteria were used. This left only 4 studies where diagnoses were reached by both sets of criteria 2 and 3 which were truly discordant.

Because results for the first performed AVS for each patient showed failed cannulation (and hence lacked a definitive subtype diagnosis) in all 62 cases using type 3 criteria and in 50 patients using type 2 criteria, we analyzed rates of subtype diagnosis for the second AVS alone (Table 4). In 30 (48.4%) of the 62 patients, the conclusion reached was the same with all three criteria. In 19 (30.6%), the conclusion was similar according to criteria type 2 and 3, but differed from that of criteria type 1. In 9 (14.5%), it was similar for criteria type 1 and 2, but differed from type 3. In 1 (1.6%), it was similar between criteria type 1 and 3, but differed from type 2, and in 3 (4.8%) patients the diagnosis was different between all the three criteria sets.

Reproducibility of subtype diagnosis

We first analysed all AVS studies using the most permissive criteria (type 1) both for success and lateralisation. With these criteria, cannulation of one or both sides was unsuccessful during at least one AVS procedure in 11 of the 62 patients, leaving 51 patients in whom AVS was successful on both occasions that it was performed, and in whom a comparison of diagnoses reached between the two studies was therefore possible. Only in 18 (35.3%) of the 51 patients was the diagnosis concordant between the first and the second AVS using type 1 criteria, leaving 33 (64.7%) in which the diagnoses differed (Table 5). Of these, in 7 (13.7%) patients who were deemed unilateral after the first AVS, the diagnosis changed to unilateral *in the contralateral gland* after the second AVS. In 19 (37.2%), a diagnosis of unilateral made after the first AVS changed to bilateral after the second. In the remaining 7 (13.7%), a diagnosis of bilateral after the first AVS became unilateral after the second (Table 6). *The minimal adrenal/PV cortisol ratio that was necessary to obtain the same diagnosis in the two AVS procedures using the type 1 lateralization criteria was ≥ 2.75.*

We also analysed reproducibility using the intermediate criteria (type 2). Because criteria for cannulation success were stricter than for type 1, they were met for both the first and second AVS study in only 8 patients (Table 5). Of these, the diagnosis was concordant between the two studies in 4 (50%), whereas in the other 4, a diagnosis of unilateral made after the first AVS became bilateral after the second (Table 6). Interestingly, even in this analysis, the minimum cut-off adrenal/PV cortisol ratio required to obtain the same diagnosis between each pair of studies was again 2.75.

DISCUSSION

Adrenal vein sampling is the only reliable approach to distinguish between unilateral, and therefore surgically-correctable, forms of PA, from bilateral forms which are usually treated with mineralocoricoid receptor antagonists. However, different, necessarily arbitrary cut-offs are used in different centers, both to define successful cannulation of the adrenal veins and for determining lateralisation of aldosterone secretion. The current study has demonstrated that the use of differing AVS cut-offs or criteria can have a profound impact on the diagnostic conclusions reached.

With regards to the effect on cannulation success rates, raising the cut-off adrenal/PV cortisol ratio from 1.1 to 3.0 dropped the proportion of studies regarded as being successful from 91% to 34%, a difference of great practical significance. It must be emphasized, however, that patients were chosen for this study on the basis of having had two AVS procedures (in order to allow an assessment of diagnostic reproducibility) because of a failed study on the first attempt, which could have exaggerated the differences in success rates observed between the three criteria sets. Taking the second AVS study into consideration alone, for example, the differences were much less marked but still considerable (98% versus 68%). It appears clear from this study that the use of permissive cannulation success criteria can lead to significant numbers of patients being given a definitive

subtype diagnosis when stricter criteria would have led to the study results being rejected (and the study repeated) or interpreted with great caution.

The impact of differing AVS criteria on subtype diagnosis was considerable. The more permissive criteria led to a much higher rate of diagnosis of unilateral PA, approximately six times (61% versus 9%) that of the least permissive criteria for all studies combined, and more than double (67% versus 26%) when only those studies considered "successful" were considered. Concordance of diagnosis between criteria sets was better between type 2 and 3 criteria than between type 1 and either of the other two types.

Because this study did not include an analysis of outcomes of surgery as a "gold standard", it is not possible from these data alone to make firm conclusions about the relative accuracy of subtype diagnosis reached by each criteria set. As an alternative approach, we examined the reproducibility of diagnostic conclusions reached by comparing results obtained between two separate studies on individual patients. Using the most permissive criteria, which resulted in studies rejected in Brisbane or Torino being included, diagnostic reproducibility was poor, with a similar diagnosis being obtained for first and second studies in only 18 (35%) of 51 patients. All combinations of discordance were observed, but of particular concern was the finding that in 7 patients lateralization actually changed from one side to the other. A somewhat higher rate of reproducibility (50%) was seen with the application of type 2 criteria, but because only eight patients satisfied those criteria in terms of successful cannulation for both first and second AVS procedures and were therefore the only subjects eligible for this analysis, it is difficult to draw firm conclusions in this respect. Similarly, we could not assess reproducibility of type 3 criteria as the nature of selection of patients for this study meant that none had successful AVS procedures during the first attempt. Importantly, however, whether type 1 or type 2 lateralization criteria were applied, reproducibility was found to be 100% only when studies in which adrenal/PV cortisol ratios of at least 2.75 were considered. This would argue for the adoption of the stricter type 3 criteria (which uses a cut-off of 3) for defining cannulation success in order to minimize diagnostic inaccuracy.

In agreement with our results is the reported lower cure rates (30%) among operated patients diagnosed as having "APA" using "permissive" criteria[24], which is about half the rate reported by the Torino and Brisbane groups[3]. In this context it should be considered that cure reported by Sukor N[25] et al in 15% of patients with BAH who underwent adrenalectomy was mainly restricted to patients who only had milder forms of PA and milder hypertension[25].

AVS is a difficult procedure, especially for the cannulation of the right adrenal vein which is small and usually empties directly into the IVC (unlike the left, which usually empties into the left renal vein, making it easier to cannulate). The success rate depends on the experience and dedication of the radiologist. It has been argued that because using higher cortisol cut-off ratios will lower the percentage of usable AVS studies, a more permissive ratio should be recommended.[23] However, as the current study demonstrates, this would likely be at the cost of risking incorrect subtype diagnoses. In one report, in which four radiologists performed AVS in 60 patients, the success rate of bilateral adrenal vein cannulation was only 44%[26]. By contrast, with experience and focusing the expertise to one or two radiologists at each center, the AVS success rate can rise to 96%[16] or at least to more than 80%[10,14,20]. Recently, the introduction of a method for real-time rapid cortisol assay[27] during AVS, which provides the radiologist with an assessment as to the success or otherwise of attempted cannulation at the time of the procedure, offers the radiologist a new tool to improve the performance of AVS.

In a recent paper, the use of AVS as the "gold standard" has been questioned, mainly because of the lack of standardization among different groups with regards to the criteria used to define cannulation success and to define lateralization[28]. We agree entirely that this reduces the usefulness of AVS as a diagnostic tool. Our contention is that criteria which incorporate less stringent requirements to define cannulation success and lateralization will result in reduced diagnostic accuracy. This may help to explain the lower sensitivity and specificity rates reported in some studies.

Coexisting autonomous aldosterone and cortisol production has been rarely reported among patients with APA[29] and this would be expected to result in absence of an adrenal/peripheral venous cortisol gradient on the side contralateral to the lesion. While this may have accounted for the absence of a cortisol gradient in some of our patients, we would expect the number to be very small. Furthermore, this would not account for the change in diagnosis between the first and second AVS as both studies should have been affected similarly.

We believe that the difference between the diagnosis obtained with the first and the second AVS is not due do the fact that both centers use a sequential cannulation technique and not a simultaneous cannulation of the adrenal veins: in fact, because of the level of experience of our radiologists, there was usually no more than 10-15 minutes gap between sampling of the two adrenal veins. Importantly, in no patient were there significant alterations in peripheral aldosterone or cortisol levels (of which at least five were available for analysis for each procedure) during either of the two AVS studies, which would argue against there being significant ACTH- or AngII-induced fluctuations in adrenal steroid output during those periods.

In our study, AVS was performed without the use of ACTH stimulation. Others have employed continuous cosyntropin infusion (50 mcg per hour started 30 minutes before sampling and continued throughout the procedure) during AVS[16,27] in order to minimize stress-induced fluctuations in aldosterone secretion during non-simultaneous AVS, to maximize the gradient in cortisol from adrenal vein to inferior vena cava (IVC), and to maximize the secretion of aldosterone from an aldosterone-producing adenoma. However, it has been suggested that in some cases this could result in stimulation of the gland contralateral to an APA, blunting the difference between the sides and leading to a mistaken diagnosis of bilateral autonomous aldosterone production. This is perhaps most likely to happen when renin suppression has not been of long duration, and aldosterone production by the contralateral gland is not thoroughly suppressed. The use of a high dose ACTH bolus (250 mcg) during simultaneous adrenal vein catheterisation has been recently reported not to result in a significant improvement in diagnosis accuracy[24]. However, we propose

that our results are also applicable to AVS performed during ACTH infusion, although an adrenal/peripheral cortisol gradient would be expected to be obtained more easily with this protocol. In a recent study published by Ceral and co-workers[28] in which AVS was performed during ACTH infusion (160 μg/hour), reproducibility in lateralization diagnosis (determined by comparing results among multiple samples collected during the same AVS procedure and using those with cortisol gradients of at least 10 as the "reference" samples) was found to be critically dependent on adrenal/peripheral cortisol gradients. Interestingly, the optimum cut-off again appeared to be at gradients of at least 3.0.

The results of the present study are of importance for the interpretation of a technique that is considered to be the gold standard for the diagnosis of PA subtypes. Indeed, as a result of our findings, the Torino center has adopted the policy to increase the adrenal vein/IVC cortisol cut-off to 3 for defining adrenal venous cannulation success.

PERSPECTIVES

Recent Guidelines on diagnosis and treatment of PA clearly stated that AVS is the reference standard test to differentiate unilateral from bilateral disease in patients with PA and thus should be performed in all patients that are candidate for surgery. However, incorporation of AVS into diagnostic protocols has been hampered for different reasons, one of the most important being the lack of standardization among different groups with regards to the criteria used to define cannulation success and to define lateralization. Our study shows that the use of more stringent criteria may result in a more consistent reproducibility of diagnosis among groups and in a higher success rate of adrenalectomy in patients with unilateral disease at AVS.

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Table 1. Clinical and biochemical parameters of patients.

Parameters	Torino (n=17)	Brisbane (n=45)
Age, years (mean ± SE)	53 ± 2	53 ± 2
Sex (M/F)	13/4	14/31
SBP, mmHg (mean ± SE)	176 ± 8	158 ± 2
DBP, mmHg (mean ± SE)	104 ± 5	91 ± 1
Plasma Potassium, mEq/L (mean± SE)	3.7 ± 0.14	3.8 ± 0.1
PAC, pmol/L (median, range)	757 (521-1559)	520 (264-1950)
PRA, ng $ml^{-1}h^{-1}$, (median, range)	0.15 (0.1-0.8)	0.2 (0.1-0.4) [n=9]
Direct Active Renin, mU/L (median, range)	-	3.7 (2.0-10.0) [n=36]
PAC/PRA Ratio (median, range)	5204 (1110-12760)	1940 (1207-11150) [n=9]
PAC/Direct Active Renin Ratio (median, range)	-	175.5 (65-520) [n=36]

Table 2. Criteria for successful cannulation and lateralisation after AVS.

Criteria	Criterion Type 1	Criterion Type 2	Criterion Type 3
Cannulation Criteria	$C_{adrenal\ vein}/C_{IVC} \geq 1.1$	$C_{adrenal\ vein}/C_{IVC} \geq 2$	$C_{adrenal\ vein}/C_{peripheral\ vein} \geq 3$
Lateralisation Criteria	$[(A/C)_{adrenal\ vein}/(A/C)_{contralateral\ adrenal\ vein}] \geq 2$	$[(A/C)_{adrenal\ vein}/(A/C)_{contralateral\ adrenal\ vein}] \geq 4$ or $[(A/C)_{adrenal\ vein}/(A/C)_{contralateral\ vein}] \geq 3$ + $[(A/C)_{contralateral\ adrenal\ vein}/(A/C)_{IVC}] < 1$	$[(A/C)_{adrenal\ vein}/(A/C)_{PV}] \geq 2$ + $[(A/C)_{contralateral\ adrenal\ vein}/(A/C)_{PV}] < 1$

Table 3. Success and final diagnosis after AVS with the three different criteria

Criterion	Type 1	Type 2	Type 3
Successful AVS	113 (91.1%)	63 (50.8%)	42 (33.9%)
APA right	39 (31.5%)	16 (12.9%)	9 (7.3%)
APA left	37 (29.8%)	7 (5.6%)	2 (1.6%)
BAH	37 (29.8%)	40 (32.3%)	31 (25%)
Unsuccessful AVS	11 (8.9%)	61 (49.2%)	82 (66.1%)

Table 4. Concordance of the diagnosis with the three different criteria.

Concordance of the diagnosis	All AVS	First AVS	Second AVS
diagnosis 1 = 2 = 3	40/124 (32.2%)	10/62 (16.1%)	30/62 (48.4%)
diagnosis 1 = 2 ≠ 3	18/124 (14.5%)	9/62 (14.5%)	9/62 (14.5%)
diagnosis 1 ≠ 2 = 3	59/124 (47.6%)	40/62 (64.5%)	19/62 (30.6%)
diagnosis 1 = 3 ≠ 2	1/124 (0.8%)	0/62 (0%)	1/62 (1.6%)
diagnosis 1 ≠ 2 ≠ 3	6/124 (4.8%)	3/62 (4.8%)	3/62 (4.8%)

Table 5. Final diagnosis after first and second AVS according to the three different criteria.

AVS		Criterion Type 1		
	Not selective	BAH	APA left	APA right
First AVS	10	10	23	19
Second AVS	1	27	14	20
		Criterion Type 2		
First AVS	50	4	3	5
Second AVS	11	36	4	11
		Criterion Type 3		
Fist AVS	62	0	0	0
Second AVS	20	31	2	9

Table 6. Variation in the diagnosis between first and second AVS using criterion type 1 and 2.

Criterion Type 1	
Concordant diagnosis	18/51 (35.3%)
Not concordant diagnosis	33/51 (64.7%)
BAH → APA	7/51 (13.7%)
APA → BAH	19/51 (37.2%)
APA left → APA right or APA right → APA left	7/51 (13.7%)

Criterion Type 2	
Concordant diagnosis	4/8 (50%)
Not concordant diagnosis	4/8 (50%)
BAH → APA	0
APA → BAH	4/8 (50%)
APA left → APA right or APA right → APA left	0