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THE ADRENAL VEIN SAMPLING INTERNATIONAL STUDY (AVIS): MAIN RESULTS OF PHASE 1 AND 2 OF THE STUDY

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RIASSUNTO

Introduzione. L'iperaldosteronismo primario (PA) è la forma più comune di ipertensione arteriosa secondaria da causa endocrina. Per distinguere le forme chirurgicamente guaribili da quelle che esigono la sola terapia medica, le attuali linee guida dell'Endocrine Society raccomandano l'esecuzione del cateterismo venoso surrenalico (AVS), un'indagine ritenuta invasiva, rischiosa, tecnicamente difficile e di ardua interpretazione. Tuttavia non è ancora del tutto noto, se e come queste linee guida vengano applicate ed interpretate nella pratica clinica. Questo studio, the Adrenal Vein sampling International Study (AVIS), è stato ideato con l'intento di chiarire le modalità di utilizzo ed interpretazione dell' AVS nei centri mondiali di riferimento.

Materiali e metodi. E' uno studio retrospettivo, osservazionale, multicentrico, internazionale ed i centri partecipanti sono stati scelti tra quelli che hanno pubblicato dati su PA e AVS nella letteratura scientifica inglese nel periodo compreso tra il 2005 e il 2010. Lo studio prevede 2 fasi: la prima ha lo scopo di raccogliere i dati relativi alle modalità di esecuzione ed interpretazione dell'AVS, mentre la seconda di raccogliere i dati individuali dei pazienti sottoposti al AVS.

Obiettivi dello studio. I principali obiettivi della prima fase dello studio sono stati quelli di chiarire una serie di quesiti irrisolti inerenti all'utilizzo e alla performance diagnostica dell'AVS per la diagnosi di PA. In particolare, ci si è proposti di accertare: 1) la percentuale di pazienti con PA che vengono sottoposti ad AVS ed il numero di AVS eseguiti presso i maggiori centri internazionali di riferimento; 2) il tasso di complicanze (intese come rotture delle vene surrenaliche verificatesi durante l'AVS) e gli eventuali predittori delle stesse; 3) le modalità d'esecuzione dell'AVS; 4) le modalità di interpretazione ed utilizzo dei suoi dati; 5) la percentuale di utilizzo degli indici di selettività, di lateralizzazione e soppressione

controlaterale ed i loro valori di riferimento; 6) il costo di AVS per il paziente ed il sistema sanitario dei diversi paesi.

Nella seconda parte dello studio sono stati raccolti i dati individuali dei pazienti sottoposti all'AVS per: valutare il tasso di AVS bilateralmente selettivi in funzione dei diversi valori soglia dell'indice di selettività; identificare il miglior valore soglia che identifica la lateralizzazione dell'aldosterone; chiarire se la soppressione controlaterale permette la diagnosi di lateralizzazione in presenza di AVS solo unilateralmente selettivi; valutare il tasso dei pazienti con ipersecrezione unilaterale dell' aldosterone curata con la surrenectomia; stabilire se la terapia medica induce ad un miglioramento della pressione arteriosa in soggetti con ipersecrezione bilaterale del aldosterone; valutare la concordanza / discordanza tra test radiologici ed AVS nella diagnosi di lateralizzazione di aldosterone. Per motivi di tempo e di brevità di questa analisi preliminare, i dati sulla soppressione controlaterale non saranno inclusi.

Risultati. Fase 1: sono stati raccolti dati su 2604 AVS eseguiti presso 20 centri mondiali di riferimento sparsi in Europa, Asia, Nord America e Australia. Dall'analisi dei risultati è emerso che la percentuale di pazienti con PA, sistematicamente sottoposti all'AVS, è pari al 77%, con un'ampia variabilità tra i diversi centri (dal 40% al 100%). La percentuale di complicanze è risultata pari allo 0.61%, con correlazione significativa tra il numero di complicanze ed il numero di AVS eseguiti presso ogni singolo centro ($p=0.002$) e correlazione inversamente proporzionale al numero di AVS eseguiti dal singolo radiologo ($p=0.007$).

Il numero di radiologi che eseguono l'AVS nei singoli centri è mediamente di 2 radiologi/centro (variabile tra 1 e 7) ad eccezione di 2 centri, dove rispettivamente 6 e 7 radiologi erano coinvolti nell'esecuzione della procedura. La maggior parte dei centri (13/20) utilizza il cateterismo sequenziale e solo 7 centri il cateterismo

simultaneo bilaterale. Per quanto riguarda l'impiego del test di stimolazione con ACTH i centri si sono distribuiti quasi equamente tra quelli che utilizzano tale stimolazione (55%) e quelli che non la utilizzano (45%). Dalle analisi non è emersa una correlazione tra il cateterismo sequenziale e l'utilizzo del test di stimolazione con ACTH.

L'indice di selettività (SI) viene calcolato nel 90% dei centri ed i suoi valori di riferimento variano da 1.1 a 3 in condizioni basali e da 2 a 10 dopo la stimolazione con ACTH. Un centro utilizza solo i valori ormonali assoluti, mentre un altro centro non calcola il SI ma solo gli indici di lateralizzazione e soppressione controlaterale. L'indice di lateralizzazione (LI) è calcolato nel 95% dei centri ed i suoi valori di riferimento variano da 2 a 5 in condizioni basali e da 2 a 4 dopo la stimolazione con ACTH. L'indice di soppressione controlaterale (CSI) viene calcolato in 65% dei centri e i suoi valori di riferimento variano da 0.5 a 1 in condizioni basali e da 0.9 a 1 dopo la stimolazione con ACTH. Non è emersa una correlazione significativa tra l'utilizzo della stimolazione con ACTH ed il calcolo del CSI. In caso di non disponibilità di AVS bilateralmente selettivi, il 60 % dei centri utilizza per diagnosi anche gli AVS non selettivi bilateralmente. E' stata trovata una correlazione significativa ($p=0.05$) tra l'uso di AVS non selettivi ed il calcolo del CSI. Per quanto riguarda i costi dell'AVS, i risultati di questa analisi hanno evidenziato un'ampia variabilità tra i diversi centri ed i diversi paesi. Il costo del singolo AVS varia da 80 a 10.532 euro per il sistema sanitario nazionale e da 0 a 1.357 euro a carico del paziente.

Fase 2: l'analisi dei dati della seconda fase dello studio è stata eseguita solo su dati parziali, a causa del ritardato inserimento degli stessi da parte di alcuni centri. In questa analisi preliminare sono state raccolte informazioni su 1.030 pazienti con PA sottoposti all' AVS, provenienti da 15 centri differenti. Il 43% dei pazienti

presentava ipertensione arteriosa resistente, il 59% era di sesso maschile e la razza caucasica era quella predominante.

L'analisi del miglioramento della pressione arteriosa, indipendentemente dal tipo di trattamento (medico o chirugico), al follow up ha evidenziato il 19% di pazienti normotesi, il 49% con significativo miglioramento della pressione - definita come pressione arteriosa normale con lo stesso o ridotto numero di farmaci e/o pressione arteriosa simile a quella basale, ma con una marcata diminuzione di farmaci (> 2 farmaci) - , il 25% ha presentato solo un lieve miglioramento della pressione arteriosa - riduzione della pressione arteriosa sistolica o distolica >10%, senza il raggiungimento della normotensione con lo stesso o ridotto numero di farmaci - , mentre, l' 8% dei casi non ha presentato alcun miglioramento. Le donne ed i soggetti di razza caucasica o africana presentavano un outcome migliore. Per quanto riguarda l'outcome pressorio nei pazienti surrectomizzati, al follow up, il 34% dei pazienti era normoteso, il 45% con significativo miglioramento della pressione, il 16% ha presentato solo un lieve miglioramento e nel 5% dei casi non c'era alcun miglioramento. Dall'analisi è stata evidenziata anche la bassa percentuale di pazienti sottoposti alla surrenectomia, in media il 53% dei pazienti, con ampia variabilità tra i diversi centri. Per quanto riguarda il controllo della pressione arteriosa con la sola terapia medica nei soggetti con ipersecrezione bilaterale di aldosterone, la maggioranza (circa il 75%) ha presentato solo un lieve miglioramento del controllo pressorio, mentre nel 24% dei casi non c'era alcun miglioramento.

La valutazione del tasso di AVS bilateralmente selettivi in funzione dei diversi valori-soglia dell'indice di selettività, ha evidenziato che, l'aumento del numero di AVS bilateralmente selettivi, era inversamente proporzionale al valore soglia del SI, sia in condizioni basali che dopo lo stimolo con ACTH. Per identificare il miglior valore soglia che identifica la lateralizzazione dell'aldosterone, sono stati

esaminati i pazienti con AVS bilateralmente selettivi, con evidenza di ipersecrezione unilaterale di aldosterone, sottoposti a surrenectomia, che al follow up presentavano l'ipertensione arteriosa curata o significatamene migliorata. Questa analisi ha evidenziato che, ad ogni SI, maggiore è il valore soglia che identifica la lateralizzazione dell'aldosterone, più alto è il tasso di pazienti con ipertensione arteriosa curata o significatamene migliorata.

Per quanto riguarda la concordanza tra test radiologici ed AVS nella diagnosi di lateralizzazione di aldosterone, l'analisi condotta nel gruppo di pazienti con AVS bilateralmente selettivo, con evidente lateralizzazione dell'aldosterone, sottoposti alla surrenectomia, ha evidenziato concordanza tra i 2 test diagnostici in solo il 57% dei casi a destra e il 65% dei casi a sinistra.

Conclusioni. Nonostante il fatto che il PA sia la forma più comune di ipertensione arteriosa secondaria da causa endocrina e che l'AVS sia ritenuto indagine "gold standard" per la diagnosi differenziale tra i suoi due principali sottotipi, e quindi per la scelta del trattamento più appropriato (medico o chirurgico), i risultati della prima fase dello studio AVIS hanno documentato l'esistenza di marcate diversità nella percentuale di utilizzo, nelle modalità d'esecuzione e d'interpretazione nei diversi centri di riferimento mondiali.

Lo studio ha evidenziato un tasso di complicanze globalmente minimo, pari allo 0.61%. Quindi nonostante l'AVS sia generalmente considerato un'indagine invasiva, in realtà in presenza di mani esperte, esso risulta essere sicuro. Questo dato conferma le raccomandazioni delle attuali linee guida dell'Endocrine Society di sottoporre ad AVS tutti i pazienti con PA in assenza di controindicazioni al trattamento chirurgico.

La fase 2 dello studio attraverso l'analisi dei dati individuali ha evidenziato che la scelta di valori più restrittitivi dell'indice di selettività e dell'indice di

lateralizzazione si traduce in maggiori tassi di guarigione e miglioramento dell'ipertensione arteriosa, al prezzo dell'esclusione di un numero maggiore di pazienti dalla surrenectomia.

ABSTRACT

Context. Primary aldosteronism (PA) is the most common form of secondary endocrine hypertension. To make a distinction between surgically-curable and surgically-non curable causes, the Endocrine Society guidelines recommend the use of adrenal venous sampling (AVS), which is considered invasive, technically challenging, difficult to interpret, and commonly held to be risky. However, whether and how these guidelines are interpreted in clinical practice is still unknown. Hence, the purpose of this PhD work was to set up a study, the Adrenal Vein sampling International Study (AVIS), to answer several questions concerning AVS.

Design and settings. AVIS is an observational, retrospective, multicenter international study. The eligible centers were identified among those that had published data about PA and/or AVS into English scientific literature during the period between 2005 and 2010. The study is designed in two phases, the first one is aimed to collect the data on AVS while the second one, the data on single patients.

Objective. In the first phase of the study, the main outcomes were to determine the complication rate of AVS and the ways in which it is performed and interpreted at major referral centers. Specifically, the rate of PA patients in whom AVS is performed and the number of AVS studies performed yearly between 2005 and 2010 per each center; the number of radiologists that perform AVS at each center and the rate of complication; the use of bilaterally simultaneous or sequential AVS catheterization and the use of cosyntropin stimulation during AVS; the use of AVS studies not bilaterally selective for diagnosis; the interpretation of AVS results by calculation of the selectivity, lateralization and contralateral suppression index and

minimum cutoff value used; the cost of AVS for the National Health System/Insurance and for patients.

The second phase is aimed to collect individual patients data to assess the rate of bilaterally selective AVS studies as a function of the different cutoff values of the selectivity index; to identify the best cutoff value for the identification of the lateralized aldosterone excess; to clarify if contralateral suppression allows diagnosing lateralization when only unilateral (usually left-sided) AVS is successful; to assess the rate of the patients with lateralized aldosterone excess secretion cured by adrenalectomy; to establish whether medical therapy induces improvement of high blood pressure (BP) in PA subtypes characterized by bilateral aldosterone excess secretion; assess the rate of AVS concordant /discordant with results of imaging tests. For reasons of time and brevity in this preliminary analysis the data on contralateral suppression will not be included.

Results. Phase 1: Twenty out of 24 eligible centers from Europe, Asia, Australia and North America participated and provided information on 2604 AVS studies performed between 2005 and 2010. The percentage of PA patients systematically submitted to AVS was 77% (median, range: 19%-100%). The overall rate of adrenal vein rupture was 0.61% and it correlated directly with the number of AVS performed at a particular center (p=0.002) and inversely with the number of AVS performed by each radiologist (p=0.007). The total number of radiologists who performed AVS at the 20 centers was 51 (range, 1 to 7; median,2). Thirteen of the 20 centers used sequential and 7 bilaterally simultaneous catheterization; cosyntropin stimulation was used in 11 centers. No significant correlation has been found between the use of ACTH stimulation and sequential catheterization.

The cutoff values for selectivity and lateralization indices varied markedly among those centers that systematically used them. Selectivity index (SI) results to be

calculated in 90% of the centers, and the cut off values varied under non-stimulated condition from 1.1 to 3, and under stimulate conditions between 2 and 10. Lateralization index (LI) is used in 95% of the centers and its cut off values varied under non-stimulated condition from 2 to 5, and under stimulate conditions between 2 and 4. Contralateral suppression index (CSI) was calculated in 65% of the centers and its cut off values varied under non-stimulated condition from 0.5 to 1, and under stimulate conditions between 0.9 and 1.

It was found out that 60% of centers used unilaterally selective studies when bilateral results were unavailable for the diagnosis and there was significant correlation between use of unilateral selective AVS and contralateral suppression (p=0,05). In contrast, no significant correlation was found between the use of ACTH stimulation and contralateral suppression. The costs of AVS showed a wide variability among centers and countries, both for the patient and for the insurance or national health care system. The cost ranged from 80 (€) to 10,532 € for health insurance systems and from 0 to 1,357 € for the patient.

Phase 2: Due to the delay of some centers in providing the data, we performed a preliminary analysis only with the available data. We collected information on 1.030 PA patients who underwent AVS in 13 referral centers worldwide. Resistant hypertension was found in 43% of the patients, 59% were men; the majority were Caucasians.

The analysis of overall improvement at follow up of high BP with either pharmacological or surgical treatment evidenced 19% of patients with cured high BP, 49% of patients with a markedly improved BP control, 25% with only a mild improvement of BP and 8% with no improvement of BP. The analysis evidenced that women, Caucasian and African had better outcomes.

As regards the outcome of BP in the adrenalectomized patients, BP was cured in 34% of cases, markedly improved in 45% of patients, mildly improved in 16% of patients and no improvement in 5% of the patients. Moreover, the rate of patients who underwent adrenalectomy was lower than expected (median 53%) with a high variability among centers. The analysis evidenced that in the patients with bilateral aldosterone excess no cases showed cured o markedly improved control of high BP while on pharmacological treatment. At follow up, the majority of the patients (about 75%) showed only a mildly improved control of BP, and 24% of the patients had no improvement.

The analysis of the rate of bilaterally selective AVS studies as a function of different cutoff values showed that lower SI cutoff values were associated with a higher rate of bilaterally selective studies. To evaluate the best cutoff value for identifying a lateralized aldosterone excess we examined the bilaterally selective studies (by different SI cutoffs), the performance of the different LI used across the centers using as reference index cure or marked improvement of BP at follow up after the adrenalectomy. This evidenced that, at each SI, the higher the LI cutoff the higher the rate of patients who were cured or markedly improved as far as BP control.

As regards the rate of concordance/discordance between AVS results and CT imaging results for the diagnosis of lateralized aldosterone excess, we performed the analysis in patients with bilaterally selective AVS, with evidence of lateralized aldosterone excess, who undergo to adrenalectomy and at follow up presented cured o markedly improved BP. This analysis evidence that in this subgroup of patients the rate of concordance between AVS results and CT imaging results was low, 65% on the left side and 57% on the right side.

Conclusions. Despite the high prevalence of PA, and the fact that AVS is crucial for discriminating between its two major subtypes, and therefore for selecting the

most appropriate treatment, this study documented marked dissimilarities in the percentage of use, protocols, interpretation and cost of AVS even among the major referral centers around the world. Importantly, overall the rate of major complications was minimal, 0.61%, which demonstrates that although being generally regarded as a risky procedure, AVS is in truth safe in experienced hands. This observation therefore supports the Endocrine Society recommendation that AVS should be used in all patients with confirmed PA who are candidate for adrenalectomy and seek surgical cure.

In the second phase of the study, analysis of the individual patients data, evidence that a choice of more restrictive values of the selectivity and the lateralization index, translates to increased rates of cured and markedly improved BP at follow up, but this at the price of the exclusion of a greater number of patients from adrenalectomy.

1. INTRODUCTION

1.1 Definition and consequences of primary aldosteronism

Primary aldosteronism (PA) is a syndrome characterized by overproduction of aldosterone, independent of angiotensin II. Patients present with hypertension, a suppressed plasma rennin activity (PRA), varying degrees of metabolic alkalosis, while hypokalemia and hypomagnesemia are not consistent findings. Patients may have a variable presentation, many subjects will have symptoms that include muscle aches, cramping and weakness, headaches, palpitations, and polyuria.(1) However, other subjects have minimal or no symptoms at all, and in some rare cases, the subject can present with normal blood BP.(2-4)

PA is the most common form of secondary endocrine hypertension. It was first described in 1953 in Polish literature by Litynski,(5) who reported about two patients with progressive hypertension and adrenal masses and suggested that mineralocorticoids were responsible for the hypertension. This report went unrecognized in the English language scientific literature until 1991.(6) Hence, the credit for the first report of PA is usually given to Jerome Conn who in 1959(7) reported the case of 34-year-old woman who had a syndrome of 'moderate hypertension', hypokalemia, metabolic alkalosis, mild hypernatremia and normal renal function.

Evidence exists that PA is associated with an excess target organ damage. This is because hyperaldosteronism produces oxidative stress(8, 9) oxidative damage to DNA,(10) inflammation,(11) cardiovascular remodeling, hypertrophy, and fibrosis.(12, 13) All of these effects explain why primary aldosteronism was found to impair left ventricular filling and diastolic function,(14) induce fibrosis of the left ventricular wall,(15) prolong the ECG PQ interval,(16) induce the endothelial

dysfunction,(17-19) stiffening of the large arteries,(20-21) remodeling of resistance arteries,(21) stroke,(22) and micro-albuminuria.(23-26) These changes translate into an elevated rate of cardiovascular events of PA patients, including atrial fibrillation, ischemic stroke, cerebral hemorrhage,(27, 28) "flash"pulmonary edema, and myocardial infarction.(29)

Milliez et al.(29) reported a higher found of cardiac events in subjects with PA compared to essential hypertension (EH). In fact, by evaluating retrospectively patients with PA compared to patients with hypertension, they found that the odds ratio of incidence of stroke, non-fatal myocardial infarction and atrial fibrillation was times higher in those with PA compared to those with EH.

In a recent study, Savard et al.(30) compared the cardiovascular event rates in patients with PA to control patients with EH, who were matched for age, gender and systolic BP. Patients with PA had a 2-fold higher likelihood of having left ventricular hypertrophy than subjects with EH in this study. Subjects with PA had a higher prevalence of coronary artery disease, nonfatal myocardial infarction, congestive heart failure and atrial fibrillation. These data underscore the adverse effects of excess aldosterone and indicate that the higher rate of cardiovascular complications in PA are at least in part independent of blood pressure.

There are evidence that within the excess cardiovascular damage, left ventricular hypertrophy, is reversible upon instauration of specific treatment: in a recent clinical trial(31) 180 patients with PA were prospectively followed using serial echocardiogram and compared to 143 patients with EH. At baseline, the subjects with PA had greater LV mass than matched patients with EH. At a mean follow up of 36 months, the subgroup with APA who underwent adrenalectomy had similar BP control as those treated with mineralocorticoid antagonists and those with EH. This was accompanied by a significant regression of LVH and LV mass

by reverse inward LV remodeling in both the surgically and medically treated group of patients with PA during the study.

Aldosterone excess also has been shown to contribute to structural damage in the kidney and its implication in the pathogenesis of renal injury.(33, 34) Moreover, in the PAPY study(24) (PA Prevalence in Hypertensives) patients with PA had higher urinary albumin excretion than comparably matched subjects with EH. In a multicenter German Conn's Registry study(35) was results that chronic renal insufficiency was more common in subjects with PA than in hypertensive controls (29% vs. 10%).

Hence, early identification of PA is crucial for preventing complications, and reversing pre-existing cardiovascular damage by specific therapy.(14, 26)

Removal of aldosterone excess by adrenalectomy normalizes BP despite withdrawal or tapering of antihypertensive therapy in up to 82% of the patients and prevents the development and/or induces regression of adverse cardiovascular changes in most patients.(36, 37) Similar regression of target organ damage can be probably achieved by mineralocorticoid receptor blockade (spironolactone or eplerenone) at the price of life-long drug treatment.(38) These considerations support an aggressive strategy aimed at making a nearly diagnosis and instituting specific treatment of PA.

1.2 Prevalence of PA

For many years after its discovery, PA was held to be a "rare bird", even though Dr. Conn claimed that its prevalence was probably about 7,3% of all hypertensive patients.(39) This because initially as case detection of PA was usually limited only to hypertensive patients with hypokalemia, though not all patients with PA have had hypokalemia. The first case of patient with PA who was eukalemic was

reported by Conn(40) in 1965 and afterwards, Hiramatsu's group reported that six of nine patients diagnosed with PA, using the Aldosterone-to-Renin Ratio (ARR, see below) as the initial screening method, had normokalemia.(41) Over the last two decades, it has been recognized that the majority of patients with primary aldosteronism do not have hypokalemia.(41-43) However, the debate on its prevalence went on for many decades and Gordon et al.(44) were one of the first groups to report that PA was more prevalent than previously realized. They estimated that the incidence of PA the 'essential hypertensive' population is between 5 and 15%, and is probably around 10%.

Nowadays there is consensus among experts that the PA may be far more prevalent than usually held.(45-47) In one review(48) from 2003 by most estimates, 5 to 13% of all patients with hypertension have PA. In 2004, a meta-analysis(49) of the rates reported over a decade showed estimates of prevalence from 1.4% to 32% (median, 8.8%) thus demonstrating that the true prevalence rate was unknown.(50)

In 2005, the results of the first large prospective study, the PAPY Study(53), PA was found in over of 11% of patients and 4.8% of all patients who were screened had a surgically curable subtype. This results evidence that PA is the most common curable form of hypertension in hypertensive patients referred to specialist centers (with or without drug-resistant hypertension).

Also, in general practice it has been reported that an elevated ARR involved 32.4% of the hypertensive individuals (with increased prevalence in females and in people over 55 year old), randomly selected from the general population, even though only a minority of them might have PA.(54)

Moreover, patients with resistant hypertension have an even higher incidence of PA with the prevalence of PA between 11 and 23%. Mosso et al.(55) evaluated over 600 patients and they reported that 13% of patients with stage 3 hypertension had

PA. In Birmingham, 88 consecutive patients with resistant hypertension underwent testing and 20% were found to have PA.(56) Gallay et al.(57) screened 90 consecutive patients with resistant hypertension and found a 17% prevalence of PA and similar results were found by Eide et al.(58) In a prospective study of 100 consecutive patients with type 2 diabetes and resistant hypertension, Umpierrez et al.(59) found that 14% had primary aldosteronism and in a large retrospective study of 1616 patients with resistant hypertension, Douma et al.(60) found that 11% were confirmed as having PA.

1.3 Classification of PA

Although there are several classifications of PA, from the practical standpoint, the most useful classification of form of PA is that presented in Table 1, which distinguishes surgically-curable from surgically-non curable causes.(61)

	Surgically curable	Not surgically curable
1	Aldosterone-producing adenoma (APA) - unilateral - bilateral	Bilateral adrenal hyperplasia (BAH)
2	Primary unilateral adrenal hyperplasia (PAH)	Unilateral APA with BAH
3	Multinodular unilateral adrenocortical hyperplasia (MUAN)	Familial type I hyperaldosteronism (FHI) or Glucocorticoid-remediable aldosteronism (GRA)
4	Ovary aldosterone-secreting tumor	Familial type II hyperaldosteronism (FHII)
5	APA or bilateral adrenal hyperplasia (BAH) with concomitant pheochromocytoma	Familial type III hyperaldosteronism (FH III)
6	Aldosterone-producing carcinoma (APC)	Apparent mineralocorticoid excess (AME) - Chronic licorice intake - Carbenoxolone (antacid) use

Table 1. Classification of PA.

It is crucial to identify surgically-curable from surgically-uncurable form for selecting the most appropriate treatment. PA deriving from bilateral aldosterone excess (predominantly from bilateral adrenal hyperplasia, IHA) are optimally treated with life-long mineralocorticoid receptor blockade (with spironolactone,

canrenone, potassium canrenoate, or eplerenone), and PA form due to unilateral overproduction of the hormone (predominantly aldosterone-producing adenoma, APA) may be treated with unilateral adrenalectomy.(62)

1.4 Screening strategy for PA

The Endocrine Society guidelines(63) suggest that screening for PA should be undertaken in all patients with a higher pre-test probability of PA (Table 2).

	Higher pre-test probability of PA
1	Unexplained hypokalemia (spontaneous or diuretic-induced)
2	Resistant hypertension and hypertension grade 2 or 3
3	Early onset (juvenile) hypertension and/or stroke (<50years)
4	Incidentally discovered apparently non functioning adrenal mass ("Incidentaloma")

Table 2. Patients with high pre-test probability of PA.

Some author(62) added other three categories of patients that were not included in this classification because accumulating evidence indicates that these patients carry a higher risk of PA: the patients with evidence of organ damage that is disproportionate for the severity of hypertension, those with obstructive sleep apnea and the obese patients with hypertension.

The evidence that there is a relationship between obesity, the metabolic syndrome and PA were confirmed in an prospective evaluation of consecutive hypertensive patients referred to specialized hypertension centers, where was found that body mass index (BMI) correlated with plasma aldosterone concentration (PAC)

independently of age, sex, and sodium intake in EH, but not in PA patients. This association of BMI is particularly evident in overweight-obese EH patients, and suggests a pathophysiological link between visceral adiposity and aldosterone secretion.(64) Moreover, in one prospective study of 466 patients with hypertension, the prevalence of the metabolic syndrome was 41.1% in subjects with PA and 29.6% in those with essential hypertension.(65) In a recent study,(93) PA patients were compared to patients with essential hypertension, and there are evidence that aldosterone excess has a direct negative effect on β-cell function in patients with PA, and after adrenalectomy, glucose-induced first-phase insulin secretion improves significantly. Obstructive sleep apnea was present in 85% of subjects with resistant hypertension in one study(66) and additionally, there was a significant correlation between PAC and the severity of sleep apnea. In another study that involve 109 patients with resistant hypertension, 77% of the subjects had obstructive sleep apnea and 28% had hyperaldosteronism.(67)

1.5 Diagnostic strategy for PA

The first step for diagnosis of PA is demonstration of excess aldosterone secretion autonomous of RAAS. To this end the screening test is based on the aldosterone–renin ratio (ARR), which is calculated as the ratio between plasma renin activity and aldosterone concentration.

Dunn and Espiner(68) were the first to report using ARR for screening of PA. The validity of using an aldosterone to plasma renin ratio as well as the aldosterone level in screening patients for PA was confirmed by Weinberger's group, they evaluated the ARR in a study of patients with PA and compared to subjects with EH and to normotensive controls and confirmed that the ratio was valid.(69) In an retrospective analysis of the data from five PA centers that used a ARR as a

screening test reported that this test lead to a 5- to 15-fold increase in the number of patients diagnosed with PA.(43)

However, the use of ARR requires careful consideration of several issues. First of all, the ARR depends not just on the PAC, but also on the plasma renin activity (PRA), which means that all the patients with suppressed PRA will have an increased ARR even if the PAC is not elevated. Hence, the ratio must be interpreted in light of the PAC itself, which should be higher than 15ng/dL, and of the lowest detectable level of the renin assay that is being used. Therefore, it is common to arbitrarily fix minimum values, 0.2ng/mL/h for the PRA and 0.6mIU/dL (0.36ng/mL) for the direct renin activity (DRA) where used.(50, 51)

These precautions are crucial in the elderly and black populations, which usually exhibit low PRA values.(70) Aldosterone levels also rise during the luteal phase of the menstrual cycle(71) and increase up to 10-times normal during the third trimester of pregnancy.(72) Moreover, aldosterone concentrations are also elevated in secondary aldosteronism due to increased renin and angiotensin levels (renal artery stenosis and in renin-secreting tumors). Secondary aldosteronism also occurs in chronic edematous states (such as congestive heart failure), cirrhosis and the nephrotic syndrome.

Other important consideration is that the PAC and renin values, and thereby the ARR, are markedly affected by many antihypertensive drugs (Table 3) and therefore before measuring the ARR, drug treatment must be appropriately modified before performing this test. There are antihypertensive drugs that does not significantly affect the renin-angiotensin aldosterone system are doxazosin and the long-acting calcium channel blocker.(51) Hence, these agents can be used (alone or in combination) to control BP when ever it is harmful to interrupt antihypertensive treatment during screening.(51) Beta blockers must be

withdrawn at least 4 weeks before the test to avoid greatly increasing the false positive rate. Diuretics should be withdrawn at least 3 weeks before, and MR antagonists should be withdrawn 6 weeks before the test, to lower the rate of false negative diagnoses. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) increase false negative results and they should be withdrawn at least 3 weeks before performing the ARR assay.

Drug	PAC	PRA	ARR	Results
β -Blockers	↓	↓↓	↑	FP
α2-Blockers	↓	↓↓	↑	FP
Diuretics	→↑	↑↑	↓	FN
MR antagonists	↑	↑↑	↓	FN
ACEi	↓	↑↑	↓	FN
ARBs	↓	↑↑	↓	FN

Table 3. Effects of antihypertensive drugs on PAC, PRA and ARR. PAC=plasma aldosterone concentration, PRA=plasma renin activity, ARR= Aldosterone-Renin-Ratio, ACEi= angiotensin I converting enzyme inhibitors, ARBs= angiotensin II type 1 receptors blockers. FP: false positive, FN: false negative.

However, before altering the antihypertensive regimen, it is important to consider the risks for an individual patient with hypertension. Following a change in antihypertensive medications, there is a risk of development of a hypertensive crisis, heart failure or atrial fibrillation. In a study by Fischer et al.(73) only 60% of their cohort could be switched off medications or have the medications adjusted during screening and confirmation of PA.

As for all quantitative tests, as the cut off value for the ARR becomes more stringent, the number of false positives decreases. The choice of the optimal cut off value, which has a profound impact on the diagnostic performance of the ARR, was based on a careful valuation of the performance of the test in only a few studies.(51, 74) Using the diagnosis of APA as reference, the PAPY study(51)

investigators determined experimentally that the optimal cutoff was 26, which corresponded to a sensitivity of 80.5% and a specificity of 84.5%. Moreover, they also showed that if the patients adequately prepared and if the minimum value of renin has been fixed, a markedly elevated ARR usually indicates the presence of PA. In fact, when repeated twice in the same patient under carefully standardized conditions, the ARR was found to be reproducible.(75)

For identification of false positive cases and exclusion of this cases before the patient selection for AVS, after the identification of patients with possible aldosterone overproduction with definition of ARR, four confirmatory testing procedures are in common use: oral sodium loading, saline infusion, fludrocortisone with salt loading, and the captopril challenge.(45, 47) All these tests are aimed at demonstrating a non suppressible aldosterone excess after blunting the RAAS (renin angiotensin aldosterone system) and they rely on the assumption that PA is autonomous from the RAAS.(76-78) Of note, all this test serve to identify false positive results and therefore to exclude these patients from AVS. As they are intended to show a fall of PAC, given that hypokalemia blunts aldosterone secretion and therefore may prevent detection of the blunting effect of the test, these maneuvers must be performed only after correction of the hypokalemia with oral or intravenous potassium supplementation. Of note, all those test serves to identify false positive results and therefore to exclude these patients from adrenal vein sampling (AVS). Moreover, at the prevalence rate of PA typically scan at referral centers they have a negative predictive value much higher than their positive predictive value. Therefore, they should be defined as exclusion tests instead of confirmatory tests.

1.6 Differentiation of Subtypes

A. **Anatomic Imaging**

All patients who have confirmed PA should undergo to anatomic imaging of the adrenal glands. High-resolution Computed Tomography (CT) of the upper abdomen is the best available technique for identifying adrenal nodules that can be an APA, primary unilateral adrenal hyperplasia (PAH) or bilateral adrenal hyperplasia (BAH). Magnetic resonance imaging (MRI) is another option, particularly if the patient cannot receive contrast. MRI can be slightly more sensitive, but it is less specific and more susceptible to motion artifacts. Although CT scans and MRI are useful to identify the large, rare, and usually fatal aldosterone-producing carcinoma(79, 80) they miss up to 42% of the APA that are smaller than 6 mm. Likewise, PAH and multinodular unilateral adrenocortical hyperplasia (MUAN)(81) are undetectable with these imaging technologies. Moreover, a non functioning adrenal mass can coexist with a small, CT-undetectable APA by chance in a hypertensive patient with a biochemical picture of PA. Furthermore, in a patient with PA an adrenal nodule can be an APA but also a macronodule of hyperplasia in a patient with idiopathic hyperaldosteronism (IHA)(82) micronodules in a patient with MUAN(81) or an apparently non functioning, incidentally discovered adenoma ("incidentaloma"), which is common at autopsy even in normotensive individuals.(83)

For all these reasons, adrenal imaging is not appropriate to achieve discrimination between APA and IHA, as shown by a study of PA patients who underwent both CT and AVS, which was used as the "gold standard" for the diagnosis. In a study the CT scans mistakenly suggested an APA in 24.2% of the patients, correctly identified a unilateral or bilateral aldosterone excess in only 53%; falsely suggested BAH in 21.2 % of the patients with a unilateral source of aldosterone excess, and

showed the presence of an APA in the wrong adrenal in 6% of the patients.(84) Similar data on the incorrect diagnosis of CT for diagnosing the surgically curable subtypes of PA have been reported in one meta-analysis where was found that when AVS is used as the criterion standard test for diagnosing laterality of aldosterone secretion in patients with PA, CT/MRI misdiagnosed the cause of primary aldosteronism in 37.8% of patients.(85) Thus, overall CT results are confounding in about half of the patients, can lead to useless or inappropriate adrenalectomy in one quarter of patients, and can exclude from adrenalectomy roughly one quarter of patients who are potentially curable with this procedure. Because of these limitations of imaging, there is a consensus that the "gold standard" test for showing the lateralization of aldosterone secretion is the measurement of PAC and plasma cortisol concentration (PCC) in adrenal venous blood.(86, 87)

B. **Adrenal Vein Sampling (AVS)**

The use of AVS test for diagnosing laterality of aldosterone secretion and therefore for differentiate unilateral from bilateral form of PA, started by Melby et al.(88) in 1967. AVS consist in sampling of venous blood draining from the adrenal veins (right and left) and the inferior cava vein for the measurement of aldosterone and cortisol levels. The adrenal veins are catheterized through the percutaneous femoral vein approach in local anesthesia, Figure 1.

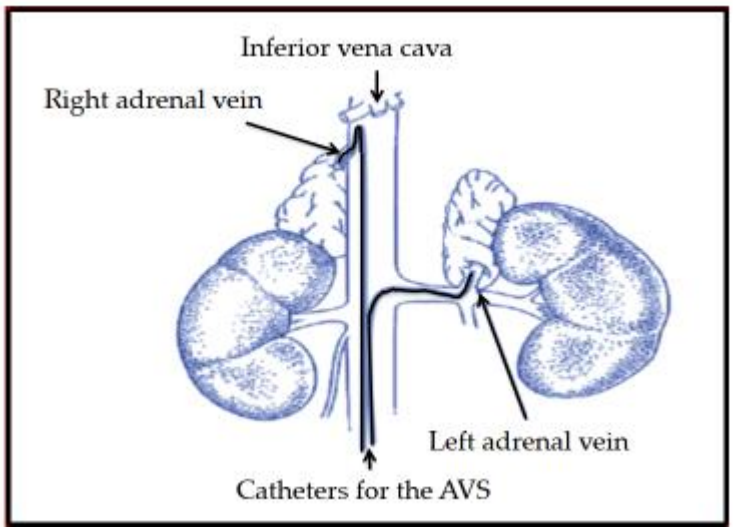

Figure 1. Position of the catheters for blood sample during the AVS.

Since both the performance and the interpretation of AVS require considerable experience, this test should be performed only at major referral centers. Potential complications include adrenal vein dissection, adrenal hemorrhage or infarction and extravasations of contrast.

1) Selection of Patients for Adrenal Venous Sampling

Considering that AVS is an invasive and expensive test the appropriate patient selection is essential. Since AVS is aimed at subtyping of PA and not at confirming this diagnosis, an unequivocal biochemical diagnosis of PA should be made before considering AVS. Since AVS helps to distinguish between unilateral and bilateral aldosterone excess, both the U.S.Endocrine Society(63) and the Japan Endocrine Society guidelines(89) recommend that AVS be performed in all patients who are reasonable candidates for general anesthesia and surgery and who wish to undergo adrenalectomy if indicated. AVS is not indicated where the patient prefers life-long medical treatment with a MR antagonist or surgery is already mandated by the size of the adenoma and/or other radiological features suspicious

of adrenocortical carcinoma. Considering that nonfunctioning adrenocortical adenoma ("incidentaloma") is infrequent in young people, a subgroup of patients in which AVS might not be needed includes young patients (<40years old) with marked PA of recent onset, as evidenced by spontaneous hypokalemia, and a clear-cut unilateral cortical adenoma on computed adrenal imaging.(90) However, even for young patients, bilateral aldosterone secretion, which occurs in those with IHA or familial hyperaldosteronism type I (FH-I), cannot be excluded without AVS. Therefore, in the patients with proven FH-I AVS is not indicated. Moreover, unilateral aldosterone excess from a small CT-undetectable APA in the adrenal gland contralateral to a CT-detectable adrenal mass cannot be reliably identified without AVS.

There is evidence that some preoperative characteristics of the patients and their history of hypertension are associated with cure of hypertension following unilateral adrenalectomy. The surgical cure of hypertension has been associated with young age,(91, 92) shorter duration of hypertension (<5 to 10 years), fewer antihypertensive medications (e≤2), higher preoperative BP, preoperative normal renal function, normal BMI (≤25 kg/m2), female gender, lack of a family history of hypertension,(36, 94-96) and no evidence of vascular remodeling.(31) These preoperative characteristics also serve to guide the clinician and the patient in discussing realistic expectations of surgical outcomes.

2) Preparation of the patient

Careful preparation of the patient for the procedure and standardization of the conditions for its performance are key steps to the success of AVS. Emotional and pain-related stress, which activates the hypothalamic pituitary adrenal axis with ensuing adrenocorticotropin-induced cortisol release from both adrenal glands, can be a major confounder of AVS results as it might lower the PAC to PCC ratio,

and thus has the potential to obscure lateralization to one of the adrenals when AVS is perform without cosyntropin (ACTH) administration. The stress reaction occurs in most patients when starting AVS, waned rapidly (e.g. over 15 minutes), increases the SI on both sides at the beginning of the procedure, is likely to influence also the LI values when using the sequential AVS sampling.(97) Allowing the patient to rest quietly for at least 15 minutes before the blood sampling in a friendly environment with psychological assistance can also be useful during the procedure. If cosyntropin is not used, AVS may be best performed in the morning, to avoid false-negative results due to diurnal fluctuation in ACTH having a more variable effect on many APAs than on the contralateral adrenal. Some centers conduct AVS in outpatients, in which case time should, if possible, allow for the patient to be kept in the supine position possibly for one hour prior to AVS.(86, 98)

AVS should be performed only after correction of the hypokalemia and timely withdrawal of confounding drugs. Hypokalemia, if present, should be corrected with oral or intravenous potassium supplements before AVS, since hypokalemia decreases aldosterone secretion, and may potentially mask a unilateral aldosterone producing adenoma. Careful adjustment of the antihypertensive agents before and during AVS is important. Peripheral α1-adrenergic receptor blockers (e.g. doxazosin mesylate, prazosin hydrochloride, and terazosin hydrochloride), and/or the long-acting dihydropyridine or non-dihydropyridine calcium channel blockers (verapamil) are recommended as these agents have not confounding effect on renin secretion, but short-acting calcium channel blockers can blunt aldosterone secretion and raise the PRA and, therefore, can cause false-negative results. Beta-blockers by decreasing the PRA and leaving the PAC relatively unaffected raise the ARR; therefore, they should be stopped at least 2 weeks before the measurement of the PAC and renin. Conversely, diuretics and mineralocorticoid receptor

antagonists should be withdrawn before 2 and 6 weeks, respectively, because they raise the PRA. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) have an even more marked effect because they not only raise the PRA but also blunt aldosterone secretion, thus reducing the ARR and increasing false-negative results. Therefore, they should be withdrawn at least 2 weeks before performing the ARR. However, in stage 3, and/or drug-resistant hypertensive patients, multiple agents may be necessary for achieving BP control before AVS and successful AVS can be achieved in these patients. Thus, in these patients ACEIs, ARBs, diuretics, and beta-adrenergic blockers may be used as long as renin is suppressed.

3) Performance and interpretation of adrenal venous sampling

Catheterization of the left adrenal vein is achieved in almost 100% of the cases, in contrast, that of the right adrenal vein is more difficult owing to its small size and its draining directly into the inferior vena cava at various angles or directly into a small accessory hepatic vein.(98-100) Hence, prior knowledge of the right adrenal vein anatomy can facilitate catheterization in difficult cases.(100) The placement of the catheter tip closer to or farther from the adrenal gland can lead to spurious results showing concentrations of several-fold difference. This implies that often just a single, absolute aldosterone and cortisol concentration can be meaningless. Therefore, the measurement of cortisol concentrations with calculation of the ratio of the hormone concentration in the blood taken from the adrenal vein over that in the infra-adrenal inferior vena cava, selectivity index (SI) (Table 4), is used as a measure of the adequacy of the cannulation and as a correction for the different degree of selectivity. The right adrenal vein often shares egress with an accessory hepatic vein, where the concentration of cortisol and aldosterone is lower than in peripheral venous blood, owing to hepatic metabolism of both hormones and to dilution of the relatively low flow from the adrenal effluent. Hence, on this side

underestimation of aldosterone and cortisol levels is the rule rather than the exception. Prior identification on CT of the right adrenal vein draining into accessory hepatic vein, selective cannulation of the right adrenal vein by using suitable catheters should be undertaken instead of the common trunk of accessory hepatic vein and right adrenal vein.(97, 100)

Unfortunately, there is no consensus on the cutoff value that determines the success of the adrenal sampling.(82, 101-103) A thorough study in which the accuracy of different cutoff values of the SI was assessed by receiver operating characteristic (ROC) curve analysis showed that if cortisol can be precisely measured (eg, with coefficient of variation < 6%), SI of 1.1 or greater provides identification of APA with an accuracy that is not significantly worse than that attained with higher cutoff values.(104) With such a criterion, selective AVS results can be obtained in close to 100% of cases on the left side, and between 85% and 95.6% on the right side.(84, 101, 102, 104) The use of more higher cutoffs(82, 84, 98, 101-103, 105) results in a marked decrease in the number of AVS tests that are bilaterally selective. With a cutoff for the SI of 1.10, the percent of bilaterally selective studies is around 80%; with a cutoff of 2.0, it decreases to 61%; and with a cutoff of 3.0, it falls to only 50%. This issue is crucial because the availability of bilaterally selective AVS data is essential for a proper diagnostic use of the results.(84, 104)

When bilaterally selective samples are available, most centers use the calculation of the lateralization index (LI) (Table 4) for establish whether a lateralized aldosterone excess exists. This index is calculated from the PAC and PCC in both adrenal veins and defined as the ratio of the higher (dominant) over the lower (non-dominant) PAC/PCC ratio, over the simultaneously measured cortisol values to correct for the degree of selectivity (dilution). Unfortunately, there is no consensus on the cutoff value that mandates adrenalectomy. The only study that

prospectively explored the use of a wide range of cutoffs by performing adrenalectomy in patients with LI down to 2 and by formally assessing the performance of different cutoffs, with ROC curve analysis, supports the use of a cutoff value of 2.(104)

Definition	Formula	Clinical significance
Selectivity index (SI)	PCC_{side}/PCC_{IVC}	Values > the cut-off confirms that the blood sample was obtained from the adrenal vein.
Lateralization index (LI)	PAC_{dom}/PCC_{dom}: $PAC_{nondom}/PCC_{nondom}$	Values > the cut-off evidence lateralized aldosterone excess.
Contralateral suppression index (CSI)	$PAC_{nondom}/PCC_{non\ dom}$: PAC_{IVC}/PCC_{IVC}	Values < the cutoff indicate ipsilateral suppression and suggest contra-lateral aldosterone overproduction.

Table 4. Definition of SI, LI and CSI. PAC= plasma aldosterone concentration; PCC= plasma cortisol concentration; PAC dominant and PAC non dominant are PAC on the side with higher and lower aldosterone secretions, respectively. PCC dominant and PCC non dominant are PCC on the side with higher and lower aldosterone secretions, respectively. IVC= inferior vena cava.

When only unilaterally selective AVS results are available for diagnostic of lateralization of aldosterone production, contralateral suppression is used, instead of a lateralization index. This contralateral suppression, contralateral suppression index (CSI), is calculated from the ratio of PAC over PCC of each side and PAC over PCC of the infrarenal inferior vena cava (Table 4). This index is used to determine if the aldosterone concentration in the adrenal vein blood is, or is not, higher than expected based on the peripheral arterial level of the hormone.

4) Intra-procedural PCC assay

Some centers have exploited the use of extemporaneous, intra-procedural PCC measurement for definition of selectivity of samples. This has the advantage of furnishing to the radiologist the immediate feedback on whether selective blood

sampling from each adrenal vein was achieved. In the latter case further attempts of selective catheterization could therefore be undertaken before removing the catheters, thus avoiding the need for a future catheterization.(106, 107) While this approach can improve the success rate, particularly during the radiologist's learning curve, it is feasible only at centers where PCC can be measured rapidly, which implies a suitable logistic organization and a dedicated laboratory technician standing by.(108, 109)

5) Use of stimulation test during AVS

Aldosterone and cortisol are released in bursts from the adrenals, which can bias the results of AVS when sequential AVS (incanulation of adrenal veins one at time) is performed, due to difference in timing of blood sampling. Therefore, to circumvent the problems it has been proposed to perform AVS after ACTH stimulation of aldosterone secretion, to facilitate the demonstration of lateralization in APA. Stimulation with a continuous cosyntropin infusion during AVS was introduced in 1979 and is currently used at many centers.(84, 110, 111) The reasoning for use of an ACTH infusion is that the infusion may minimize any stress-induced perturbations in aldosterone and cortisol during the procedure. This stimulation enhance the PCC gradient between the adrenal vein and the inferior vena cava, and thus increasing the SI values and confidence of successful sampling, reduce stress-induced fluctuations in cortisol and aldosterone secretion during sequential AVS and increase aldosterone secretion from APA.(84, 110, 111) But there are evidence that ACTH stimulation markedly increases the selectivity index and exerts a confounding effect on the lateralization index.(87, 112-114) Use of bilaterally simultaneous catheterization (bilateral simultaneous cannulation of both adrenal veins)(112), avoids generating differences between sides owing to the different timing of the blood sampling under the stressful condition represented by AVS, but it might slightly increase the risk of adrenal vein thrombosis, as it

increases the time of catheter's obstructing the vessel lumen until the contralateral vein is successfully catheterized.

1.7 Purposes for performing the AVIS Study

Considering this background, we decided to perform Adrenal Vein International Study (AVIS) because despite the high prevalence of PA, and the fact that AVS is crucial for discriminating between its two major subtypes and therefore for selecting the most appropriate treatment, marked differences remain in the use of AVS even at major referral centers. This under-utilization is likely due to the misconception, mostly based on anecdotal experiences and/or on retrospective observational studies,(86) that AVS is technically challenging, invasive, risky, and not always necessary. Moreover, still today there are not accepted international standards for the performance of AVS and neither established criteria for interpretation of its results. In particular, some centers use the bilateral simultaneous technique, while others perform the sequential catheterization. Moreover, some centers perform AVS during or after using ACTH stimulation, albeit with different doses, and others do not use any stimulation. In addition, some are utilizing the absolute values of plasma cortisol and aldosterone to determinate the selectivity and lateralization, whereas others are relying on calculation of the selectivity index followed by calculation of the lateralization index. Moreover, large differences in the cutoffs for establishing selectivity and lateralization exist.

The creation of one large database, comprising data from international referral centers on performance e interpretation of the AVS and information of patients' outcome after the treatment decision based and AVS results, using the "four corners" approach(51) (Table 5), will form the basis for providing evidence-based guidelines in a this still controversial field.

	"FOUR CORNERS" CRITERIA for diagnosing APA
1	Biochemical evidence of PA
2	Unequivocal evidence of lateralized aldosterone secretion at bilaterally selective AVS
3	Evidence of adrenocortical nodule on CT/MRA imaging and/or surgery and/or histopathology
4	Post-adrenalectomy follow-up data (at least 120 days after adrenalectomy) a)Cure or improvement of hypertension b)Correction of the biochemical picture of PA

Table 5. Four Corner Criteria for diagnosis of APA. Cure: SBP<140 mmHg and/or DBP<90 mmHg without medications; Improvement: SBP and/or DBP <140/90 mmHg, respectively, on the same or reduced number of medications and/or a reduced number of defined daily doses.

2. AIMS OF THE STUDY

Aim 1

The first aim of the study was collect data on how AVS is being performed across the refferal centers worldwide to answer the following questions:

1. How many AVS studies were performed yearly from 2005 to 2010 per center;
2. What is the rate of PA patients in whom AVS is performed;
3. How many adrenal vein ruptures occurred during the AVS;
4. Use bilaterally simultaneous or sequential AVS catheterization;
5. Number of radiologists that perform AVS at each center;
6. Use of cosyntropin stimulation during AVS;
7. Calculation of the selectivity, lateralization and contralateral suppression index and minimum cutoff value used;
8. Use of AVS studies not bilaterally selective for diagnosis;
9. Cost of AVS for the National Health System/Insurance and for patients.

Aim 2

In the second phase of the study we collected information on individual data of PA patients who were submitted to AVS. In particular we collected information on the following variables:

- Demography data (sex, age, race);
- Weight and height of patients;
- Presence of resistant hypertension;
- Baseline pharmacological treatment;
- Baseline BP and heart rate values;
- Serum K+, plasma aldosterone and plasma renin level before AVS;
- Dynamic test during the AVS if any;

- Plasma aldosterone and cortisol concentration in the infraadrenal inferior vena cava and in the right and left adrenal vein;
- Final diagnosis (unilateral or bilateral APA, unilateral or bilateral hyperplasia, APA with hyperplasia)
- Treatment (adrenalectomy or pharmacological therapy);
- Post-treatment BP values;
- Post-treatment sK+, plasma aldosterone and plasma renin level;
- Diagnosis concordance/discordance between imaging (CT or RM) and AVS.

As no consensus exists on the performance and interpretation of the AVS results, in the second phase of the Study we have the following aims:

1) to assess the rate of bilaterally selective AVS studies as a function of the different cutoff values of the selectivity index;

2) to identify the best cutoff value for the identification of the lateralized aldosterone excess;

3) to clarify if contralateral suppression allows diagnosing lateralization when only unilateral (usually left-sided) AVS is successful.

4) To assess the rate of the patients with lateralized aldosterone excess secretion cured by adrenalectomy;

5) to establish whether medical therapy induces improvement of high BP pressure in PA subtypes characterized by bilateral aldosterone excess secretion;

6) to assess the rate of AVS concordant /discordant with results of imaging tests.

For reasons of time and brevity in this preliminary analysis the data on contralateral suppression will not be included.

3. METHODS

3.1 Study design

Retrospective, multi-center, observational study.

3.2 Center selection

Eligible centers were identified from those that had published in English on PA and/or AVS in the last decade following the PICO strategy ((P, population = adults with PA; I, intervention = AVS; C, comparator = simultaneous AVS vs sequential catheterization technique, use of cosyntropin testing vs non-stimulated condition, use of bilaterally vs unilaterally selective AVS results, use of absolute hormonal data vs selectivity and lateralization indices; O, outcome = the ways AVS was performed and interpreted, adrenal vein rupture).(115) Suitable studies were identified by computer-assisted database searches (PubMed database, U.S. National Library of Medicine using the key-words: aldosterone; primary aldosteronism; endocrine hypertension; adrenal vein sampling), scanning of reference lists, hand-searching of relevant journals, correspondence with authors of relevant reports and meeting presentations, and consultation with experts in the field. Moreover, during the study recruiting centers belonging to the European Network for the Study of Adrenal Tumors the study size was enlarged. Four centers that participated in the first phase did not take part in the second phase of the study.

3.3 Recruitment

Invitation of the corresponding Author of each article to participate in the study. The flow chart of the study is shown in Figure 2.

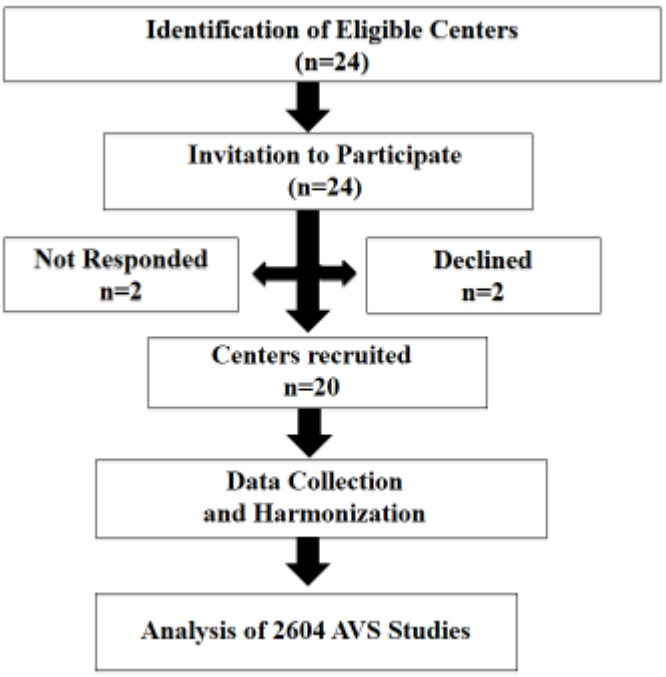

Figure.2. Flow chart of AVIS phase 1

3.4 Inclusion/exclusion criteria

After identification of the eligible centers the only inclusion/exclusion criteria were the leading investigator's agreement or unwillingness to participate in the study, respectively.

3.5 Data collection

Data were collected in a predefined form and stored securely and anonymously on a server protected by firewalls and passwords at the coordinating center. Each participating center filled the database online with their own data or they sent the data via mail to the coordinator center. The data were collected and handled at the maximum level of confidentiality under the requirement of the Declaration of Helsinky. Information on categorical variables were systematically re-coded to maximize comparability among studies. Data obtained from each participating study were checked for internal consistency and any queries then referred back, in

confidence, to the study collaborator(s), before harmonization to a standard format. The content of the data were unchanged by this process, and computer generated detailed summary tabulations based on the converted data were reviewed and confirmed by collaborators.

3.6 Statistical analyses

Statistical analysis was performed by SPSS for Mac (vers 21.0), GraphPad and the MedCalc softwares. For phase 1 of the study a stepwise regression analysis (backward, Wald) with a p-in=0.05 and p-out=0.10 was used to identify the predictors (number of AVS performed by each radiologist, number of AVS performed by each center, use of cosyntropin stimulation, use of bilateral simultaneous vs sequential AVS technique) of adrenal vein rupture. For phase 2 specifics algorithms were used in SPSS for Mac (vers 21.0) for the analysis of the data. Exploratory analysis followed by X^2 were performed.

3.7 Definitions

Adrenal vein rupture was defined as lumbar pain occurring during or after AVS plus fluoroscopic evidence of adrenal gland and/or retroperitoneal haemorrhage. Post-procedure imaging (eg, ultrasound, CT, or MRI) was performed to document this complication whenever clinically required, as judged by the lead investigator of each participating center.

Selectivity index (SI), which is used to assess the selectivity of the adrenal catheterization based on the step-up cortisol between the adrenal vein and the inferior vena cava plasma, was defined as the ratio of cortisol in each adrenal vein blood and in the infrarenal inferior vena cava.

Lateralization index (LI), which is used to establish whether a lateralized aldosterone excess exists, was defined the ratio of aldosterone to cortisol on the dominant side, e.g. the side with higher aldosterone secretion, over aldosterone to cortisol on the non dominant side, e.g. the side with lower aldosterone secretion.

Contralateral suppression index (CSI), which is used to determine if the aldosterone concentration in the adrenal vein plasma is higher then expected based on the peripheral arterial level of the hormone, was defined as the ratio of aldosterone over cortisol of each side and aldosterone over cortisol of the infrarenal inferior vena cava.

Blood pressure cured, blood pressure at follow up < 140/90 mmHg without therapy.

Blood pressure markedly improved, normotension at follow up on the same or reduced number of medications and blood pressure similar to baseline but with a marked decreased (> 2 drugs or >daily dose drug) of medication.

Blood pressure markedly improved, at follow up a fall of systolic and/or diastolic BP > 10%, but without achievement of normotension with the same or reduced therapy.

Blood pressure no improved, at follow up no fall of systolic and/or diastolic BP and/or need for increased number and/or dose of antihypertensive medications.

4. RESULTS AVIS PHASE 1

4.1 Recruitment of centers

After preliminary identification of eligible centers, 20 of 24 invited centers from Asia, Australia, North America, and Europe agreed to participate (Table 6). Of the remaining centers, two declined and two did not respond to the invitation (Figure 2).

Center	City	State	Principal Investigator/Invetigators
Università degli studi di Padova, DIMED	Padova	Italy	GP.Rossi, M.Barisa, D.Miotto, T.M..Seccia, A.C.Pessina
Hospital of The University of Pennsylvania	Philadelphia	PA, USA	S.Trerotola, D.Cohen
Yokohama Rosai Hospital	Yokohama	Japan	T.Nishikawa, M.Omura
Foothills Medical Centre, University of Calgary	Calgary	Canada	G.Kline
General Faculty Hospital, Prague	Prague	Czech republic	J.Widimsky jr.
University of Paris, Hopital Européen Georges Pompidou	Paris	Francia	P.F.Plouin, L.Amar
University of Texas Southwestern Medical Center	Dallas	Texas, USA	R.Auchus
Tohoku University Hospital	Sendai	Japan	F.Satoh
Heinrich Heine Universität Düsseldorf	Düsseldorf	Germany	L.C.Rump, O.Vonend
National Taiwan University Hospital	Taipei, Taiwan	Taiwan	K.D.Wu, V.Wu
National Hospital Organization, Kyoto Medical Center	Kyoto	Japan	M.Naruse
Medical College of Wisconsin	Menomonee Falls	Wisconsin, USA	S.B.Magill
Centre Hospitalier de l'Université de Montreal	Montreal	Quebec, Canada	A.Lacroix
Medizinische Klinik Innenstadt	Munich	Germany	M.Reincke, C.Degenhart, E.Fischer
Charité Campus Mitte	Berlin	Germany	M.Quinkler
University hospital Wuerzburg	Wuerzburg	Germany	B.Allolio. R. Kickuth
Endocrine Hypertension Research Centre, University of Queensland School of Medicine	Brisbane	Queensland, Australia	M.Stowasser, R. Gordon, E.Pimenta
Tokyo Women's Medical University	Tokyo	Japan	A.Tanabe
Radboud University Nijmegen Medical Center	Nijmegen	The Netherlands	J.Deinum, L. Schultze Kool
Dipartimento di Medicina Interna e Specialità Mediche, Medicina 2	Reggio Emilia	Italy	E.Rossi

Table 6. List of the participating centers and investigator in AVIS phase 1

4.2 Number of AVS performed

The database of the first phase was locked 1st October 2011. Upon locking we collected information on 2604 AVS studies performed in the period from 2005 and

2010 at 20 referral centers worldwide. The average total number of AVS performed over the 6 years at each center was 130.2 (s.e.m.= 24.4). Figure 3 show the total number of AVS performed by each center in 6 years period. The number of AVS performed yearly at each center showed a trend toward increasing use over time. The average numbers of AVS performed annually for each center was generally lower than 40 and ranged between 16 in 2005 and 34 in 2010 (Figure 4).

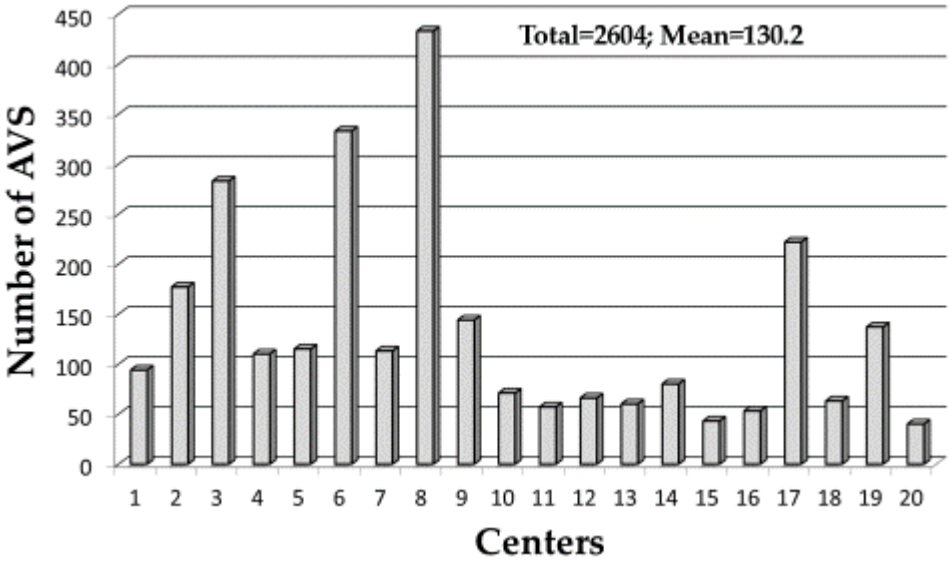

Figure 3. Number of AVS performed over the 6 years period at each center.

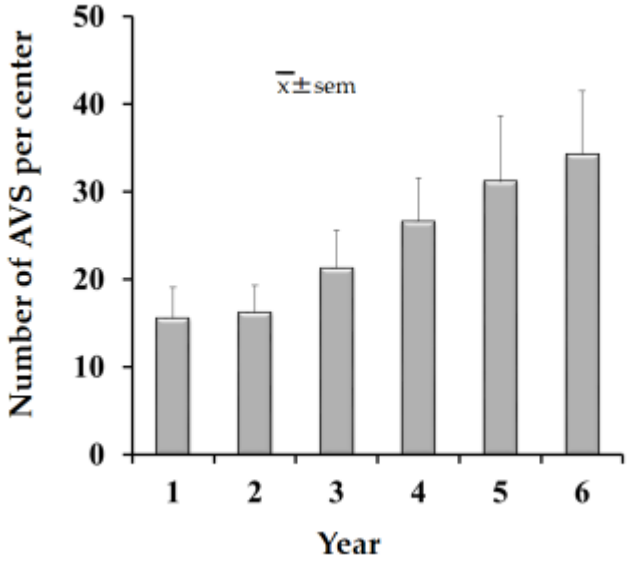

Figure 4. Average number of AVS performed for each center yearly.

4.3 Rate of performance of AVS

From analysis of the data results that AVS was not offered to all patients eligible for adrenalectomy: the percentage of patients with confirmed PA in whom AVS was performed was 77% (median) but ranged widely between 19% and 100% across the centers (Figure 5).

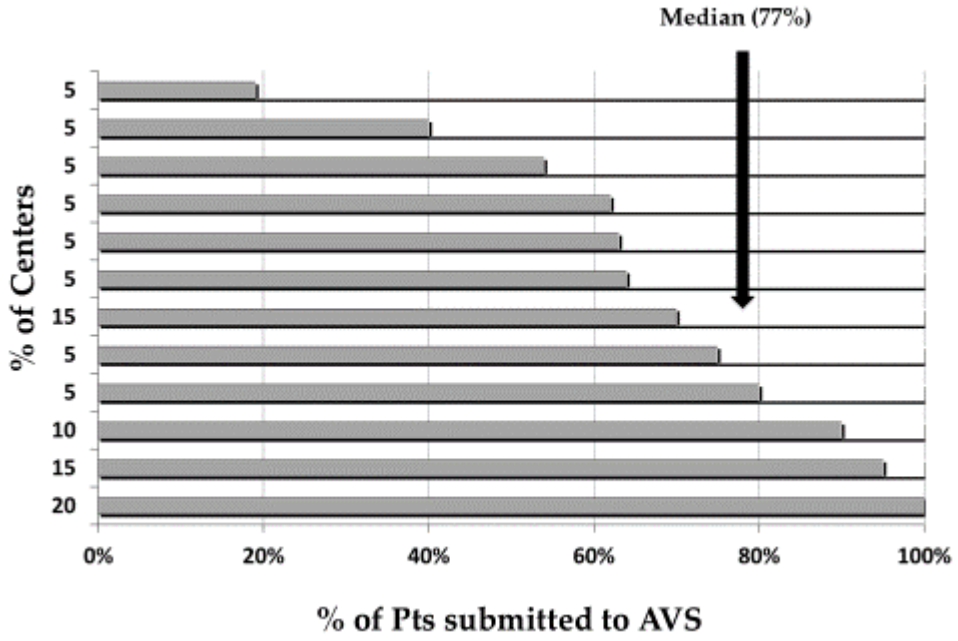

Figure 5. Rate of AVS use in PA patients.

4.4 Performance of AVS

Almost two-thirds (13/20) of the centers reported using sequential catheterization technique (cannulation of the adrenal veins one at a time), while the remaining 7 centers used bilateral simultaneous cannulation. There were no differences in rate of adrenal vein rupture between centers using either catheterization technique.

In terms of dynamic testing, 11 of 20 centers performed AVS with ACTH stimulation, and the rest without any stimulation. Use of ACTH was not more common among centers that used sequential catheterization.

4.5 Number of radiologists performing AVS and rate of complications

The number of radiologists performing the procedure at each center was low, median 2 radiologist/center, range 1 to 7 per center (Figure 6). The number of radiologist at the 20 centers was in total 51, and only at two centers were more than three radiologists (6 and 7, respectively) performing AVS.

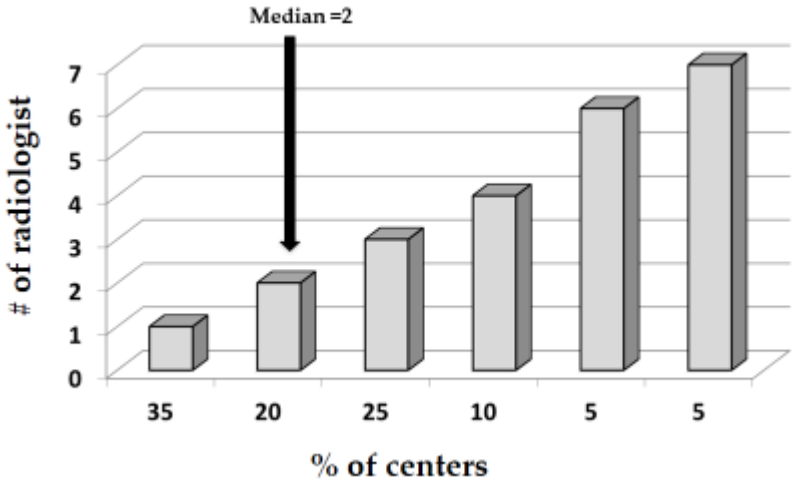

Figure 6. Number of Radiologist performing AVS per center.

The number of ruptures of an adrenal vein during or after AVS, was 16, corresponding to an overall rate of 0.61% (Figure 7). All resolved with conservative treatment and there were no deaths. The rate differed significantly among centers: at one center for instance it was 10-fold higher than the average rate recorded by all others. In this center the procedure was performed by radiologists in training and in rotation. After exclusion of this outlier center from the analysis the rate of adrenal vein rupture fell to 0.51%.

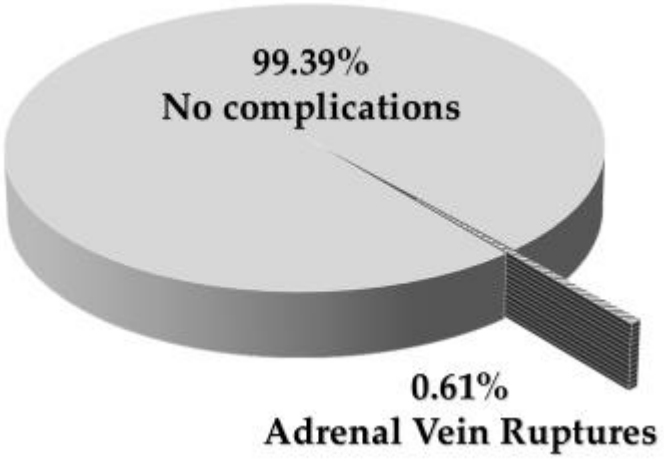

Figure.7. Rate of complications during AVS.

Regression analysis showed that adrenal vein rupture was predicted by the number of AVS performed by each radiologist (β= -0.683; p=0.007) and the number of AVS performed per center (β= 0.831; p=0.002), but not by use of ACTH stimulation, bilateral simultaneous vs sequential AVS technique, or by the continent on which the performing center was located. A regression model with these two predictors (F=7.72, p=0.005) accounted for 40% of adrenal vein ruptures.

4.6 Use of bilaterally vs unilaterally selective AVS results

AVS is aimed at obtaining adrenal venous blood and therefore its results are interpreted based on the assumption that selective blood samples have been obtained from each side. Notwithstanding this, 8 of 20 centers used bilaterally selective studies for diagnosis, while the majority (12/20) used unilaterally selective studies when bilateral results were unavailable. Use of a unilateral study is based on the assumption that if the patient has unequivocal evidence of PA and one side is suppressed (by volume expansion and high BP) then the excess aldosterone should come from the contralateral side. This might be important because it can allow diagnosis even when the right adrenal vein cannot be successfully catheterized; unfortunately, it is largely theoretical and yet unsupported by specific

studies.

4.7 Interpretation of AVS results

Wide variations in the way results are used and interpreted across centers were evident: one center used absolute hormonal values instead of selectivity and lateralization indices, while another center calculated only the lateralization index even for non-selective studies.

The cutoff values for selectivity and lateralization indices varied markedly among those centers that systematically used them. Figure 8 shows the proportion of centers using the different cutoffs for selectivity and lateralization indices under both non-stimulated condition and with ACTH stimulation. Under non-stimulated condition cutoffs were lower than during stimulated sampling for selectivity but not for lateralization. In terms of the selectivity index, the majority of the centers used a cutoff of 2 under non-stimulated conditions and 3 - 5 under ACTH stimulation. For the lateralization index most centers used 2 - 4 under non-stimulated conditions and 2.6 - 4 for ACTH-stimulation. For the selection of the SI and Li cutoffs 67% of the centers relied on values reported in the literature; 11% reported selecting their cutoffs based on a formal assessment of diagnostic accuracy with ROC curves alone, and a further 11% also used the Youden index. In 11% of the centers the selection was made empirically with no formal analysis.

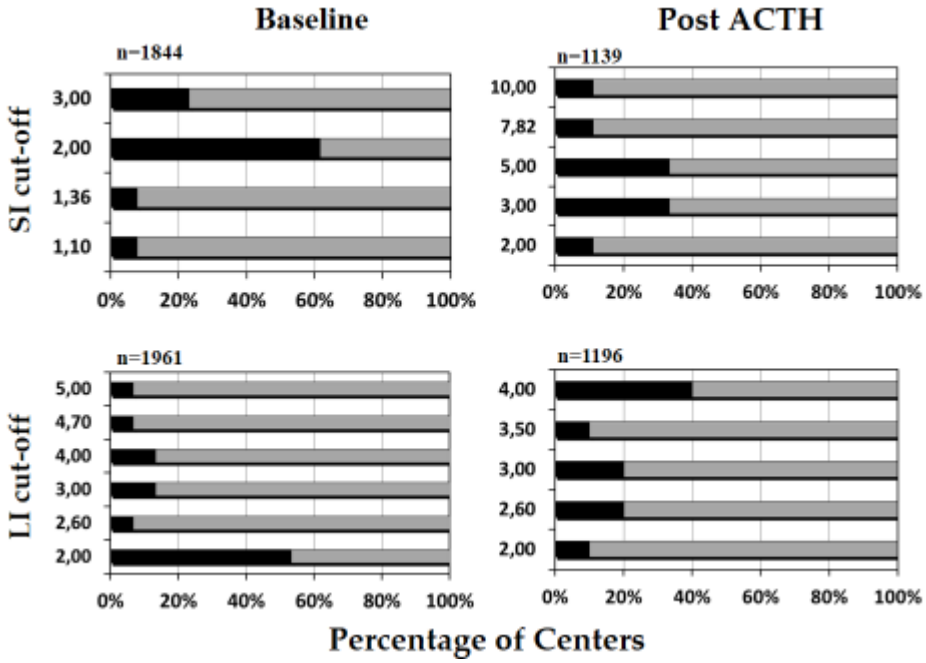

Figure 8. Cut off values used for the SI e LI, baseline and during ACTH stimulation

Use of unilaterally selective AVS results for diagnostic purposes would imply reliance on contralateral suppression, instead of a lateralization index. We therefore next examined how many centers used the former index and whether there was an association between use of unilateral selective AVS and of contralateral suppression. We found that those centers that systematically relied on non-bilaterally selective AVS also used contralateral suppression more frequently than expected by chance (X^2=5.90, p= 0.05). In contrast, no significant correlation could be found between the use of ACTH stimulation and contralateral suppression.

4.8 Costs of AVS

The costs of AVS showed a wide variability among centers and countries, both for the patient and for the insurance or national health care system. The cost ranged

from 80 to 10532 € for health insurance systems and from 0 to 1357 € for the patient, Figure 9.

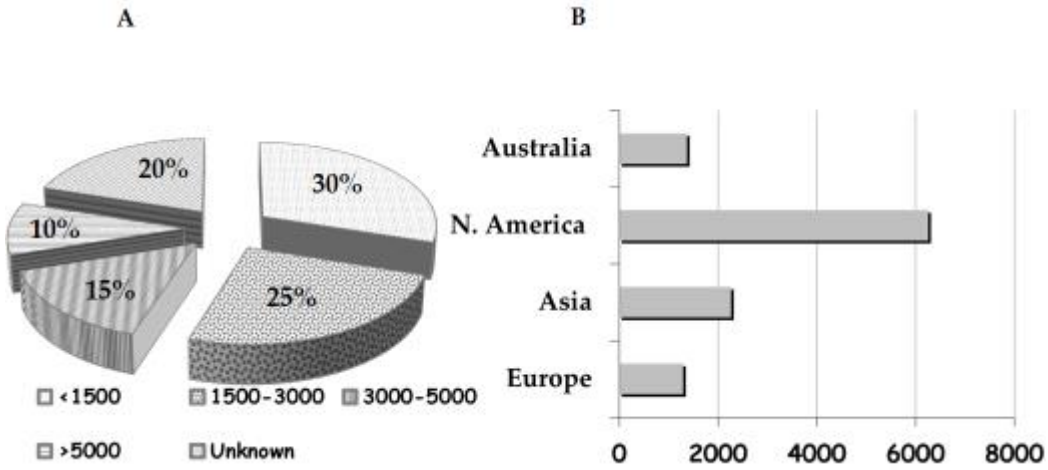

Figure 9. Cost of AVS in euro. Panel A. Cost of AVS across the different centers. Panel B. difference in the cost between different continents.

5. RESULTS AVIS PHASE 2

5.1 Baseline Characteristics of the Patients

Due to the delay of some centers in providing the data, we performed a preliminary analysis with the data available upon locking the database on December, 31st 2013. When we locked the database there was information on 1030 PA patients that undergo to AVS in 15 referral centers worldwide, Table 7.

Center	City	State	Principal Investigator/Invetigators
Università degli studi di Padova, DIMED	Padova	Italy	GP.Rossi, M.Barisa, D.Miotto, T.M..Seccia, A.C.Pessina
Foothills Medical Centre, University of Calgary	Calgary	Canada	G.Kline
General Faculty Hospital, Prague	Prague	Czech republic	J.Widimsky jr.
University of Paris, Hopital Européen Georges Pompidou	Paris	Francia	P.F.Plouin, L.Amar
Tohoku University Hospital	Sendai	Japan	F.Satoh
Heinrich Heine Universität Düsseldorf	Düsseldorf	Germany	L.C.Rump, O.Vonend
National Taiwan University Hospital	Taipei	Taiwan	K.D.Wu, V.Wu
National Hospital Organization, Kyoto Medical Center	Kyoto	Japan	M.Naruse
Medical College of Wisconsin	Menomonee Falls	Wisconsin, USA	S.B.Magill
Medizinische Klinik Innenstadt	Munich	Germany	M.Reincke, C.Degenhart, E.Fischer
Charité Campus Mitte	Berlin	Germany	M.Quinkler
Tokyo Women's Medical University	Tokyo	Japan	A.Tanabe
Radboud University Nijmegen Medical Center	Nijmegen	The Netherlands	J.Deinum, L. Schultze Kool
Dipartimento di Medicina Interna e Specialità Mediche, Medicina 2	Reggio Emilia	Italy	E.Rossi
University Hospital Duesseldorf, Dept. of Endocrinology, Diabetes and Rheumatology	Düsseldorf	Germany	H.S. Willenberg

Table 7. List of the participating centers and investigator in AVIS phase 2.

The baseline data of the patients are shown in Table 8. The average age of the patients was 51 years, 59% were men and 41% women. As regards race, the majority were Caucasian. At baseline the mean value of blood pressure were 155/93 mmHg for systolic and diastolic blood pressure, respectively, and 43.3% of patients were defined with resistant hypertension (defined as blood pressure that remained above treatment goals despite administration of an optimal three drug regimen that included a diuretic). In addition, at baseline, the median serum potassium level was 3.6 mmol/L.

Variable	Patients, N=1030
Age (yrs)	51.4 (16-79)
Gender (male/female), %	59/41
Race: Caucasian	77.7
Asian	18.6
African	2.9
Hispanic, %	0.8
Body Mass Index	28.4
Systolic BP, mmHg	155
Diastolic BP, mmHg	93
HR, beats/min	73
Serum K+ baseline, mmol/L	3.6
Resistant Hypertension, %	43

Table 8. Baseline data of the Patients.

5.2 Blood pressure evaluation on follow up

Overall, at follow up about 19% of patient were cured from high BP while 49% patients had a markedly improved control of their high BP with either pharmacological or surgical treatment. About one fourth of the patients showed only a mild improvement of BP; in 8% there was no improvement of BP.

The analysis evidenced significant differences of BP outcome between gender and race: women had a better outcome when compared to men, $p<0.005$. As regards race, Caucasian and African have better outcome then the Asian and Hispanic, $p<0.005$.

We performed the analysis of BP outcome dividing the patients in 2 group by pharmacological or surgical treatment. In the medically treated group BP was cured in only 1% of cases, markedly improved BP in 53%, and mildly improved BP in 34 %. In 12% of the patients there was no improvement of BP (Table 8). In the adrenalectomized patients, BP was cured in 34% of cases, markedly improved in 45% of patients, and mild improvement in 16% of patients. In 5% of the patient there was no improvement of BP (Table 9).

BP Outcome	% of Patients		Definition
	Adrenalectomy	Pharmacological Treatment	
Cured	34	1	BP < 140/90 mmHg without therapy.
Marked Improvement	45	53	Normotension on the same or reduced number of medications and BP similar to baseline but with a marked decreased (> 2 drugs or >DDD) of medication.
Mild Improvement	16	34	A fall of systolic and/or diastolic BP > 10%, but without achievement of normotension with the same or reduced therapy.
No Improvement	5	12	No fall of systolic and/or diastolic BP and/or need for increased number and/or dose of antihypertensive medications.

Table 9. Blood Pressure Outcome at follow up, with pharmacological treatment or after the adrenalectomy; BP=blood pressure, DDD= daily dose drug.

Moreover, the median percentage of the patients that underwent adrenalectomy was lower (53%) than expected (range: 17% - 90%), with a high variability among centers ($p<0.0001$, $X^2= 82,73$), Figure 10. There was no significant difference between the genders, adrenalectomy was performed in 51.7% of the women and 54.9% of the men. In addition, we found significant differences among races: Caucasian were more frequently submitted to adrenalectomy when compared to Asian, African and Hispanic ($p<0.0001$, X^2 30.36), Figure 11. However, the number of Hispanic e African patients was small, thus exposing this finding to a potential bias.

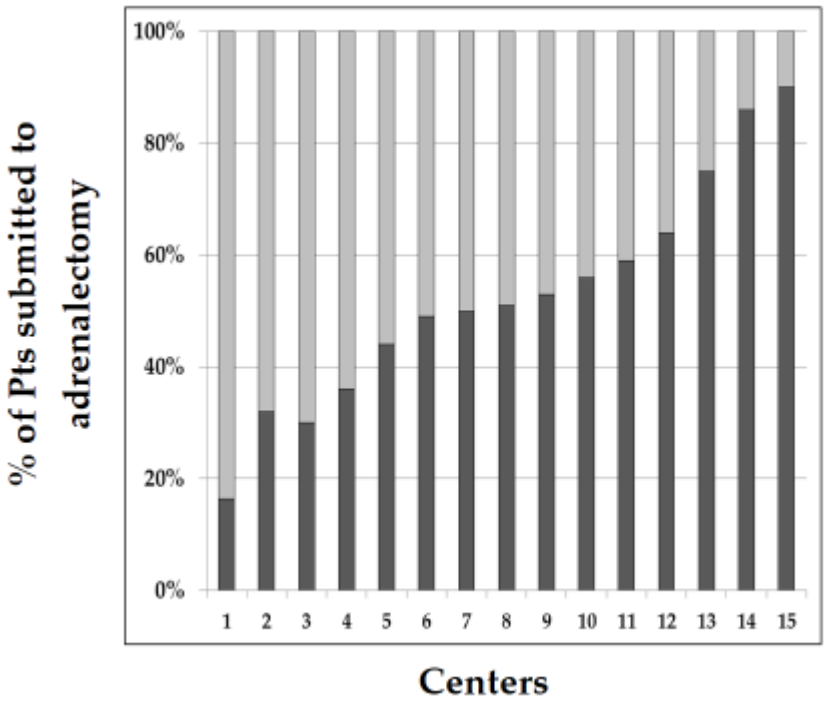

Figure 10. Percentage of patients submitted to adrenalectomy in each center.

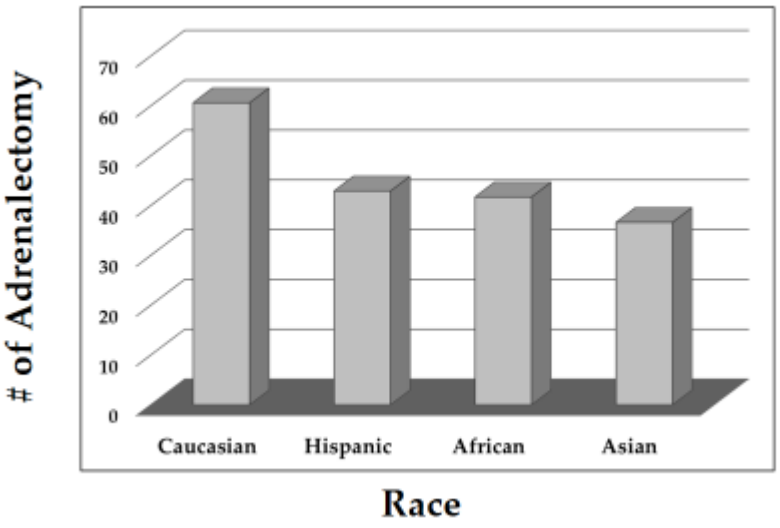

Figure 11. Different rate of adrenalectomy performance by race.

5.3 Improvement of high BP with medical therapy in PA with bilateral aldosterone excess

The analysis evidenced that in the patients with bilateral aldosterone excess there were no cases that showed cured o marked improved control of high BP with pharmacological treatment. At follow up, the majority of the patients (about 75%) showed only a mildly improved control of BP, and 24% of the patients had no improvement.

5.4 Bilaterally selective AVS studies as function of SI cut-off

The cut off values used at the different centers to assess the selectivity of the studies ranged between the 1.1 and 3.0 under non-stimulated conditions and between the 1.1 and 7.82 under ACTH stimulation. We analyzed the rate of bilaterally selective AVS studies as a function of different cutoff values and, as expected, found that the lower SI cutoff values were associated with a higher number of bilaterally selective studies (Figure 12), with no significant difference between the genders.

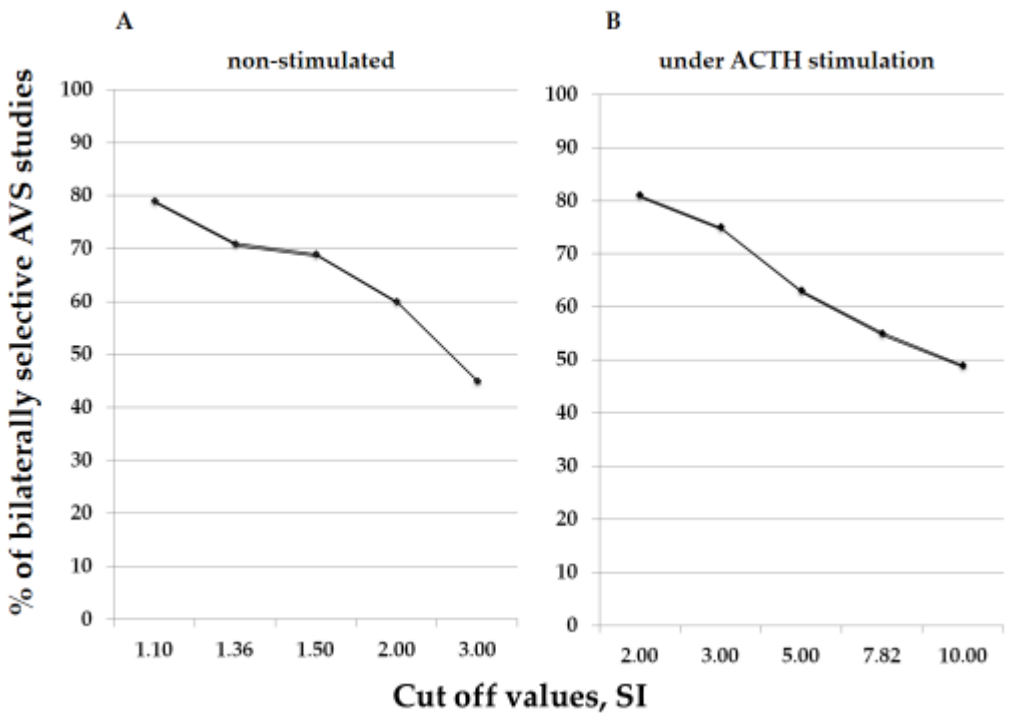

Figure 12. Bilaterally selective AVS studies as a function of the different cutoff values of the SI, baseline (A) and under ACTH stimulation (B).

5.5 Best cut off value for identification of the lateralized aldosterone excess and rate of the patients with lateralized aldosterone excess cured by adrenalectomy

The cutoff values used across the centers to assess the lateralized aldosterone excess ranged between the 2.0 and 5.0 under non-stimulated conditions and between the 2.0 and 4.0 under ACTH stimulation. The analysis of the of rate of lateralized AVS studies as a function of the different cutoffs evidenced that higher LI values was related to lower rate of patients diagnosed with lateralized aldosterone excess (Figure 13).

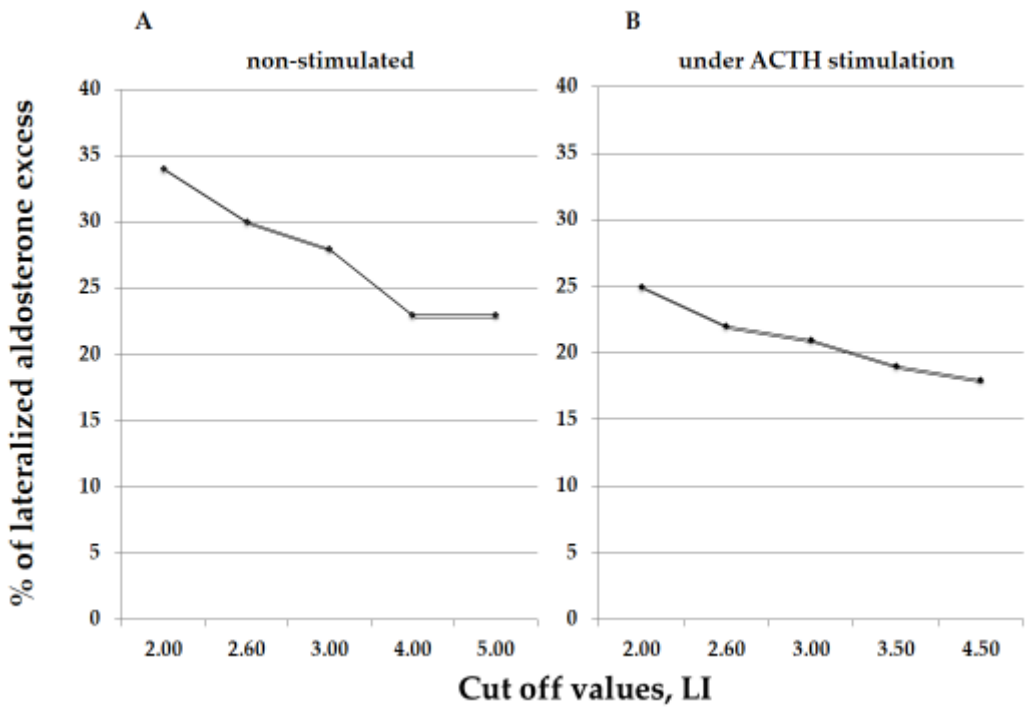

Figure 13. Lateralized aldosterone excess as a function of the different cutoff values of the LI, baseline (A) and under ACTH stimulation (B).

To evaluate the best cutoff value for identifying a lateralized aldosterone excess we examined in the bilaterally selective studies, as determined using different SI cutoffs (1.10. 1.36, 1.50, 2.0 and 3.0), the performance of the different LI used in different centers (2.0, 2.6, 3.0, 4.0, 4.7 and 5.0) for the identification of the patients who were cured o markedly improved at follow up after the adrenalectomy. This analysis evidenced that, at each SI, the higher the LI cutoff value the higher was the rate of patients with cured or markedly improved BP control. However, as already mentioned, the higher the SI cutoffs the lower the number of bilaterally selective patients and therefore the lower the number of patients who could be selected for adrenalectomy.

5.6 Rate of AVS concordant /discordant with results of imaging tests

We performed a Chi square analysis to establish the rate of concordance/discordance between AVS results and CT imaging results for the

diagnosis of lateralized aldosterone excess. Using the cut off value of LI= 4.0 under unstimulated (baseline) conditions we found the rate of concordance was low, both on the right side (26%) and on the left side (45%). After ACTH stimulation the concordance rate was 28% on the right and 49% on the left side.

Moreover, we performed the analysis in patients who had bilaterally selective AVS, evidence of lateralized aldosterone excess, who were submitted to adrenalectomy and were cured or presented markedly improved BP at follow up. This analysis evidence that in this subgroup of patients the rate of concordance between AVS results and CT imaging results was low, 65% on the left side and 57% on the right side. However, the limitation of this analysis entails the low number of patients who could be included, e.g. 202 patients for the left side, and 116 patients for the right side.

6. DISCUSSION

By performing Phase 1 of the AVIS study we were able to gather novel information on the way in which AVS is performed and interpreted at 20 referral centers worldwide. There are several important findings in this study. The first concerns the rate with which the AVS is performed in PA patients at these referral centers worldwide. To some surprise, despite the Endocrine Society guidelines(63) recommendation that AVS should be used in all patients with confirmed PA who are candidate for adrenalectomy and seek surgical cure, the Phase 1 of AVIS showed that AVS was systematically performed in only about 80% (median 77%; range: 19% to 100%) of patients with confirmed PA. Thus, it is altogether evident that even at major referral centers the therapeutic decisions for PA are not based on AVS in about one every five, e.g. a substantial proportion of the PA patients. Such a low AVS rate might depend on several factors including reliance on imaging tests, costs, and lack of expertise in AVS or surgery, or preference to use medical management alone. However, it is of concern that adoption of an imaging-based strategy in the rest of the patients may well be suboptimal, because it bears the risk of denying curative adrenalectomy in about 20% of patients, performing unnecessary adrenalectomy in 15% - 25%, and removing the wrong adrenal in almost 4%, as shown in the Mayo clinic series and in a recent systematic review.(84, 85) The low accuracy of CT alone for diagnosing APA was recently confirmed,(116) even though two retrospective studies claimed that an imaging-based strategy did not perform significantly worse than an AVS-based strategy for selecting the patients for surgery, at least when assessed in terms of blood pressure lowering and correction of hypokalemia,(117, 118). The Mayo Clinic approach is currently to avoid AVS in patients who have unequivocal PA, are younger than 40 years and have a single node in one adrenal and an entirely normal contralateral gland at CT.(127) This is based on the premise that non functioning adrenocortical

adenoma (incidentaloma) are rare before 50 and therefore a single node in a young PA patient should be the culprit of PA. More recently, recognizing the fallacies of this approach a strategy based on score calculated from CT findings, eGFR, and hypokaliemia was proposed to avoid the need of AVS (90). Of note, the results of this study were not confirmed in two following studies, one already published (119) and another one in publication (Scott Trerotola et al, personal communication). Hence, it can be concluded that there are no valid substitutions to AVS at present and therefore the test should be more widely used given that it is safe.

In fact, a second major finding of this study is the minimal (0.61%) overall rate of adrenal vein rupture. Moreover, this rate was probably an overestimate in that one center reported a much higher rate than all the other centers. This anomaly reflected assignment at that center of radiologists in training and in rotation, to perform the procedure, which might account for this unusual rate. The finding that the number of AVS performed by each radiologist is a strong predictor of adrenal vein rupture supports this. Thus, these results disprove the concept that AVS per se is a risky procedure, and do not contradict the Endocrine Society Practice Guidelines recommending that AVS should be offered to all PA patients who are candidates for adrenalectomy and seek a surgical resolution of PA.(63)

The average numbers of AVS performed annually for each center was generally lower than 40 and ranged between 16 in 2005 and 34 in 2010 (Fig. 4), but it showed a trend toward increasing use over time. The number of radiologists performing the procedure at each center was low, on average 2,6. It is likely that the low number of radiologists involved at each procedure, by promoting the development of enhanced expertise in those individuals, contributed to the low rate of complications.

AVS technique and stimulation test

A further important result of this survey is that each center uses their own guidelines and criteria, which testifies the little uniformity in the way each center performed AVS and interpreted its data. Initially AVS involved sequential catheterization - catheterization of one adrenal vein followed by the other adrenal vein. However, since aldosterone and cortisol are release in bursts from the adrenals, which can bias the results of AVS due to difference in timing of blood sampling if sequential catheterization is used Weinberger et al.(110) in 1979 proposed the strategy of maximally stimulating hormone secretion with ACTH infusion. Simultaneous bilateral catheterization was then introduced in 1980 to minimize differences between the sides due to timing (120) and then improved with use of differently shaped catheters on each side.(112) This technique might therefore be performed without cosyntropin stimulation. In spite of this, only 7/20 centers reported adopting simultaneous bilateral AVS, with the majority still relying on sequential catheterization with or without cosyntropin stimulation. Reasons for this may include the added technical difficulties inherent with attempting to successfully catheterize both adrenal veins at the same time.

The Phase I AVIS also showed that centers were almost equally divided between those using and not using cosyntropin stimulation. As stimulation might appear to be necessary when the sequential catheterization is used, we hypothesized that the use of cosyntropin stimulation might be associated with sequential catheterization but this was not the case: performing the sequential sampling was not more common at the centers that used the cosyntropin stimulation.

Clinical use and interpretation of AVS

A basic assumption underlying the appropriate diagnostic utility of AVS is obtaining adrenal vein blood with minimal dilution from extra-adrenal sources.

This means that the proper placement of the catheter into the adrenal vein should be verified. This is generally accomplished by measuring the cortisol step-up between blood from the infrarenal inferior vena cava and blood from each adrenal vein to thereby calculate the selectivity index.(87) Moreover, as the level of both cortisol and aldosterone in adrenal vein blood is highly dependent upon the proximity of the catheter tip to the adrenal cortex, it is necessary to correct aldosterone levels against those of cortisol when assessing lateralization. These reasons support using the selectivity index and the lateralization index for optimal benefit for patients undergoing AVS. Several centers did not use one or both indices; this was unexpected, and of concern that it may lead to sub optimally informed clinical decisions. Of interest, use of plasma metanephrines, which are more stable of the parental catecholamines and showed a much larger concentration step-down between the adrenal vein and the inferior vena cava and therefore could be better than cortisol for assessing selectivity.(126) Prospective studies are necessary to confirm the usefulness and practical feasibility of this approach.

Finally, given that unilateral aldosterone excess would be expected to turn off aldosterone synthesis in (and secretion from) the contralateral adrenal gland, contralateral suppression has also been clinically used to lateralize PA.(121) However, the adrenal gland of subject receiving DOC and salt, e.g. placed in conditions that mimic PA and the adrenal cortex surrounding the APA,(122) can continue to produce aldosterone.(123) Even though it was reported that plasma aldosterone concentrations are 129±60 and 498±84 ng/dl at 0 min and 30 min after ACTH stimulation, respectively, in ten essential hypertensive patients,(81) data on aldosterone plasma levels in adrenal veins or, more importantly, aldosterone/cortisol ratios and concurrent peripheral ratios of healthy volunteers subjected to similar conditions are lacking, presumably for ethical reasons. Thus,

normal values of aldosterone production, and thereby the normal cut-off values, remain uncertain. Accordingly, the use of the contralateral suppression index remains empirically based. In spite of all these considerations contralateral suppression is being used in a two-thirds of the centers; in one center demonstration of contralateral suppression was a sine qua non indication for adrenalectomy.

This study also showed that slightly less that on third of centers systematically used nonselective AVS for diagnostic purposes. Even more worrying an additional 6/20 occasionally used unilaterally selective studies, necessitating a finding of contralateral suppression. Those centers that systematically relied on non-bilaterally selective AVS also used contralateral suppression more frequently than expected by chance.

Cutoff values for AVS

The Phase I of the AVIS study documents a wide variability in the cut-off values used for selectivity, lateralization, and contralateral suppression, both under basal conditions and cosyntropin stimulation. This variability is due in large part to choices of the cut-off values that were experience-guided rather than based on formal assessment of diagnostic performance.

Overall the majority of the centers selected their cutoffs values for both the SI and the LI from the literature and only a minority based their selection on a formal assessment of diagnostic accuracy using ROC curve analysis alone (11%) or combined with the Youden Index (11%). The only studies that have prospectively explored the performance of these indices under non-stimulated conditions to date support the choice of low values for both the selectivity and the lateralization index, but a recent study on patients who required a repeated AVS because of failure on the first attempt would suggest use of more stringent cutoffs for the

selectivity index.(124) The choice of higher cut-off values for lateralization adopted in many centers presumably identifies patients with an increased chance of cure but might exclude from adrenalectomy some patients who are curable.

Costs of AVS

Surprisingly when specifically enquired the lead investigator at many centers was unaware of cost of the procedure: only 12/20 were able to provide accurate information on costs for insurance and/or the patient. Cost varied widely between centers and countries largely reflecting the wide variability in organization of health care system and the minimum level of care provided in different countries.

Rate of bilaterally selective AVS studies as a function of the different cutoff values of the selectivity index

There is no consensus on the cutoff value that determines the success of the adrenal sampling, neither across the referral centers. The cut off value of selectivity index across the participating centers ranged between the 1.1 and 3.0 under non-stimulated conditions and between the 1.1 and 7.82 under ACTH stimulation. We analyzed the rate of bilaterally selective AVS studies as a function of different cutoff values.

As expected, we found that the lower was the cutoff value of SI , the higher was the number of bilaterally selective studies. This evidenced that use of increasingly restrictive cutoffs, markedly decreased the number of AVS studies that are bilaterally selective.

Best cutoff value for the identification of the lateralized aldosterone excess

There is no consensus on the cutoff value of lateralization index, which assesses the lateralized aldosterone excess. Across the centers participating in this study this cut off ranged between the 2.0 and 5.0 under non-stimulated conditions and between the 2.0 and 4.0 under ACTH stimulation. The analysis of the rate of lateralized AVS studies as a function of the different cutoffs evidenced that higher LI values were related to lower rates of patients diagnosed with lateralized aldosterone excess. This analysis confirmed that at each SI, the higher was LI cutoff value the higher was the rate of patients with cured or markedly improved BP control.

However, the higher the value of SI cutoffs the lower was the number of bilaterally selective patients, and therefore the lower the number of patients who could be selected for adrenalectomy. Adoption of a high cutoff for defining a lateralized aldosterone excess resulted in exclusion of a fairly large number of patients, who could be cured, from adrenalectomy.

Improvement of hypertension with medical therapy or adrenalectomy

We analyzed AVS data of 1.030 patients to establish the improvement of high BP at follow up, obtained with medical therapy in patients with bilateral aldosterone excess or with adrenalectomy in patients with lateralized aldosterone production. We distinguished four possible BP outcome: cured, markedly improved, mildly improved and no improvement. We found that in the patients with bilateral aldosterone excess treated with pharmacological therapy, at follow up, three-quarters of the patients showed only a mildly improved control of BP, and 24% of the patients had no improvement. Moreover, medical treatment, did not lead to cured or markedly improved outcome of BP.

At variance, in the patient submitted to adrenalectomy the BP was cured in 34% of cases, markedly improved in 45% of patients, and mild improved in 16% of patients. In only 5% of the patients there were no improvement of BP. This finding confirm the already known fact that removing of aldosterone excess by adrenalectomy normalizes BP despite withdrawal or tapering of antihypertensive therapy in up to 82% of the patients.(36, 37)

Moreover, the analysis shown that only about half of the patients submitted to AVS underwent adrenalectomy. There are no significant differences of gender, but significant differences by race: Caucasians were most frequently treated with surgery then Asians (X^2=30.36, p<0.0001). In some Asian centers of this study the SI was not used and the cut off values used to determine lateralized aldosterone excess were higher then in other centers, a finding that can explain this differences.

All these considerations support the recommendation of Endocrine Society guidelines to perform the screening for PA and if indicated to be submitted to AVS, gold standard exam for distinction between surgically-curable and surgically-non curable causes. The evidence that adrenalectomy cure or markedly improve BP in about 80% of the cases, evidence the possibility to suspend or at least to reduce the medical treatment. Considering that PA is the most common cause of endocrine hypertension, and since PA patients are at increased risk of target organs damage and cardio-renal complications, their early identification is crucial for preventing these complications and for reversing preexisting cardiovascular damage by specific therapy. This will be also correlated to a lower cost for the national health system.

Rate of AVS concordant /discordant with results of imaging tests.

The analyses performed in phase 2 of the study confirm the well known low accuracy of imaging test for diagnosing lateralized aldosterone excess. This study evidenced that the rate of concordance between imaging data e AVS data was low: using the cut off value of LI = 4, we found the concordance was less than 50% on the left side and less than 30% on the right side, both at baseline and after stimulation with ACTH.

The low accuracy of imaging data alone was confirmed by analyses of subgroups of patients with confirmed diagnosis of APA by the four corner criteria approach. In this APA patients the imaging data not identified about 35% of APA on left side and about 43% on the right side. However, the limitation of this analysis entails the low number of patients who could be included.

6. CONCLUSIONS

Despite the high prevalence of primary aldosteronism, and the fact that AVS is crucial for discriminating between its two major subtypes and therefore for selecting the most appropriate treatment, marked differences remain in the use of AVS even at major referral centers. The results of Phase 1 of the AVIS study with the strength of a large size entailing data from 2604 AVS procedures documented the existence of a low complication rate and marked dissimilarities in the percentage of use, protocols, and interpretation and cost of AVS among the major referral centers around the world.

Importantly, overall the rate of major complications was minimal which demonstrates that although being generally regarded as a risky procedure, AVS is in truth safe in experienced hands. This observation therefore supports the Endocrine Society recommendation that AVS should be used in all patients with confirmed PA who are candidate for adrenalectomy and seek surgical cure. Despite the introduction of bilateral simultaneous catheterization technique by some centers, almost two thirds of the centers continue to use the sequential technique and perform catheterization during cosyntropin stimulation, while only few centers use ACTH stimulation with bilateral simultaneous catheterization.(125) Moreover, some centers rely on measurement of absolute hormonal values rather than indices of selectivity, lateralization, and contralateral suppression.

In the second phase of the study, analysis of the individual patients data, evidence that a choice of more restrictive values of the selectivity and the lateralization index, translates to increased rates of cured and markedly improved BP at follow up, but this at the price of the exclusion of a greater number of patients from adrenalectomy.

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