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Imaging by CT and MR in Primary Aldosteronism: in a Pilot Study, the AVIS-2-IM study and the AVIS-2-Young study

Coordinatori: Ch.mo Prof. Claudio Letizia (Sede di Roma); Ch.mo Prof. Gian Paolo Rossi (Sede di Padova)

Supervisore: Ch.ma Prof.ssa Raffaella Motta

Dottorando: Filippo Crimì

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Chapter 1

Introduction and outline of the thesis

INTRODUCTION

High blood pressure is one of the most frequently detected condition in the general population, with a supposed prevalence in the world of 1 billion people, that is expected to rise in the next years [1].

Usually arterial hypertension does not induce any symptoms but it can lead to cardiovascular diseases, such as myocardial infarction, stroke, heart failure, renal failure and peripheral artery disease; therefore, its diagnosis and treatment is fundamental to avoid these harmful complications[2].

Arterial hypertension can be classified as primary/essential, if the cause is unknown, or secondary, if there is a underlying cause of disease [3,4].

The reported prevalence of secondary arterial hypertension varies from 10% in the general population studies to 30% in hypertensive patients evaluated in referral centers; these differences are probably due to the not widespread application of specific tests in the population in a general care setting [4].

It is crucial to identify secondary causes of hypertension, especially if these are curable and therefore the organ damages provoked by the high blood pressure can be stopped. The causes of secondary hypertension are: renal parenchymal disease, renovascular disease, primary aldosteronism, obstructive sleep apnea, drug or alcohol induced hypertension, pheochromocytoma or paraganglioma, Cushing's syndrome, hypothyroidism, hyperthyroidism, aortic coarctation, primary hyperparathyroidism, congenital adrenal hyperplasia, mineralocorticoid excess other than primary aldosteronism and acromegaly [5].

Primary Aldosteronism (PA) is characterized by an increased production of aldosterone without activation of the renin-angiotensin-aldosterone system and is the most common form of curable endocrine hypertension, with a reported prevalence in hypertensive patients of 11% [6]. In this disease, the inappropriately high plasma levels of aldosterone cause sodium retention, increased extraction of potassium and suppression of plasma renin leading to an increase in blood volume and consequently high blood pressure, cardiovascular damage and hypokalemia [7]. Moreover, aldosterone excess causes fibrosis, oxidative stress and tissue inflammatory changes, independently from the levels of blood pressure, thus leading to a cardiovascular morbidity and mortality higher than sex and age-matched

essential hypertensive patients that show the same blood pressure levels [8]. The PA can be sporadic or familial, with the first being much more frequent than the second. Four types of familial PA have been reported, involving mutations of CYP11B1/CYP11B2, CLCN2, KCNJ5 and CACNA1H [9]. In the sporadic form of PA, the source of aldosterone hypersecretion can be an aldosterone producing adenoma (APA), an adrenal hyperplasia with aldosterone-producing cell clusters, an aldosterone producing carcinoma or an ovarian aldosterone-secreting tumor [7]. The PA can be furtherly subdivided in unilateral PA in case of a hypersecretion of aldosterone in a single adrenal gland (unilateral adrenal hyperplasia, aldosterone producing carcinoma, unilateral APA) or bilateral PA in case of hyperproduction of aldosterone from both adrenal glands (bilateral adrenal hyperplasia, bilateral APA, etc.) [7].

The patients who present the following characteristics should undergo work-up for PA diagnosis: I) blood pressure higher than 150/100 mmHg or blood pressure higher than 140/90 mmHg resistant to 3 anti-hypertensive drugs or controlled blood pressure (<140/90 mmHg) by using 4 or more anti-hypertensive drugs; II) high blood pressure and incidental adrenal nodule; III) high blood pressure and a family history of precocious high blood pressure or cerebrovascular events in young age (< 40 years); IV) high blood pressure and sleep apnea; V) high blood pressure in first degree relatives of patients affected by PA [10].

The first step in the work-up for PA diagnosis is based on demonstration of inappropriately high excretion of aldosterone and low renin levels. In the clinical practice, the more accurate and widely used test for the identification of PA is the calculation of the aldosterone to renin ratio (ARR), starting from the measurement of the plasma aldosterone concentration renin activity [7]. The measurement of plasma potassium is not specific for the diagnosis of PA, indeed only less than one third of PA patients show hypokalemia at diagnosis [6]. After the detection of an elevated ARR, in some centers confirmatory tests as captopril challenge test and saline infusion test are required, even if in a large study was demonstrated that they do not add any diagnostic gain over ARR [11]. Afterwards, the guidelines suggest to perform a second level imaging technique (contrast-enhanced CT or MRI) in

order to exclude adrenal masses especially aldosterone producing carcinoma, to help interventional radiologists in the adrenal veins sampling (AVS) depicting the anatomy of adrenal glands venous drainage and to guide surgeons for treatment planning [10,12].

The AVS is the key test to distinguish between unilateral and bilateral type of PA and all PA patients that can be candidate for surgery should undergo this kind of test [7].

In this radiologic interventional procedure two catheters are introduced in the femoral vein to reach the left and right adrenal vein, thus blood samples from right adrenal vein, inferior vena cava and left adrenal vein are collected and, therefore, any lateralization of the hypersecretion of aldosterone can be detected. In case of a unilateral hypersecretion of aldosterone the patient can be submitted to adrenalectomy, thus removing the cause of aldosterone elevation, while if a bilateral hypersecretion of aldosterone is detected the patient can be addressed to medical therapy with mineralocorticoid receptor antagonists [7].The main issue about AVS is that this test is expensive and requires an expert radiological team to be performed and, therefore, it is not widely available. Hence, different methods for the subtyping of PA have been proposed to skip AVS, such as functional imaging [13,14] and clinical prediction scores [15–17], with variable success. Different authors studied imaging reliability in the identification of unilateral and bilateral forms of PA, drawing the conclusion that it is not accurate enough to refer patients directly to surgery only on the basis of imaging results. Young WF et al observed that in showed unilateral disease in 22% of patients with normal CT finings AVS found a unilateral PA while in 25% of cases with a unilateral nodule at CT AVS detected bilateral or contralateral disease at AVS [18]. Kempers MJE et al in a systematic review found that in 37.8% of PA patients CT/MRI results were discordant with AVS [19]. Similarly, other groups detected a not satisfying accuracy of imaging in PA subtyping [20,21] indicating that a nodule detection at CT or MRI is not always correlated to a ipsilateral hypersecretion of aldosterone and vice-versa normal appearing adrenal glands at imaging can hide anyway a small APA or clusters of aldosterone producing cells. Nevertheless, based on the premises that the prevalence of non-secreting adrenal nodules is low in young patients [22], Young WF [23] and Lim V [20] proposed to directly address to surgery skipping AVS

patients younger than 40 years old [23] or younger than 35 years old with a unilateral hypodense nodule at CT 10-20 mm in size and a contralateral normally appearing adrenal gland [20]. This proposal, although based on a small sample of patients, was reported in the Endocrine Society Practical Guidelines [10].

OUTLINE OF THE THESIS

The present thesis is a collection of the main research studies concerning imaging in PA, conducted during the PhD Course.

In the **second** and **third chapters**, starting from the large cohort of PA patients recruited in the AVIS-2 study, we investigated respectively: a) if imaging by CT and/or MR could permit an accurate detection of PA and identification of unilateral PA; b) if a unilateral hypodense adrenal nodule alongside a contralateral normally appearing adrenal gland at imaging, can allow an accurate identification of unilateral surgically curable PA in patients aged 45 years or younger.

In the **fourth chapter** I discussed the limitation of imaging in the diagnosis of PA and in its subtyping and which are the new possible algorithms and applications of imaging in PA, starting from the results obtained in the studies shown.

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Chapter 2

Imaging for Identification of Surgically Curable Primary Aldosteronism in AVIS-2 IM, a Large International Study

Gian Paolo Rossi[1]; Filippo Crimi[2]; Giacomo Rossitto[1]; Laurence Amar[3,4]; Michel Azizi[3,5]; Anna Riester[6]; Martin Reincke[6]; Christoph Degenhart[6]; Jiri Widimsky Jr.[7]; Mitsuhide Naruse[8]; Jaap Deinum[9]; Leo Schultze Kool[10]; Tomaz Kocjan[11]; Aurelio Negro[12]; Ermanno Rossi[12]; Gregory Kline[13]; Akiyo Tanabe[14]; Fumitoshi Satoh[15]; Lars Christian Rump[16]; Oliver Vonend[16]; Holger S. Willenberg[17]; Peter J. Fuller[18]; Jun Yang [18]; Nicholas Yong Nian Chee[18]; Steven B. Magill[19]; Zulfiya Shafigullina[20]; Marcus Quinkler[21]; Anna Oliveras[22]; Vin Cent Wu[23]; Zuzana Kratka[7]; Giulio Barbiero[2]; Teresa Maria Seccia[1]; Michele Battistel[2].

[1] University of Padova, Department of Medicine-DIMED, International PhD Program in Arterial Hypertension and Vascular Biology (ARHYVAB), University Hospital, Padova, Italy;

[2] University of Padova, Department of Medicine-DIMED, Institute of Radiology, University Hospital, Padova, Italy;

[3] AP-HP, Hôpital Européen Georges Pompidou, Hypertension Unit, Paris, France;

[4] Faculty of Medicine, Université de Paris, INSERM, UMR970, Paris-Cardiovascular Research Center, France;

[5] INSERM, CIC1418, F-75015, Paris, France;

[6] Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, LMU München, Germany;

[7] 3rd Department of Medicine, Charles University Prague, General Hospital, Czech Republic;

[8] Department of Endocrinology, National Hospital Organization Kyoto Medical Center, Clinical Research Institute, Japan;

[9] Radboud University Medical Center, Department of Internal Medicine, Nijmegen, Netherlands;

[10] Radboud University Medical Center, Department of Radiology and Nuclear Medicine, Nijmegen, Netherlands;

[11] Department of Endocrinology, Diabetes and Metabolic diseases, University Medical Centre, Ljubljana, Slovenia;

[12] Department of Internal Medicine, Azienda Unità Sanitaria Locale, IRCCS Arcispedale S. Maria Nuova, Hypertension Unit, Reggio Emilia, Italy;

[13] Foothills Medical Centre, University of Calgary, Canada;

[14] Department of Diabetes, Endocrinology and Metabolism, National Center for Global Health and Medicine, Tokyo, Japan;

[15] Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Hospital, Sendai, Japan;

[16] Heinrich Heine University Düsseldorf, Nephrologie, Germany;

[17] Division of Endocrinology and Metabolism, Rostock University Medical Center, Germany;

[18] Endocrinology Unit, Monash Health, Clayton, VIC, Australia;

[19] Medical College of Wisconsin, Endocrinology Center, North Hills Health Center, Menomonee Falls, WI, USA;

[20] Department of Endocrinology, University of St. Petersburg, Russia;

[21]Endocrinology in Charlottenburg, Berlin, Germany;

[22] Department of Nephrology, Hypertension Unit, Hosp. del Mar Universitat Autònoma de Barcelona, Spain;

[23] Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan;

Submitted

ABSTRACT

Current guidelines recommend adrenal glands imaging for the work-up of primary aldosteronism (PA), even though its diagnostic performance has never been established in large multi-center studies. In a large international study that enrolled PA patients seeking for surgical cure (AVIS-2-IM) in 19 tertiary referral centers in Europe, Asia, Australia, and North America, we assessed the accuracy of adrenal imaging by CT and/or MR following the STARD recommendations, i.e. using as gold reference a conclusive diagnosis of unilateral PA. All AVIS-2-IM patients underwent imaging followed by adrenal vein sampling (AVS). A positive imaging result was defined as a visible adrenal nodule with a maximum diameter ≥ 5 mm. The accuracy of imaging for identification of the culprit adrenal was estimated by area under the receiver operator characteristics (ROC) curve using unilateral PA diagnosed at follow-up post-adrenalectomy as reference. Information on imaging results was judged to be adequate in 80.7% of the patients. Of them, 33.9% showed no detectable nodules and 7.1% bilateral nodules. Corresponding rates in those with unilateral surgically cured PA were 20.1% and 5.5%, respectively. Thus, overall imaging did not identify unilateral nodules in 41% of the cases and did not detect the culprit adrenal in 25.6%.

While testifying the low sensitivity of imaging for detecting nodules in PA and for identifying the culprit nodule, these data by no means support the usefulness of CT and/or MR for identification of PA and its subtypes.

INTRODUCTION

Primary aldosteronism (PA) is the most common surgically curable form of secondary arterial hypertension [1–3], but adrenalectomy requires demonstration of a unilateral cause of the disease. Because for subtype differentiation, all current guidelines recommend adrenal vein sampling (AVS) [4–7], only a small proportion of the eligible patients are surgically treated and ultimately cured, being technically challenging and difficult to interpret AVS, is available only in major referral centers worldwide [8]. To by-pass this "bottleneck" in the subtyping of PA patients, non-invasive strategies, such as functional imaging[9,10], and clinical prediction scores[11–14] have been proposed, but, on the whole, their success has been variable and inconsistent across studies[11–14]. As regards imaging, thus far its accuracy has been investigated in single-center studies[13,15–17], and a meta-analysis[18] of studies that, on the whole, did not follow the STARD criteria for assessment of diagnostic accuracy[19] in that they lacked a conclusive unambiguous diagnosis concerning the presence of unilateral PA. Moreover, the only prospective study that compared CT imaging with an AVS-based strategy to address patients to adrenalectomy failed to show differences between the groups in the intensity of antihypertensive medication required to control blood pressure, and quality of life indexes [20], but, this was an underpowered study [21].

The Adrenal Vein Sampling International Study (AVIS-2) recruited patients submitted to AVS in major referral centers in four continents, because of their wish to achieve surgical cure of PA[22]. The majority of the patients eventually received a conclusive diagnosis regarding the presence or absence of unilateral PA, based on biochemical cure after adrenalectomy. This permitted to assess the accuracy of imaging using such gold reference index, according to the STARD guidelines [19]. The clinical outcomes, and different cutoff values for assessing success, e.g. bilateral selectivity and their impact on lateralization of AVS, recorded in AVIS-2, were reported in detail elsewhere [22,23].

In the AVIS-2-Imaging sub-study (AVIS-2-IM), we investigated if imaging, as performed by CT and/or MR at expert referral centers for PA, could allow an accurate detection of PA and identification of unilateral PA.

METHODS

The AVIS-2 study was conceived in 2012 as an observational multicenter study to create a large database of individual patient AVS studies performed worldwide. After registration (at clinicaltrials.gov, NCT01234220) the protocol was amended to reach the target recruitment of 1500 PA patients by permitting inclusion of those submitted to AVS between 2000 and 2015. The recruitment ended in 2015 and the database was locked in 2017 in order to allow gathering the follow-up data. Details of the methodology used have been previously reported in details [22,23] and are recapitulated in the Supplemental material.

Inclusion/exclusion criteria

The participating centers were selected based on their prior participation in the AVIS-1 study [8] and/or publications on PA and AVS, as identified through a PubMed search [22,23].

All procedures followed the principles of the Helsinki Declaration and the protocol of the study was approved by the Institutional Ethics Committees.

The patients' inclusion criteria were: a) age ≥ 18 years; b) center's agreement to participate in the data collection; c) approval of the Ethics Committee. The only exclusion criteria were the lead investigator's unwillingness to participate in the study and/or the lack of local Ethics Committee's approval.

Definitions and assignment to treatment

Unilateral PA was diagnosed in the patients who underwent AVS-guided unilateral laparoscopic adrenalectomy and showed biochemical cure at follow-up.

Biochemical cure was defined as normalization of plasma aldosterone concentration (PAC), plasma renin activity, and serum K^+ levels at follow-up post-adrenalectomy [24]. This was used as gold diagnostic reference used to assess the accuracy of imaging in a receiver operator characteristics (ROC) curve analysis.

AVS-guided adrenalectomy was defined as surgery performed after demonstration of lateralized aldosterone excess at bilateral adrenal vein catheterization success.

For the purpose of this study, bilateral success was defined as a selectivity index (SI) ≥ 2.0 under unstimulated conditions, and/or ≥ 4.0 post-cosyntropin on both sides, as defined in a consensus of experts [25].

Patients with no lateralization defined as a lateralization index (LI) on the dominant side ≥ 2.0 on a bilaterally successful AVS, and those who were not biochemically cured after unilateral adrenalectomy, were classified as bilateral PA.

Adrenal nodules were defined as nodular lesions on imaging with a largest diameter ≥ 5 mm. This definition was based on a pilot study that showed inconsistent detection of nodules smaller than 5 mm maximum diameter when examined by different experienced radiologists (Supplemental material).

Data collection

Data were gathered with a predefined web-based platform created ad hoc for on-line data collection, which exploited anonymization to warrant privacy protection (available as Supplemental material), as described [22]. Appropriate filters were implemented to prevent input of values that were not biologically plausible and/or were in wrong unit of measures. Data were stored securely in a server protected by firewalls at the coordinating center. The PI had full access to the dataset with username and password; each local PI had access to his/her center's database for quality control purposes.

Allocation of recruited patients to surgical or medical treatment was based on decisions of investigators at each participating center. In order to depict current real-life practice in imaging at these centers the reading of the imaging results was note centralized and left to each center practice.

Data handling and statistical analysis

After locking the database, the data were checked for internal consistency; emerging queries were clarified with each center's lead investigator. Data were then harmonized to a standard format before

undertaking the statistical analysis, as described in detail [22]. Univariate and multivariate outliers were identified following the procedure of Tabachnick and Fidell [26], and excluded from the analysis. Results are expressed as mean ± SD, or median and interquartile range (IQR), as appropriate. Significance was set at $p<0.05$. Continuous variables were tested for normal distribution with Kolmogorov-Smirnov test; in case of skewed distribution, log-transformed data were used. Comparisons were performed with parametric or nonparametric tests (Wilcoxon), as appropriate. Pearson's χ^2 test was used for analysis of categorical variables.

SPSS for Mac™ (vers. 26 for Mac, IBM-SPSS Bologna, Italy), Prism™ (vers. 8.4 for Mac, GraphPad Software, La Jolla California USA), and MedCalc™ (MedCalc Software Ostend Belgium, vers. 15.8) were used for the statistical analysis.

RESULTS

The PA patients were recruited in 19 centers located in 4 continents Europe (including Russia), Asia (including Japan), North America, and Australia. Upon database locking on January 15th 2017, individual data were available for 1820 patients; however, in order to examine a cohort reflecting current practice, it was decided to limit the analysis to studies performed from 2000 to 2015, which left 1625 patients to be analyzed (Figure 1). Of them, 1067 were recruited retrospectively from 2000 to 2012 and 558 were enrolled prospectively from 2013 to 2015. The comparison of clinical features between these groups showed no statistically significant difference and is reported in the Supplemental Material.

Adequate information on the presence or absence of nodules and their size was available in 1311 patients, whose overall, characteristics did not differ significantly from those of the whole AVIS-2 study population [22]. In 178 additional patients the presence, but not the size, of the nodules was also ascertained. We performed a sensitivity analysis that included these patients, which is provided as Supplemental result.

Features of the population

Table 1 shows the demographic features of the cohort of patients with complete imaging data. Briefly, the average age of the patients was 50.8 ± 11.0 years and 60% were men; Caucasians (74.1%) prevailed over the other ethnicities (Asian 22.4%; African and African-American 3.1%; Hispanic 0.5%). The average BMI was 28.3 ± 5.4 Kg/m^2; overweight-obesity was common with 35.0% of the patients being over 30.0 Kg/m^2.

Imaging results

Per protocol and following the guidelines [4,6], imaging was performed prior to AVS in all patients. CT was performed in 1210 patients (92.3%) and MR in 169 (12.9%); 68 (5.2%) underwent both imaging tests (Figure 1). On the whole, imaging did not identify the culprit adrenal in 41% of the patients. 33.9% of the patients had no detectable nodules and 7.1% of the patients showed bilateral nodules (Figure 2). Nodules were detected more commonly (about two-fold) in the left than the right side (39.1% vs 19.9%; p<0.0001).

The results were concordant between CT and MR in 63.2% in the small subset (n= 68) of patients submitted to both imaging techniques (see Table 4 Supplemental).

Final diagnosis and concordance with imaging

Imaging data and final diagnosis were available in 70.1% (n=919/1311) of the patients. Unilateral PA was diagnosed in 57.5% (n=528) of the patients who had imaging data; 42.5% (n=391) had bilateral PA. These patients did not differ from the entire cohort, indicating that they were representative of the whole AVIS-2-IM and AVIS-2 Study population (Table 5 Supplemental).

Based on the final diagnosis, the patients with unilateral PA were divided into left (n= 328) and right (n=200) PA. Discordance between the final diagnosis and the adrenal pathology identified at imaging, occurred in 33.4% (n=307) of the patients (Table 2). Among the 528 patients with imaging data who received a final diagnosis of unilateral PA, the diagnosis was missed at imaging in 135 (25.6%) patients, who demonstrated either no (n=106, 20.1%) or bilateral nodules (n=29, 5.5%)

(Table 2). If imaging alone had been used for subtype differentiation, these patients would have been denied a potentially curative operation. Of the 391 patients with a diagnosis of bilateral PA, 147 (37.6%) showed a unilateral nodule on imaging, which posed them at risk of inappropriate operation if imaging alone would have informed the clinical decision making.

Culprit nodule size and final diagnosis

The distribution of nodules at imaging (Figure 3) showed a skewed distribution ($p<0.001$) at Kolmogorov-Smirnov normality test, and a median size of 14.0 mm (range 5.0-60.0 mm).

We sought for determining the accuracy of imaging-detected nodules and the size that provided the highest accuracy, i.e. the best combination of sensitivity and specificity by a receiver operating characteristic (ROC) curve and Youden's index analysis (Figure 4), using the final diagnosis of unilateral PA as categorical status. The area under the ROC curve, calculated as an index of overall accuracy, albeit differing from that under the identity line ($p= 0.01$), was low, i.e. 0.553 (95% CI: 0.517-0.589). The nodule size associated with the highest diagnostic accuracy at Youden's index analysis was 10 mm (95%CI: 7.0-15.0 mm). At this size, the specificity was 33% (95% CI: 28.3%-37.8%) and the sensitivity was 77% (95% CI: 72.8%-81.6%).

DISCUSSION

The imaging sub-study (AVIS-2-IM) aimed at capturing real-life practice in adrenal imaging of PA in four continents in a large population of patients likely comprising a cohort presenting with a florid clinical phenotype, because they were referred to leading centers owing to their wish to achieve surgical cure.

Of interest, CT was the imaging technique most commonly used in AVIS-2-IM: 74% of the patients underwent CT and only 10% MR, indicating that it was more widely available in most municipalities, because is less time-consuming, and less expensive. CT and MR furnished concordant results in around 63% of the PA patients, thus confirming a moderately comparable yield, in keeping with results of a smaller pilot study [27]. Conclusions on technique equivalence should, however, be drawn

with caution, because only 68 patients underwent both imaging tests, which suggests the possibility of a selection bias in that only those with equivocal CT results were submitted to MR; moreover, the comparison between imaging techniques was not a predefined aim of the AVIS-2-IM study.

The first remarkable finding of this study was that overall current imaging techniques did not detect adrenal nodules in 33.9% of the PA patients (Figure 1). Hence, imaging would miss about one third of the PA cases; moreover, bilateral nodules were observed in 7.1% of the patients. Hence, imaging did not allow identification of the culprit adrenal in about 41% of the cases even in selected PA patients with a florid PA phenotype referred to experienced centers.

Considering the high prevalence of adrenal nodules in hypertensive patients, which increases with aging [28,29], and the fact that the majority these nodules are not hormonally active [28,29], such use of imaging would likely result into a high rate of false positives.

In this study the majority (70.1%) of the PA patients eventually received a conclusive diagnosis of unilateral PA, based on demonstration of biochemical cure at follow-up post-adrenalectomy. Starting from such a gold reference, we could therefore assess, for the first time in a large multicenter dataset, the diagnostic accuracy of imaging. We found that the area under the receiver operator characteristics (ROC) curve, a proxy of diagnostic accuracy, of imaging was 0.553 (95% CI: 0.517-0.589), which albeit significantly different from the area under the identity line (p=0.01) corresponded to a tiny (5.3%) diagnostic gain over 'tossing a coin'.

The nodule's diameter that performed best for identification of the culprit adrenal, and thus of unilateral PA, was 10 mm (95% CI 7.0-15.0 mm), the size that was previously used in a clinical prediction score for identifying unilateral PA [11], and is endorsed by the Endocrine Society guidelines as cutoff for distinguishing between macro- and micro-adenoma [30]. This size, while carrying a 77% sensitivity implied only a 33% specificity, which indicates that if this size criterion were utilized to identify the culprit nodule, many false positive results would occur. As regard subtype identification, concordance between a unilateral nodule on imaging (Table 2) and the side of final diagnosis was

seen in 68.1% (368/540) of the patients; hence, based on imaging alone, 31.9% (172/540) of patients would have been submitted to useless, or wrong, adrenalectomies.

Among patients with a final diagnosis of unilateral PA, imaging would have led to removal of the wrong adrenal in 4.7% (25/528) of the patients. Furthermore, CT and/or MR would have led to denying a potentially curative surgery in 25.6% (135/528) of the patients who tested negative on imaging.

Limitations and strengths intrinsic to many real-world studies need to be acknowledged in AVIS-2-IM. First, the extrapolation of the present results to general population of PA patients might be unwarranted as, by design, this study recruited a selected cohort of PA patients, who wished to accomplish long-term surgical cure, and, thus, comprised those with the most florid (severe) PA phenotype. In addition, as Caucasians predominated over the other ethnicities and patients of African origin were under represented, extrapolation of the present findings and conclusions to patients with milder forms of PA and/or to other ethnicities is unwarranted at this stage. Second, the study, designed in 2012, was performed retrospectively from 2012 to 2000, and then prospectively, from 2012 to 2015, which might suggest selection biases due to changes of clinical practice over time. However, in our view, this possibility seems unlikely as the clinical and imaging features showed no significant differences between the retrospective and prospective cohort. Third, the lack of centralization for the reading of imaging could be seen as a limitation while, in fact, was a strength of the study as the observational design and large size of the study provided a snapshot of ongoing real-world clinical practice on imaging in PA.

Finally, it could be that some patients labelled as bilateral in reality had a unilateral cause of PA as the diagnosis of bilateral PA was an exclusion one based on predefined AVS-based criteria. However, given the lack of criteria to conclusively diagnose bilateral PA, unfortunately this is a limitation common to practically all studies in this field.

In summary, with the strength of a large dataset of PA patients subtyped by AVS, who eventually received a conclusive diagnosis of unilateral PA, these results demonstrate the poor performance of

imaging both for the detection of PA and also for identification of unilateral PA, in keeping with results of smaller single-center studies [31–34], an international study that examined only adrenalectomized PA patients [35], and a meta-analysis [18].

These observations have three important practical implications, which are recapitulated in the graphic abstract: i) negative imaging results by no means allow to exclude either PA or a unilateral surgically curable form it; ii) if the clinical decision making were based on imaging results alone, over 40% of the patients would be judged to have a bilateral form of PA and, therefore, would be denied potentially curative surgery; iii) in the adrenalectomized patients who received a final diagnosis of unilateral PA, and for whom imaging was available, the results were similarly striking: in these patients, the diagnosis was missed in 25.6% as imaging showed either no nodules or bilateral nodules; moreover, wrong adrenalectomies would been undertaken in 4.7%, of the patients if imaging had been used for clinical decision making (Table 2).

Perspectives

Considering the diagnostic performance of imaging and the 'bottle neck' in the management of PA patients represented by AVS, investigative efforts need to be devoted to developing alternative strategies for selecting the candidates for surgery. Along this way, functional imaging with radiotracers in PET/CT or PET/MR are, in our view, more promising than multivariate clinical scores [11,12]. However, thus far, their use has been limited by the very short half-life of the C^{11}-radiotracers, which confined clinical use of this technique to only few centers endowed with a cyclotron on site [9,36,37]. Longer half-life radiotracers might eventually furnish a powerful diagnostic tool, particularly for centers that have no access to AVS [38,39].

FIGURE LEGENDS AND TABLES

Figure 1. Flow-chart of the AVIS-2-IM study

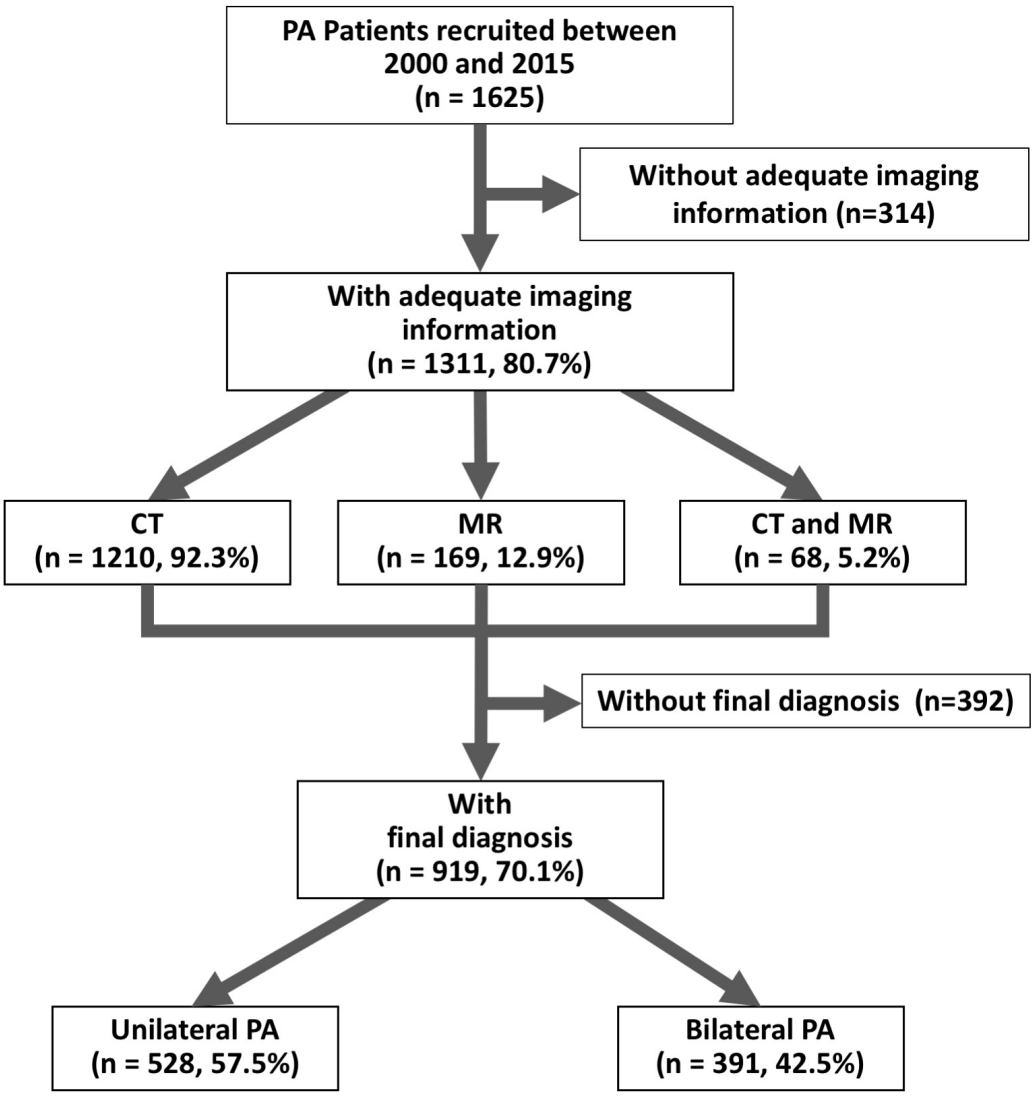

Figure 2. Results of imaging (A), CT (B) and MR (C) in the patients of AVIS-2-IM study.

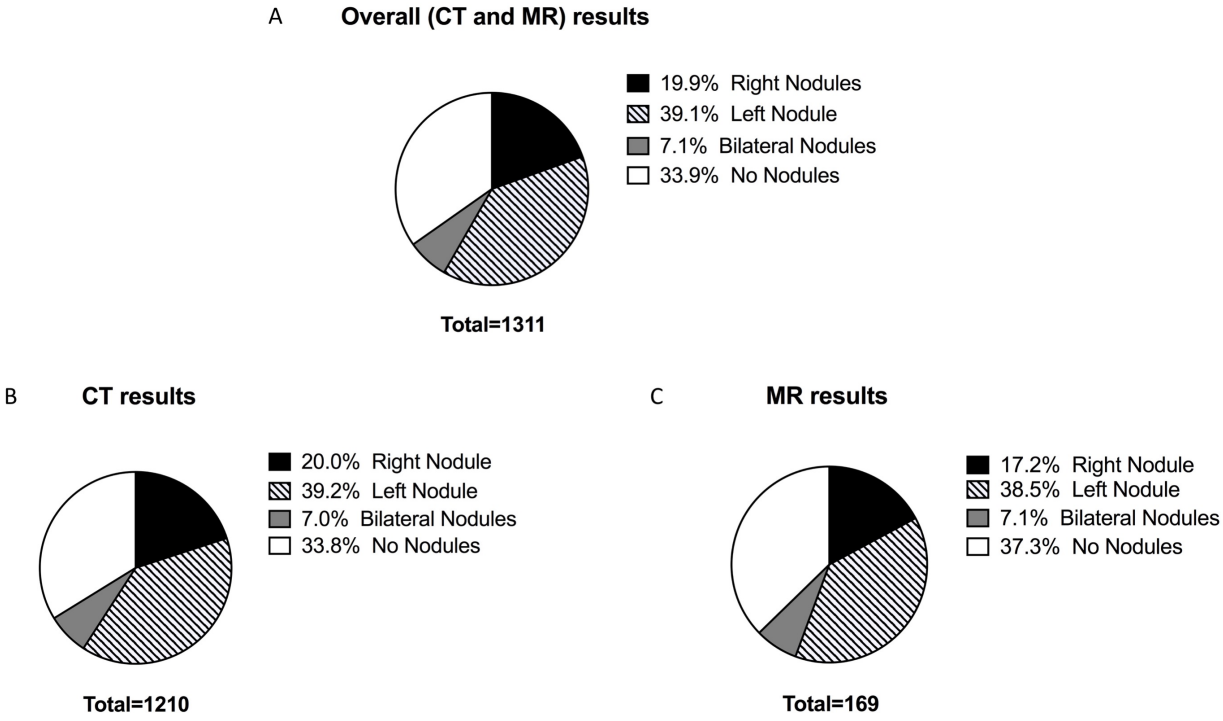

Figure 3. Distribution of size of nodules identified by imaging in the AVIS-2-IM study population.

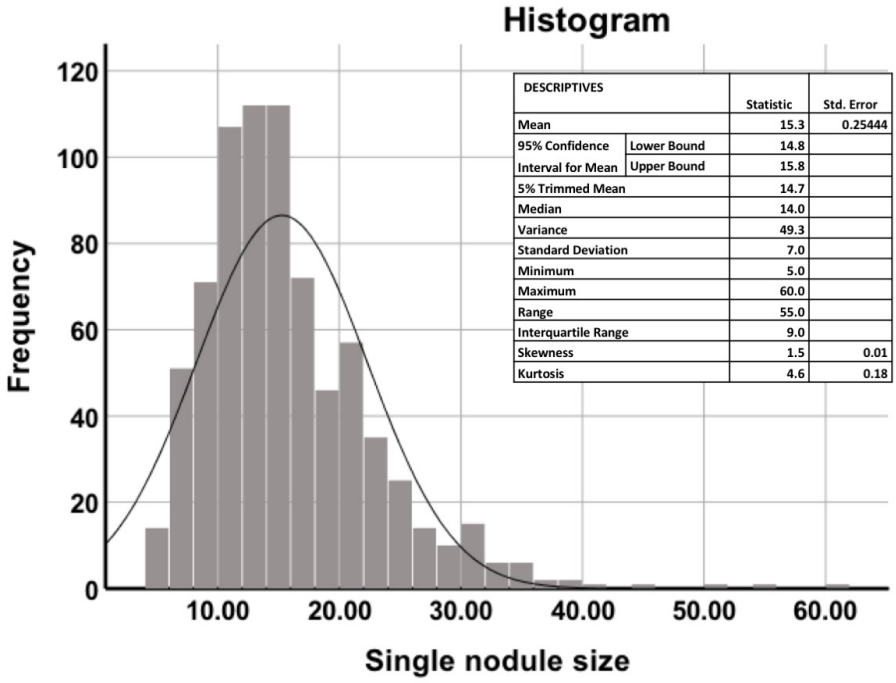

Figure 4. ROC curve analysis for the nodule size effect on the identification of the culprit side.

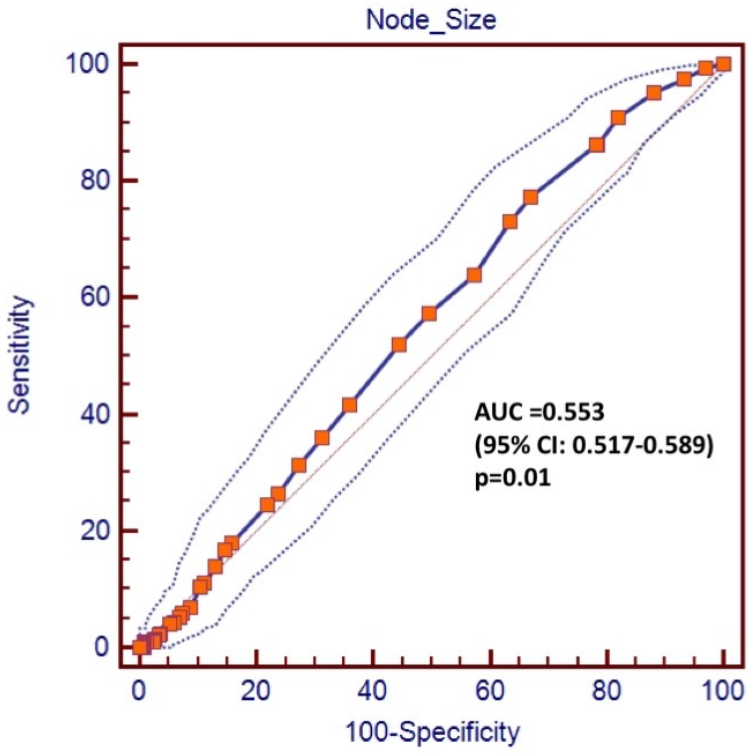

Table 1. Baseline demographic, clinical and biochemical features of the 1311 PA patients with imaging data available. M ± SD or median and IQ range. Abbreviations: PRA: plasma renin activity; PAC: plasma aldosterone concentration; ARR: aldosterone renin ratio.

Variable	Value
Age (years)	50.8 ± 11.0
Sex (M/F), n (%)	782 (59.6%)/ 529 (40.4%)
Body Mass Index (Kg/m^2)	28.3 ± 5.4
Systolic BP (mmHg)	152.2 ± 20.2
Diastolic BP (mmHg)	92.1 ± 12.7
Heart rate (beats/min)	73.0 ± 12.5
Serum K+ (mmol/L)	3.6 ± 0.5
PRA (ng/mL/h)	0.3 (0.2-0.6)
PAC (ng/dL)	22.7 (14.3-36.5)
ARR (ng/dL)/(ng/mL/h)	67.5 (36.3-121.0)
Ethnicity (%): Caucasians Asians Africans Hispanics	 74.1 22.4 3.1 0.5

Table 2. The matrix table shows the concordance between imaging findings and final diagnosis of unilateral/bilateral PA.

Cross-sectional Imaging diagnosis n (%)	**Final Diagnosis n (%)**			
	Right Unilateral PA	**Left Unilateral PA**	**Bilateral PA**	**Total**
Right nodule	122 (13.3%)	8 (0.9%)	46 (5.0%)	176 (19.2%)
Left nodule	17 (1.8%)	246 (26.8%)	101 (11.0%)	364 (39.6%)
Bilateral nodules	17 (1.8%)	12 (1.3%)	29 (3.2%)	58 (6.3%)
No nodules	44 (4.8%)	62 (6.7%)	215 (23.4%)	321 (34.9%)
Total	200 (21.8%)	328 (35.7%)	391 (42.5%)	919 (100.0%)

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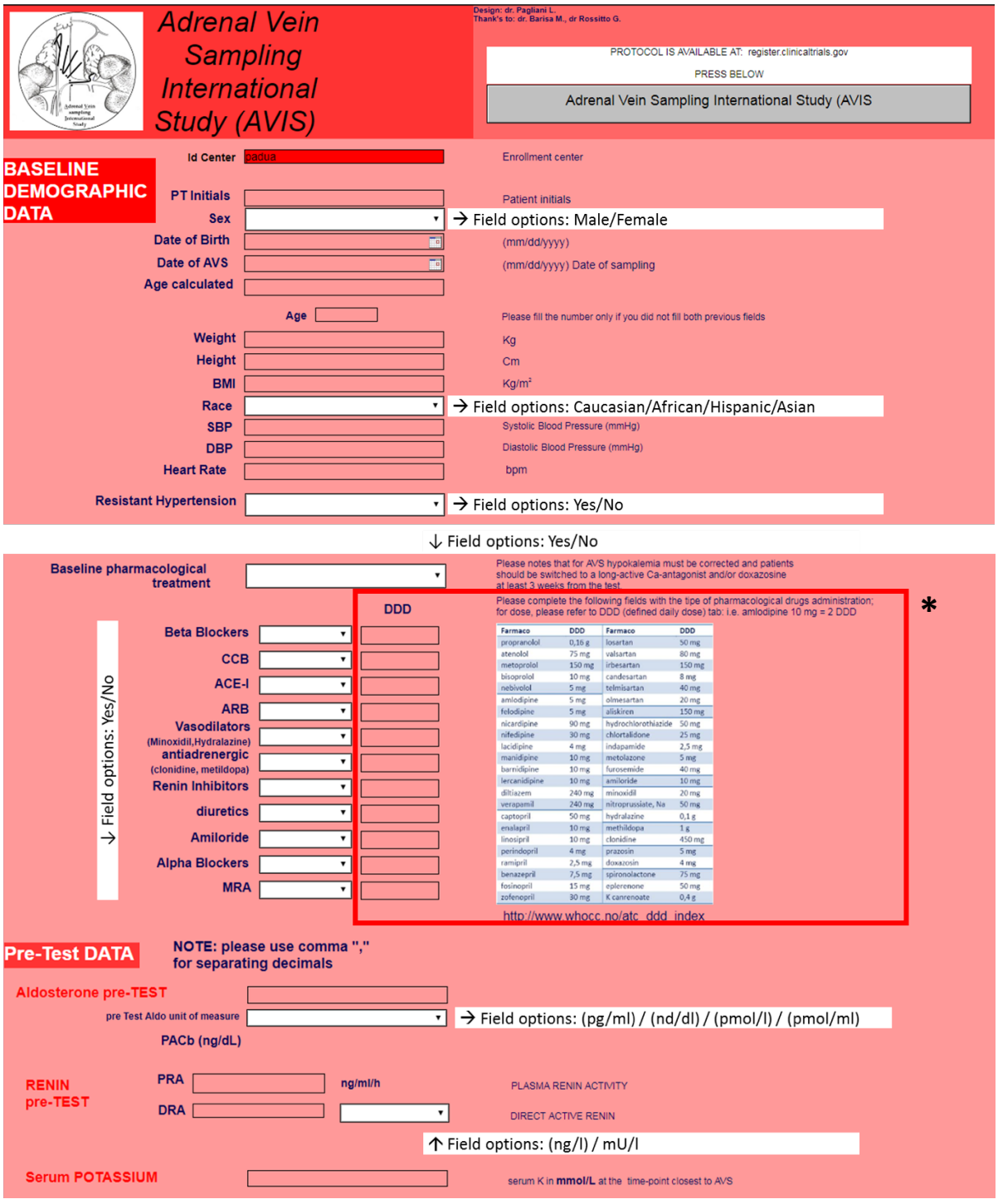
SUPPLEMENTARY DATA

Data collection form for AVIS2 (1)



* DDD was not prespecified at the beginning of the study; was later introduced but excluded from the current analysis because not available from all centers and/or all patients

Data collection form for AVIS2 (2)

BASELINE AVS DATA

NOTE: please use comma "," for separating decimals

ALDOSTERONE

Unit ALDO AVS baseline → Field options: (pg/ml) / (nd/dl) / (pmol/l) / (pmol/ml)

please note: pg/ml = ng/l

ALDOIVCb ALDOIVCb pg/ml		Aldosterone in inferior vena cava baseline
RightALDOb RightALDOb pg/ml		Aldosterone in right adrenal vein baseline
LeftALDOb LeftALDOb pg/ml		Aldosterone in left adrenal vein baseline

CORTISOL

Unit CORTISOL AVS baseline → Field options: (ng/dl) / (nmol/l) / (microg/dl) / (umol/l) / (ng/ml)

IVCCb CORTIVCb ng/ml		Cortisol in inferior vena cava baseline
RightCb RightCORTb ng/ml		Cortisol in right adrenal vein baseline
LeftCb LeftCORTb ng/ml		Cortisol in left adrenal vein baseline

STIMULATED AVS DATA

NOTE: please use comma "," for separating decimals

ALDOSTERONE

Unit ALDO AVS post stimulated → Field options: (pg/ml) / (nd/dl) / (pmol/l) / (pmol/ml)

ALDOIVCpost ALDOIVCpost pg/ml		Aldosterone in inferior vena cava post-stimulation
RightALDOpost RightALDOpost pg/ml		Aldosterone in right adrenal vein post-stimulation
LeftALDOpost LeftALDOpost pg/ml		Aldosterone in left adrenal vein post-stimulation

CORTISOL

Unit CORTISOL AVS post stimulated → Field options: (ng/dl) / (nmol/l) / (microg/dl) / (umol/l) / (ng/ml)

CortIVCpost CortIVCpost ng/ml		Cortisol in inferior vena cava post-stimulation
RightCortpost RightCortpost ng/ml		Cortisol in right adrenal vein post-stimulation
LeftCortpost LeftCortpost ng/ml		Cortisol in left adrenal vein post-stimulation

Rupture of adrenal vein → Field options: Yes/No

IMAGING DATA

↓ Field options: Yes/No

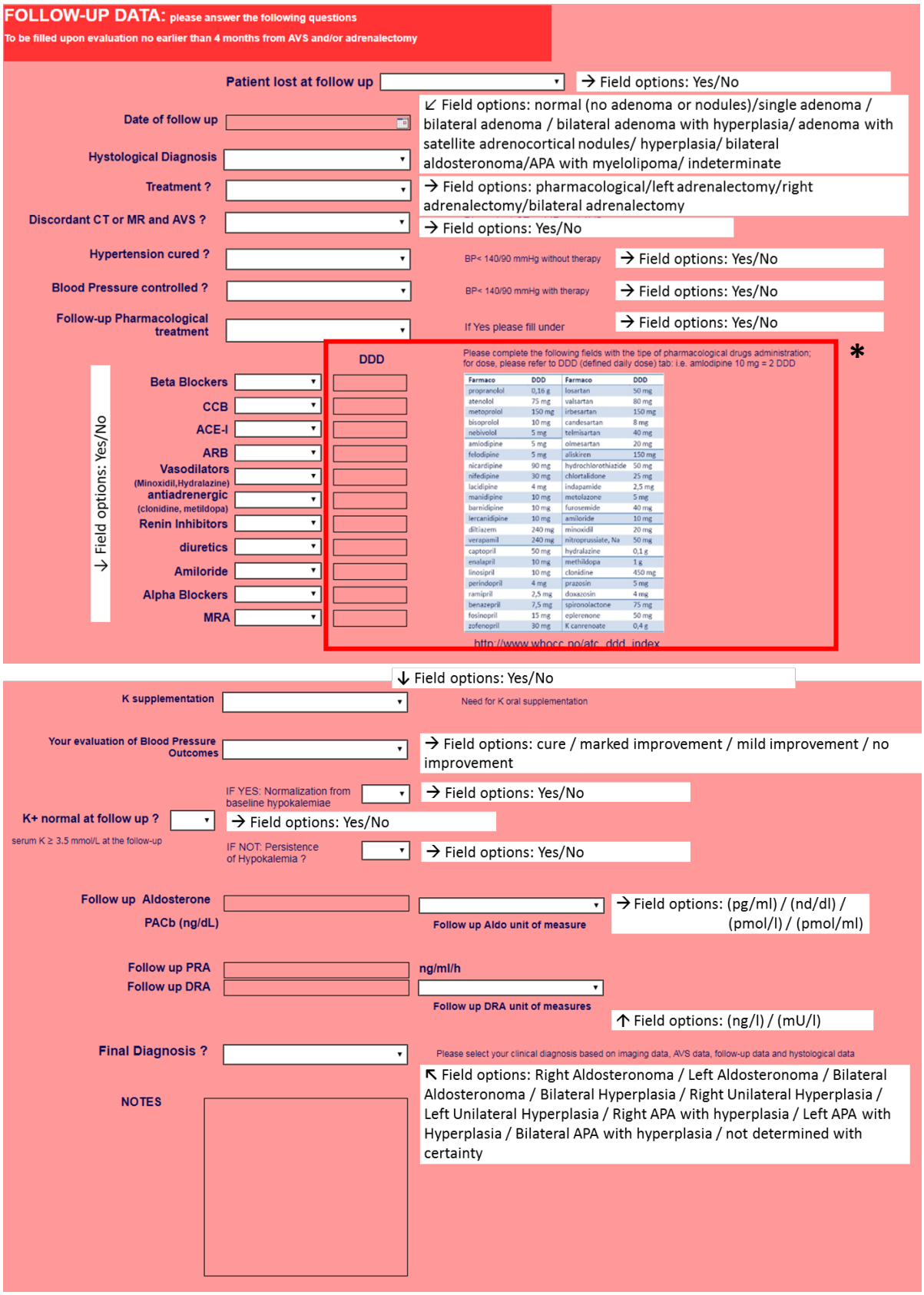
CT

CT right adrenal node		if yes indicate the maximum size of node in the field below
CT right adrenal node size mm		
CT left adrenal node		if yes indicate the size of node in the field below
CT left adrenal node size mm		

MR

MR right adrenal node		if yes indicate the size of node in the field below
MR right adrenal node size mm		
MR left adrenal node		if yes indicate the size of node in the field below
MR left adrenal node size mm		

Data collection form for AVIS2 (3)



* DDD was not prespecified at the beginning of the study; was later introduced but excluded from the current analysis because not available from all centers and/or all patients

Summary List of the collected variables

- Demography (sex 1 =M 2=F, weight, BMI, race, etc.);
- AVS date (MM/DD/YYYY);
- Birth date (MM/DD/YYYY);
- Calculated age at AVS = AVS date (MM/DD/YYYY)- Birth date (MM/DD/YYYY);
- Systolic and diastolic blood pressure values at the time of AVS;
- Ongoing medical therapy at the time of AVS;
- Biochemical profile at baseline (sK^+, plasma aldosterone concentration (PAC); plasma renin activity (PRA).
- AVS protocol (bilaterally simultaneous/sequential; stimulated/unstimulated).
- PAC and plasma cortisol concentration (PCC) in each adrenal vein and in the inferior vena cava blood;
- Concordance/discordance between imaging and AVS results.
- Treatment modality: right/left/bilateral laparoscopic adrenalectomy; medical treatment.
- Blood pressure outcome at 6-months defined as reported in Supplemental Table 2.
- Persistence /correction of hypokalaemia at follow-up.
- Serum K^+, PAC and PRA at follow-up.
- Complications: adrenal vein rupture (appearance of persistent pain during or after catheterization, confirmed at imaging).
- Diagnosis (unilateral aldosterone-producing adenoma (APA); bilateral APA, unilateral adrenal hyperplasia; bilateral adrenal hyperplasia.

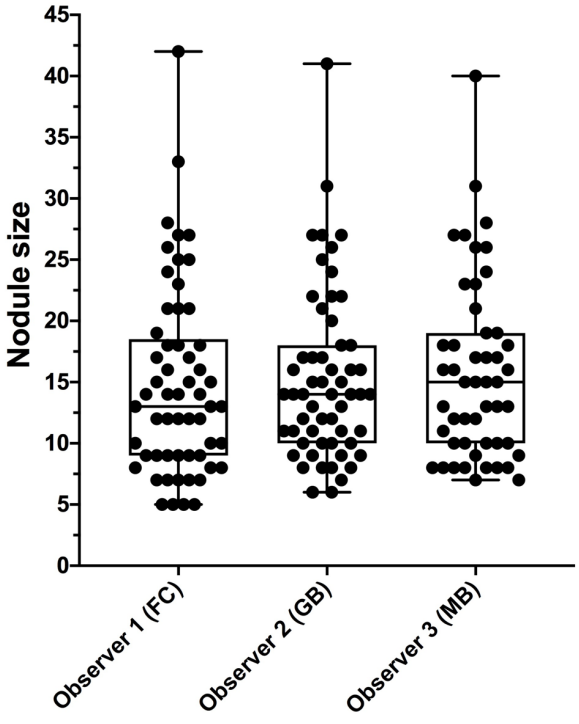
Conclusive diagnosis of unilateral PA required demonstration of biochemical cure at follow-up.

Pilot study on nodules' size at imaging

We enrolled 60 PA patients of the AVIS-2 examined in the same center with a 64 slice CT scanner (Somatom Sensation, Siemens Healthineers, Erlangen, Germany). CT protocol included unenhanced, arterial (10 seconds after the achievement of 100 HU within the abdominal aorta lumen) and venous phase (60 seconds after intravenous contrast injection) acquisitions after intravenous injection of 2 ml/kg of Iohexol 350 mg I/ml (Omnipaque, GE Healthcare, Milwaukee, USA) followed by a 50 ml saline flush. The slice thickness was 1.5 and 3 mm for all the acquisitions. Three radiologists experienced in adrenal imaging independently evaluated CT scans blinded to clinical data and to results of the other observers reporting the presence/absence of adrenal nodules and their maximum axial diameter. In case of absence of nodules, the measurement reported for the diameter was 0.

The distribution of nodules size measured by each observer are reported in Figure 1.

Figure 1. Distribution of the size of the nodules for the three observers



None of the observer was able to identify nodules below the size of 5 mm, indicating that this cut off is the resolution power of the CT for adrenal nodules detection. We compared the measurements recorded by observer 1 with those of observer 2 and the measurements of observer 1 to those of the observer 3 performing a Bland-Altman analysis (Figure 2 and 3). If one of the observers did not see any alteration of the adrenal gland and the other identified a measurable adrenal nodule the size reported in the analysis for the first observer was 0.

Figure 2. Bland-Altman analysis showing comparison between Observer 1 and 2

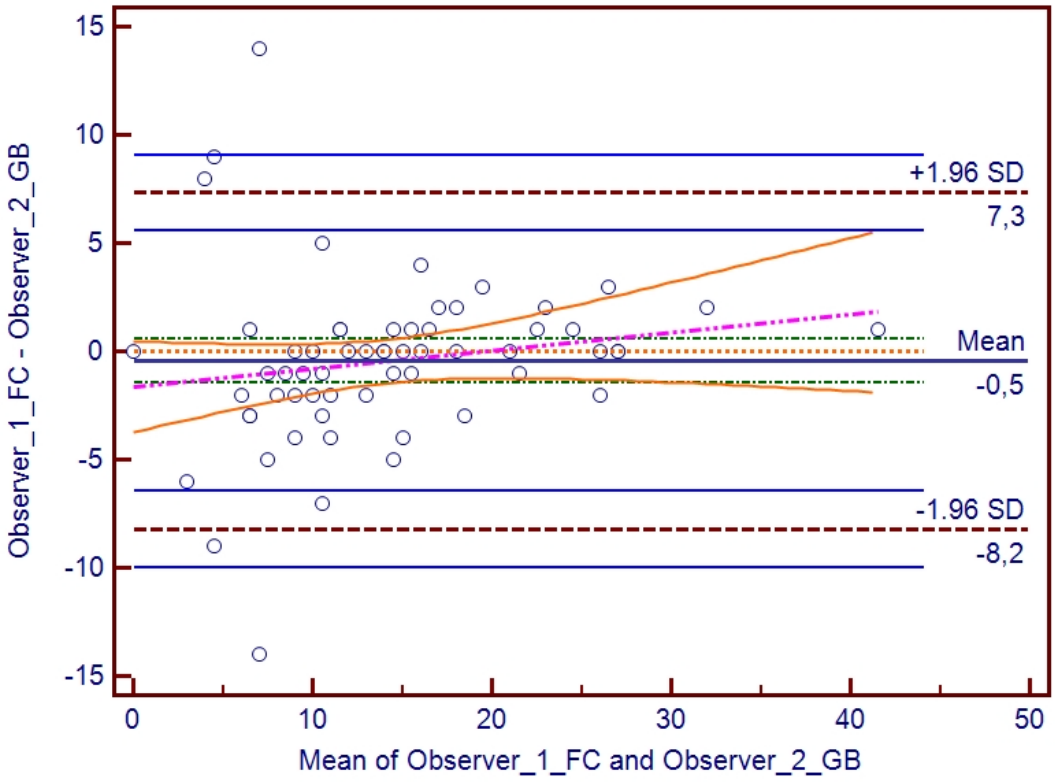
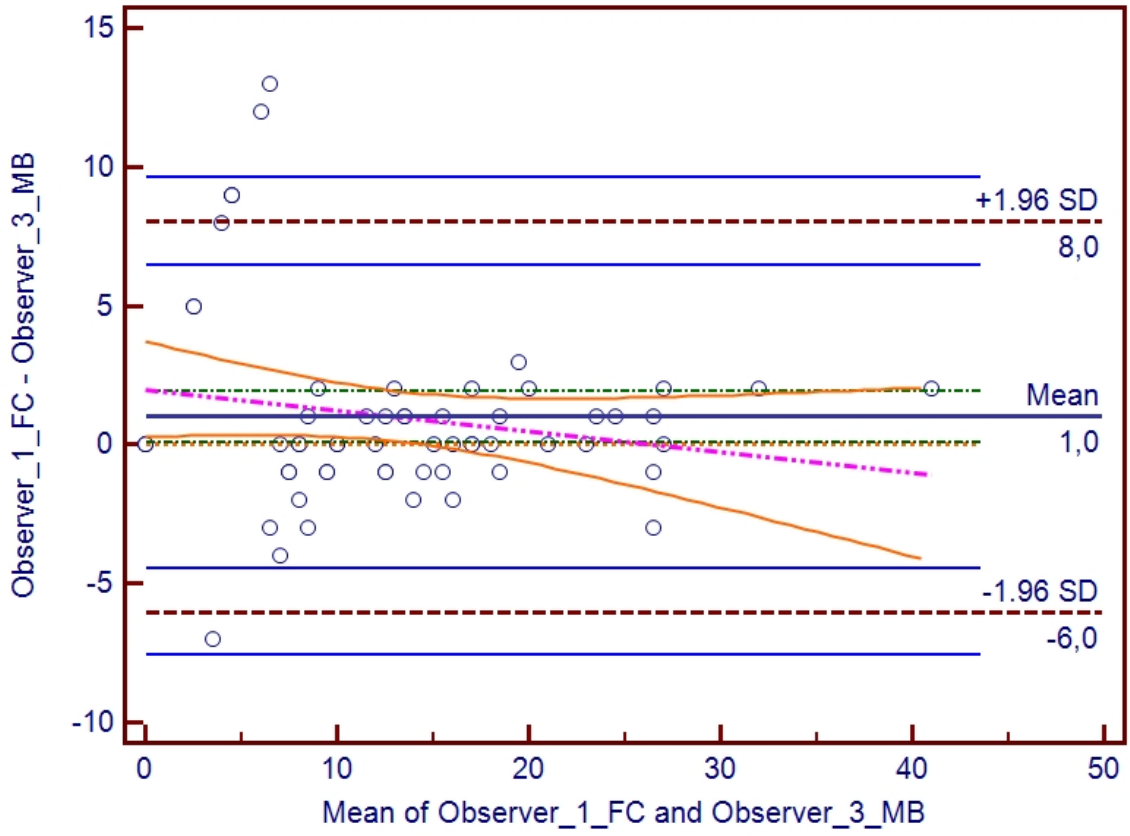


Figure 3. Bland-Altman analysis showing comparison between Observer 1 and 3



The Bland-Altman analysis showed that as the average size increases the differences between observers' measurements tend to get smaller and therefore the smaller are the nodules the wider are the discrepancies in the size of the nodules measured by the radiologists.

The Inter Class Coefficient (ICC) analysis showed a coefficient of 0.8764 (95% CI 0.8188-0.9194), revealing a good concordance among the three observers.

Starting from these evidences, we decided to exclude from the analysis of the study nodules smaller than 5 mm.

Retrospective vs Prospective Cohorts

Table 1 supplemental. Comparison of clinical features of retrospective cohort (n=1067) and prospective cohort (n=558) of AVIS-2 study

Variable	Value Retrospective Cohort	Value Prospective Cohort	P value
Age (years)	50.6 ± 10.9	51.3 ± 10.6	NS
Sex (M/F), n (%)	642 (60.2%)/ 425 (39.8%)	343 (61.5%)/ 215 (38.5%)	NS
Body Mass Index (Kg/m2)	28.2 ± 5.1	28.8 ± 5.7	NS
Systolic BP (mmHg)	152.6 ± 20.9	151.0 ± 19.3	NS
Diastolic BP (mmHg)	92.5 ± 12.9	91.0 ± 12.2	NS
Heart rate (beats/min)	72.6 ± 12.4	73.2 ± 12.6	NS
Serum K+ (mmol/L)	3.6 ± 0.5	3.6 ± 0.5	NS
PRA (ng/mL/h)	0.30 (0.20-0.60)	0.24 (0.20-0.60)	NS
PAC (ng/dL)	29.6 ± 23.5	22.8 (14.4-36.0)	NS
ARR (ng/dL)/(ng/mL/h)	62.6 (37.4-117.5)	68.4 (34.6-122.5)	NS

Sensitivity analysis including the entire dataset

The database contained the results of imaging for the presence/absence of nodules in 1489 patients: 1382 underwent CT, 180 MR and 73 both examinations. Concordance between CT and MRI was 64.4% (Table 2 supplemental).

Table 2 supplemental. Comparison MRI and CT using the entire dataset

	Right nodule at MR	Left nodule at MR	Bilateral nodules at MR	No nodules at MR	Total
Right nodule at CT	11 (15.1%)	1 (1.4%)	1 (1.4%)	4 (5.5%)	17 (23.3%)
Left nodule at CT	1 (1.4%)	21 (28.8%)	1 (1.4%)	10 (13.7%)	33 (45.2%)
Bilateral nodules at CT	0 (0.0%)	3 (4.1%)	2 (2.7%)	1 (1.4%)	6 (8.2%)
No nodules at CT	1 (1.4%)	3 (4.1%)	0 (0.0%)	13 (17.8%)	17 (23.3%)
Total	13 (17.8%)	28 (38.4%)	4 (5.5%)	28 (38.4%)	73 (100.0%)

20.6% of patients showed nodules on the right, 41.2% on the left adrenal glands, 8.4% cases bilateral nodules and imaging was negative in 29.8% of the patients. Of the 1489 who had CT and/or MR imaging, 68.8% (n=1024) had conclusive diagnosis of unilateral PA (n=607) or bilateral PA (n=417). Discordances between imaging and final diagnosis occurred in 33.1% (n=339) of the patients (Table 3 supplemental).

Table 3 supplemental. Comparison between final diagnosis and imaging results using the entire dataset

Imaging diagnosis n (%)	**Final Diagnosis n (%)**			
	Right Unilateral PA	**Left Unilateral PA**	**Bilateral PA**	**Total**
Right Nodules	148 (14.4%)	8 (0.8%)	48 (4.7%)	204 (19.9%)
Left Nodules	20 (1.9%)	288 (28.1%)	120 (11.7%)	428 (41.8%)
Bilateral Nodules or No Nodules	64 (6.2%)	79 (7.7%)	249 (24.3%)	392 (28.3%)
Total	232 (22.7%)	375 (36.6%)	417 (40.7%)	1024 (100.0%)

Comparison between MRI and CTR with 5 mm nodules cut-off

In the group of patients that had both CT and MRI performed (n= 68), the concordance between techniques was 63.2%. In the remaining group of patients, CT showed unilateral or bilateral nodules not detected by MRI in 15 cases, MR showed unilateral or bilateral nodules not identified by CT in 4 patients and in 1 case the side of a single identified nodule was discordant between CT and MRI (Table 4 supplemental). Based on these explorative results and on a much larger availability of data, for imaging definitions of adrenal nodule CT results were used as reference unless only MR was available.

Table 4 supplemental. Comparison MRI and CT with 5 mm nodules cut-off

	Right nodule at MR	**Left nodule at MR**	**Bilateral nodules at MR**	**No nodules at MR**	**Total**
Right nodule at CT	9 (13.2%)	1 (1.5%)	1 (1.5%)	4 (5.9%)	15 (22.1%)
Left nodule at CT	0 (0.0%)	19 (27.9%)	1 (1.5%)	10 (14.7%)	30 (44.1%)
Bilateral nodules at CT	0 (0.0%)	3 (4.4%)	2 (2.9%)	1 (1.5%)	6 (8.8%)
No nodules at CT	1 (1.5%)	3 (4.4%)	0 (0.0%)	13 (19.1%)	17 (25.0%)
Total	10 (14.7%)	26 (38.2%)	4 (5.9%)	28 (41.2%)	68 (100.0%)

Table 5 supplemental. Clinical features of population with final diagnosis available with 5 mm nodule cut-off (n=919)

Variable	**Value**
Age (years)	50.6 ± 11.0
Sex (M/F), n (%)	557 (60.6%)/ 362 (39.4%)
Body Mass Index (Kg/m2)	28.5 ± 5.5
Systolic BP (mmHg)	153 ± 20
Diastolic BP (mmHg)	93 ± 13
Heart rate (beats/min)	73 ± 12
Serum K+ (mmol/L)	3.6 ± 0.5
PRA (ng/mL/h)	0.3 (0.2-0.6)
PAC (ng/dL)	23.7 (14.8-37.6)
ARR (ng/dL)/(ng/mL/h)	67.5 (38.0-124.5)
Ethnicity (%): Caucasians Asians Africans Hispanics	 77.9% 18.7% 3.0% 0.4%

ROC curve

Variable	Nodule_Size
Classification variable	Unilateral_PA_on_Nodule_Side

Sample size	762
Positive group [a]	367 (48,16%)
Negative group [b]	395 (51,84%)

[a] Unilateral_PA_on_Nodule_Side = 1
[b] Unilateral_PA_on_Nodule_Side = 0

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0,553
Standard Error [a]	0,0207
95% Confidence interval [b]	0,517 to 0,589
z statistic	2,555
Significance level P (Area=0.5)	0,0106

[a] *DeLong et al., 1988*
[b] *Binomial exact*

Youden index

Youden index J	0,1030
95% Confidence interval [a]	0,05056 to 0,1572
Associated criterion	>10
95% Confidence interval [a]	>7 to >15
Sensitivity	77,38
Specificity	32,91

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Summary Table

Estimated specificity at fixed sensitivity			
Sensitivity	Specificity	95% CI [a]	Criterion
80,00	29,67	23,27 to 36,39	>9,825454545
90,00	18,75	14,11 to 23,38	>8,217647059
95,00	12,04	7,59 to 17,00	>7,023333333
97,50	6,69	3,62 to 10,89	>6,019444444
99,00	3,88	2,00 to 6,87	>5,238571429
Estimated sensitivity at fixed specificity			
Specificity	Sensitivity	95% CI [a]	Criterion
80,00	22,34	16,62 to 28,89	>19,333333333
90,00	9,47	4,95 to 15,85	>24,25
95,00	3,80	1,52 to 6,97	>29,178571429
97,50	1,09	0,00 to 0,00	>34,025
99,00	1,09	0,00 to 0,00	>36,05

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
≥5	100,00	99,0 - 100,0	0,00	0,0 - 0,9	1,00	1,0 - 1,0		
>5	99,46	98,0 - 99,9	3,04	1,6 - 5,2	1,03	1,0 - 1,0	0,18	0,04 - 0,8
>6	97,55	95,4 - 98,9	6,58	4,3 - 9,5	1,04	1,0 - 1,1	0,37	0,2 - 0,8
>7	95,10	92,4 - 97,1	11,90	8,9 - 15,5	1,08	1,0 - 1,1	0,41	0,2 - 0,7
>8	91,01	87,6 - 93,7	17,97	14,3 - 22,1	1,11	1,0 - 1,2	0,50	0,3 - 0,7
>9	86,38	82,4 - 89,7	21,52	17,6 - 25,9	1,10	1,0 - 1,2	0,63	0,5 - 0,9
>9,4	86,38	82,4 - 89,7	21,77	17,8 - 26,2	1,10	1,0 - 1,2	0,63	0,5 - 0,9
>10	77,38	72,8 - 81,6	32,91	28,3 - 37,8	1,15	1,1 - 1,3	0,69	0,5 - 0,9
>11	73,02	68,2 - 77,5	36,46	31,7 - 41,4	1,15	1,0 - 1,3	0,74	0,6 - 0,9
>12	64,03	58,9 - 68,9	42,53	37,6 - 47,6	1,11	1,0 - 1,2	0,85	0,7 - 1,0
>13	57,49	52,3 - 62,6	50,38	45,3 - 55,4	1,16	1,0 - 1,3	0,84	0,7 - 1,0
>14	52,04	46,8 - 57,3	55,44	50,4 - 60,4	1,17	1,0 - 1,4	0,86	0,8 - 1,0
>15	41,69	36,6 - 46,9	64,05	59,1 - 68,8	1,16	1,0 - 1,4	0,91	0,8 - 1,0
>16	35,97	31,1 - 41,1	68,61	63,8 - 73,2	1,15	0,9 - 1,4	0,93	0,8 - 1,0
>17	31,34	26,6 - 36,4	72,66	68,0 - 77,0	1,15	0,9 - 1,4	0,95	0,9 - 1,0
>18	26,43	22,0 - 31,3	76,20	71,7 - 80,3	1,11	0,9 - 1,4	0,97	0,9 - 1,0
>19	24,52	20,2 - 29,3	77,97	73,6 - 82,0	1,11	0,9 - 1,4	0,97	0,9 - 1,0
>20	17,98	14,2 - 22,3	84,05	80,1 - 87,5	1,13	0,8 - 1,5	0,98	0,9 - 1,0
>21	16,89	13,2 - 21,1	85,32	81,4 - 88,7	1,15	0,8 - 1,6	0,97	0,9 - 1,0
>22	13,90	10,5 - 17,9	87,09	83,4 - 90,2	1,08	0,8 - 1,5	0,99	0,9 - 1,0
>23	11,17	8,1 - 14,8	88,86	85,3 - 91,8	1,00	0,7 - 1,5	1,00	1,0 - 1,1
>24	10,35	7,4 - 13,9	89,62	86,2 - 92,4	1,00	0,7 - 1,5	1,00	1,0 - 1,0
>25	6,81	4,5 - 9,9	91,14	87,9 - 93,8	0,77	0,5 - 1,3	1,02	1,0 - 1,1
>26	5,99	3,8 - 8,9	92,66	89,6 - 95,0	0,82	0,5 - 1,4	1,01	1,0 - 1,1
>27	5,18	3,1 - 8,0	93,16	90,2 - 95,4	0,76	0,4 - 1,3	1,02	1,0 - 1,1
>28	4,36	2,5 - 7,0	93,92	91,1 - 96,1	0,72	0,4 - 1,3	1,02	1,0 - 1,1
>29	4,09	2,3 - 6,7	94,68	92,0 - 96,7	0,77	0,4 - 1,5	1,01	1,0 - 1,0
>30	2,45	1,1 - 4,6	96,46	94,1 - 98,0	0,69	0,3 - 1,6	1,01	1,0 - 1,0
>31	2,18	0,9 - 4,2	96,71	94,4 - 98,2	0,66	0,3 - 1,6	1,01	1,0 - 1,0
>32	1,63	0,6 - 3,5	97,22	95,1 - 98,6	0,59	0,2 - 1,6	1,01	1,0 - 1,0
>33	1,36	0,4 - 3,2	97,47	95,4 - 98,8	0,54	0,2 - 1,6	1,01	1,0 - 1,0
>34	1,09	0,3 - 2,8	97,47	95,4 - 98,8	0,43	0,1 - 1,4	1,01	1,0 - 1,0
>35	1,09	0,3 - 2,8	98,73	97,1 - 99,6	0,86	0,2 - 3,2	1,00	1,0 - 1,0
>37	1,09	0,3 - 2,8	99,24	97,8 - 99,8	1,44	0,3 - 6,4	1,00	1,0 - 1,0
>38	0,82	0,2 - 2,4	99,24	97,8 - 99,8	1,08	0,2 - 5,3	1,00	1,0 - 1,0
>39	0,54	0,07 - 2,0	99,24	97,8 - 99,8	0,72	0,1 - 4,3	1,00	1,0 - 1,0
>41	0,27	0,007 - 1,5	99,24	97,8 - 99,8	0,36	0,04 - 3,4	1,00	1,0 - 1,0
>45	0,00	0,0 - 1,0	99,24	97,8 - 99,8	0,00		1,01	1,0 - 1,0
>51	0,00	0,0 - 1,0	99,49	98,2 - 99,9	0,00		1,01	1,0 - 1,0
>55	0,00	0,0 - 1,0	99,75	98,6 - 100,0	0,00		1,00	1,0 - 1,0
>60	0,00	0,0 - 1,0	100,00	99,1 - 100,0			1,00	1,0 - 1,0

Chapter 3

Imaging of Primary Aldosteronism in Young Patients from a Large International Study (AVIS-2-Young)

Gian Paolo Rossi[1]; Filippo Crimi[2]; Giacomo Rossitto[1]; Laurence Amar[3,4]; Michel Azizi[3,4]; Anna Riester[5]; Martin Reincke[5]; Christoph Degenhart[5]; Jiri Widimsky Jr.[6]; Mitsuhide Naruse[7]; Jaap Deinum[8]; Leo Schultze Kool[9]; Tomaz Kocjan[10,11]; Aurelio Negro[12]; Ermanno Rossi[12]; Gregory Kline[13]; Akiyo Tanabe[14]; Fumitoshi Satoh[15]; Lars Christian Rump[16]; Oliver Vonend[16]; Holger S. Willenberg[17]; Peter J. Fuller[18]; Jun Yang [18,19]; Nicholas Yong Nian Chee[18]; Steven B. Magill[20]; Zulfiya Shafigullina[21]; Marcus Quinkler[22]; Anna Oliveras[23]; Vin Cent Wu[24]; Zuzana Kratka[6]; Giulio Barbiero[2]; Michele Battistel[2]; Teresa Maria Seccia[1].

[1] University of Padova, Department of Medicine-DIMED, International PhD Program in Arterial Hypertension and Vascular Biology (ARHYVAB), University Hospital, Padova, Italy;

[2] University of Padova, Department of Medicine-DIMED, Institute of Radiology, University Hospital, Padova, Italy;

[3] AP-HP, Hôpital Européen Georges-Pompidou, Hypertension Department and DMU CARTE, F-75015 Paris, France;

[4] Université de Paris, INSERM, CIC1418 and UMR 970, F-75015 Paris, France;

[5] Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, LMU München, Germany;

[6] 3rd Department of Medicine, Charles University Prague, General Hospital, Czech Republic;

[7] Department of Endocrinology, National Hospital Organization Kyoto Medical Center, Clinical Research Institute, Japan;

[8] Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands;

[9] Department of Radiology, Radboud University Medical Center, Nijmegen, The Netherlands;

[10] Department of Endocrinology, Diabetes and Metabolic Diseases, University Medical Centre Ljubljana, Zaloska 7, 1525 Ljubljana, Slovenia;

[11] Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana. Slovenia

[12] Department of Internal Medicine, Azienda Unità Sanitaria Locale, IRCCS Arcispedale S. Maria Nuova, Hypertension Unit, Reggio Emilia, Italy;

[13] Foothills Medical Centre, University of Calgary, Canada;

[14] Department of Diabetes, Endocrinology and Metabolism, National Center for Global Health and Medicine, Tokyo, Japan;

[15] Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Hospital, Sendai, Japan;

[16] Heinrich Heine University Düsseldorf, Nephrologie, Germany;

[17] Division of Endocrinology and Metabolism, Rostock University Medical Center, D-18057 Rostock, Germany;

[18] Endocrinology Unit, Monash Health, Clayton, VIC, Australia;

[19] Department of Medicine, Monash University, Clayton, Victoria, Australia

[20] Medical College of Wisconsin, Endocrinology Center, North Hills Health Center, Menomonee Falls, WI, USA;

[21] Department of Endocrinology, University of St. Petersburg, Russia;

[22] Endocrinology in Charlottenburg, Berlin, Germany;

[23] Department of Nephrology, Hypertension Unit, Hosp. del Mar Universitat Autònoma de Barcelona, Spain;

[24] Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan;

Submitted

ABSTRACT

Background. According to the Endocrine Society guidelines when referring for surgery it is feasible to skip adrenal vein sampling (AVS) in young PA patients with a unilateral adrenal nodule and a contralateral normally appearing gland. In this study we sought for determining the accuracy of such strategy for identification of unilateral PA.

Methods. We examined a large multiethnic cohort of patients ≤45 years-old recruited in the AVIS-2-Young study using biochemical cure of PA after adrenalectomy as the final diagnosis of unilateral PA.

Findings. Among the 1625 AVIS-2 patients 29.0%, 15.5% and 6.1% were ≤45, 40, and 35 years-old, respectively. In the same age cohorts, a unilateral adrenal nodule was found in 53.2%, 57.3% and 47.3%. However, 42.6%, 38.5%, and 50.9% respectively, showed no nodules and 4.2%, 4.2% and 1.8% bilateral nodules. The culprit adrenal was identified in 81.4%, 84.1%, and 92.3% of the patients with a unilateral nodule. We identified 38 years as the best cut-off for identification of unilateral PA; moreover, in those with a nodule size >10 mm, the overall concordance was 94%. However, in all young age cohorts bilateral or no nodules were detected in >37% of the patients with unilateral PA.

Interpretation. Basing referral for surgery on a unilateral nodule at imaging carried a likelihood of wrong/inappropriate surgery in 18.6%, 15.8% and 7.7% of PA patients ≤45, 40 and 35 years-old, respectively. By selecting patients ≤38 years with a nodule >10 mm this error rate can be minimized to 6.0%. However, up to 1/3 of the patients, who had unilateral surgically curable PA, were not detected with imaging in these age cohorts.

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INTRODUCTION

Adrenal Vein sampling (AVS), the key test recommended by consensus of experts [1] and current guidelines [2–4] for the subtype differentiation of patients with primary aldosteronism (PA), is poorly available because it is technically challenging and difficult to interpret. Alternative strategies, as functional imaging with C^{11}metomidate and clinical scores [5–8], were, therefore, proposed when referring PA patients for surgery. Moreover, it was suggested that PA patients with a unilateral hypodense nodule between 10 mm and 20 mm of size and a contralateral normally appearing adrenal gland on CT, who are younger than 40 years [9], or 35 years [10], can be referred for surgery with no need of AVS [11], based on the premises that non-functioning adrenal nodules are uncommon in patients younger than 50 years [12]. Albeit endorsed by the Endocrine Society Practical Guidelines on PA, this proposition was based on a single-center experience gained in 6 patients [10] and remained to be supported by larger studies in multiple centers.

The Adrenal Vein Sampling International study (AVIS-2) recruited PA patients ranging between 18 and 80 years of age, who were submitted to AVS in major referral centers in four continents, because of their wish for surgical cure [13]. This allowed creation of the largest available international registry reflecting current clinical practice in the diagnostic work-up of PA patients. In AVIS-2, the majority of patients eventually received a conclusive diagnosis regarding the presence or absence of unilateral PA, which allowed us to assess the accuracy of imaging using such diagnosis, as gold reference index, following the STARD guidelines [14]. As the AVIS-2 registry comprised a good deal of PA patients below 45, 40, and 35 years of age, it offered a privileged observatory to investigate if imaging alone can allow an accurate identification of unilateral PA in such young patients.

The AVIS-2-Young Study was, therefore, set out to investigate if a unilateral hypodense adrenal nodule alongside a contralateral normally appearing adrenal gland can permit an accurate identification of unilateral surgically curable PA in patients aged 45 years or younger.

METHODS

The AVIS-2 study was conceived as an observational multicenter study to create a large database of individual PA patients' data submitted to AVS worldwide. After registration (at clinicaltrials.gov, NCT01234220) the protocol was slightly amended to allow reaching the target recruitment of 1500 PA patients by permitting inclusion of those submitted to AVS between 2000 and 2015.

Details of the study methodology have been previously described in details in papers that reported on the outcome, the assessment of selectivity, the rate of drug-resistant hypertension [13,15], and in a submitted manuscript that described the overall diagnostic accuracy of imaging in the entire cohort.

Inclusion/exclusion criteria

Participating centers were selected based on prior participation in the AVIS-1 study [16] and/or publications on PA and AVS, as identified through a PubMed search.

The study was approved by the Institutional Ethics Committees and all procedures followed the principles of the Helsinki Declaration.

Inclusion criteria were: a) age ≥ 18 years; b) center's agreement to participate in the data collection; c) approval of the Ethics Committee. The only exclusion criteria were the lack of local Ethics Committee's approval and/or the refusal of the lead investigator to participate.

Definitions and assignment to treatment

Resistant Hypertension (RH) was defined according to the 2018 AHA definition [17]. RH was held to be present when pseudo-RH was excluded and blood pressure remained above 130/80 mmHg despite use of three antihypertensive drug classes, commonly including a long-acting calcium channel blocker, a blocker of the renin-angiotensin system (angiotensin-converting enzyme inhibitor or angiotensin receptor blocker), and a diuretic, each drug being administered at maximum, or maximally tolerated, daily doses.

This definition includes patients with so-called "controlled RH", i.e. whose blood pressure achieved target values but required ≥ 4 antihypertensive medications [17].

Unilateral PA was diagnosed in the patients who underwent AVS-guided unilateral laparoscopic adrenalectomy and showed biochemical cure at follow-up. This criterion was used instead of blood pressure outcome, a notoriously complex phenotype, as gold diagnostic reference to assess the accuracy of imaging. Biochemical cure was defined as normalization of plasma aldosterone concentration (PAC), plasma renin activity, and serum K^+ levels at follow-up post-adrenalectomy [18].

For consistency purposes, in this study we adopted the criteria to ascertain selectivity of catheterization and lateralization of aldosterone excess reported in a consensus of experts [1]. Bilateral success was defined as a selectivity index (SI) $\geq 2{\cdot}0$ under unstimulated conditions, and/or $\geq 4{\cdot}0$ post-cosyntropin on both sides. In case of bilaterally selective AVS lateralization, defined as a lateralization index (LI) on the dominant side $\geq 2{\cdot}0$, was assessed and used as a guide to unilateral adrenalectomy.

Patients with no lateralization on a bilaterally successful AVS, and those who were not biochemically cured after unilateral adrenalectomy, were classified as bilateral PA.

Adrenal nodules were defined as nodular lesions with a largest diameter ≥ 5 mm on imaging, based on a pilot study that showed that nodules smaller than 5 mm maximum diameter could not be consistently detected when examined independently by different experienced radiologists blind to clinical diagnosis (Supplemental material).

AVS-guided adrenalectomy was defined as surgery performed after demonstration of lateralized aldosterone excess at bilaterally successful AVS.

The decisions to allocate the patients to surgery or medical treatment was left to investigators at participating centers

Data collection and harmonization

Data collection was performed on-line with a predefined web-based platform created ad hoc, as described [13]. Appropriate filters were implemented to prevent input of values that were not

biologically plausible and/or were in wrong unit of measures. Data were stored securely in a server protected by firewalls at the coordinating center. The PI had full access to the dataset; each local PI had access to his/her center's database to allow for revision and corrections.

Data handling and statistical analysis

After locking the database, the data were checked for internal consistency; emerging queries were clarified with each center's lead investigator. Data harmonization to a standard format was carried out before undertaking the statistical analysis, as described in detail [13]. Univariate and multivariate outliers were identified following the procedure of Tabachnick and Fidell [19], those that could not be resolved with center's lead investigator excluded from the analysis.

Results are expressed as mean ± SD, or median and interquartile range (IQR), as appropriate. Significance was set at $p<0{\cdot}05$. Continuous variables were tested for normal distribution with Kolmogorov-Smirnov test; in case of skewed distribution, log-transformed data were used. Comparisons were performed with parametric or nonparametric tests (Wilcoxon), as appropriate. Pearson's chi square test was used for analysis of categorical variables.

SPSS for Mac™ (vers. 26 for Mac, IBM-SPSS Bologna, Italy), Prism™ (vers. 8·4 for Mac, GraphPad Software, La Jolla California USA), and MedCalcTM (MedCalc Software Ostend Belgium, vers. 15·8) were used for the statistical analysis.

RESULTS

The flow chart of AVIS-2-Young is shown in Figure 1: of the 1625 patients recruited in the AVIS-2 study 29·0% (n = 472) were ≤ 45 years of age, 15·5% (n = 252) were ≤ 40, and 6·1% (n = 100) were 35-year-old or younger. Table 1 shows the clinical features of the three age cohort; their imaging and final diagnosis data are described in the next sections.

PA patients 45-years-old or younger

Of the 472 patients in this age group, 263 had both imaging data and final diagnosis available. 62·4% (164/263) received a conclusive diagnosis of unilateral PA; the remining were judged to have bilateral PA. A unilateral hypodense adrenal nodule alongside a contralateral normally appearing adrenal gland was found in 53·2% of the 263 patients with imaging results and conclusive diagnosis available; 42·6% showed no identifiable nodules, and 4·2% were found to have bilateral nodules. Overall imaging did not allow identification of the culprit adrenal in 46·8% of the patients aged 45 years or younger.

The distribution of nodules size, shown in the Supplemental Results (Figure 4 supplemental), evidenced a mean size of adrenal nodules of 15·2 ± 7·3 mm (range 5 – 60 mm). Table 2 shows a matrix of imaging findings vs the final diagnosis of unilateral/bilateral PA. On the whole, the concordance between imaging results and final diagnosis of unilateral or bilateral PA was 72·6% (191/263).

Figure 2 shows a scatter plot of nodules size as a function of age only in the PA patients found to have a unilateral hypodense adrenal nodule alongside a contralateral normally appearing adrenal gland. According to the final diagnosis, 81·4% of the cases would have been sent to correct adrenalectomy on the basis of imaging; however, 15·7% and 2·9% would have received inappropriate and wrong adrenalectomies, respectively.

Furthermore, 37·4% (46/123) of the patients with an inconclusive diagnosis at imaging because they showed bilateral or no nodules received a final diagnosis of unilateral PA.

PA patients 40-years-old or younger

Of the 252 patients in this age group, 143 had both imaging data and a conclusive diagnosis, which was unilateral PA in 66·4% (95/143) and bilateral PA in the remaining cases. Of the patients with imaging and final diagnosis available, 57·3% were found to have a unilateral hypodense adrenal nodule alongside a contralateral normally appearing adrenal gland.

However, 38·5% showed no identifiable nodules, and 4·2% bilateral nodules. Thus, overall, imaging did not allow identification of the culprit adrenal in 42·7% of the aged 40 years or younger.

The distribution of nodules size identified by imaging in this age cohort, shown in the Supplemental Results (Figure 5 supplemental), identified a mean size of the nodules of 14·9 ± 7·1 mm, (range 5 – 60 mm).

Table 2 shows a matrix of imaging findings vs the final diagnosis of unilateral/bilateral PA. The concordance between imaging and final diagnosis in the cohort of patients < 40 years of age was 74·8% (107/143).

Figure 2 shows a scatter plot of nodules size as a function of age in the PA patients found to have a unilateral hypodense adrenal nodule alongside a contralateral normally appearing adrenal gland. According to the final diagnosis, the rate of correct adrenalectomies was 84·1%, while inappropriate and wrong adrenalectomies, were respectively 12·2% and 3·7%.

The rate of patients who received a conclusive diagnosis of unilateral PA without showing a unilateral nodule on imaging was 37·7% (23/61).

PA patients 35-years-old or younger

Of the 100 patients with ≤ 35 years of age, both imaging results and final diagnosis were available in 55: 65·4% of them had unilateral PA and the remaining bilateral PA (Figure 1). Of them, 47·3% were found to have a unilateral hypodense adrenal nodule alongside a contralateral normally appearing adrenal gland. The proportion of patients with negative imaging. i.e. without detectable nodules, was 50·9%, and those with bilateral nodules were 1·8%. Accordingly, imaging did not allow identification of the culprit adrenal in 52·7% of the patients in this age cohort.

The size distribution of nodules identified by imaging is shown in Supplemental Results (Figure 6 supplemental); mean size was 14·2 ± 5·2 mm (range 5 – 32 mm). The concordance between imaging

and final diagnosis in this cohort was 76·4% (42/55), as shown in the matrix of imaging findings vs the final diagnosis of unilateral/bilateral PA (Table 2).

Figure 2 shows a scatter plot of nodules size as a function of age in PA patients with a unilateral hypodense adrenal nodule alongside a contralateral normally appearing adrenal gland. The cases that received correct, inappropriate and wrong adrenalectomies, as judged by the final diagnosis, were 92·3%, 3·8% and 3·8%, respectively.

Among these 26 patients, 2 had nodules smaller than 10 mm and 3 patients larger than 20 mm (Figure 2). Out of the two patients with a unilateral nodule smaller than 10 mm, one had a left adrenal nodule of 6 mm, but a final diagnosis of right unilateral PA. All the 3 patients with nodules larger than 20 mm, had a final diagnosis of unilateral PA ipsilateral to the side of the nodule identified by imaging.

However, final diagnosis of unilateral PA was made in 37·9% of the 29 patients with bilateral or no nodules at imaging.

Receiver Operator Characteristics curve and Youden index analysis

We performed these analyses to determine the overall accuracy of a unilateral nodule and a contralateral normally appearing adrenal gland at imaging using unilateral PA at final diagnosis as reference.

In the cohort ≤45 years, the AUC was 0·664 (95% Confidence Interval (CI): 0·579-0·742) that was significantly greater than that of the identity line (p=0·0087), the Youden's index analysis identified a nodule size >10 mm as the best combination of sensitivity (82·6%) and a specificity (50·0%) (Figure 7 supplemental and Table 7 supplemental).

In the cohorts ≤40 years and ≤35 years the ROC curve AUC did not differ significantly from that under identity line; the Youden's index analysis detected as the best dimensional cut-off > 10 mm for the cohort ≤40 years and > 6 mm for the cohort ≤35 years with a sensitivity and specificity of 89·9%

and 46·1% and of 95·8% and 50·0%, respectively (Figures 8-9 supplemental and Tables 8-9 supplemental).

We performed a ROC curve analysis to establish the age cutoff the furnished the best combination of sensitivity and specificity using the final diagnosis of unilateral/bilateral PA as category status in the patients with a unilateral nodule at imaging with a contralateral normal appearing adrenal gland.

The AUC was 0·592 (95% CI: 0·506-0·674), which did not differ significantly from that under the identity line (p=0·1263). The Youden's index analysis identified as the age cut-off ≤38 years corresponding to a sensitivity of 45·6% and a specificity of 73·1% (Figure 3 and Table 10 supplemental).

A further ROC curve analysis was performed to identify the nodule size that could furnish a higher concordance between single adrenal nodule at imaging and ipsilateral diagnosis of unilateral PA in the cohort of patients ≤38 years of age (Figure 10 supplemental and Table 11 supplemental). The AUC detected was 0·688 (IC 95%: 0·554-0·802), which did not differ significantly from the identity line (p=0·1950). The size cut-off identified at the Youden's index analysis was > 10 mm with a sensitivity and specificity of 90·4% and 57·1%.

By restricting the analysis to the patients ≤ 38 years with a unilateral adrenal nodule within this size range and a contralateral normally appearing adrenal, 94·0 % (47/50) would have been correctly classified; only two would have received a wrong adrenalectomy and another patient an inappropriate surgery (Figure 2).

DISCUSSION

In spite of its being the most common curable form of arterial hypertension, PA is usually overlooked and mostly diagnosed after the fifth or sixth decade [20,21]. Young patients, i.e. below age 45 years, and particularly below 35 years, entailed a minority of the reported cases [10], which suggests that the diagnosis, and consequently targeted treatment, are delayed or never accomplished in many young patients, at variance with the guidelines recommendation [2–4] that emphasize the importance of an

early screening for PA and also with the notion that PA has a long natural history [22]. This is of further worries as young age and a short history of high blood pressure are strong predictors of complete cure of PA with surgery [23]. Considering that AVS, the key test for PA subtyping, is so poorly available, with entire countries that do not perform the procedure even within the EU, likely the proportion of the young PA patients who can be referred for curative adrenalectomy is unacceptably low.

To overcome the bottle - neck represented by AVS, it was proposed to skip this test in PA patients younger than certain cutoffs of age, if they have a unilateral nodule, and a contralateral normally appearing adrenal gland on imaging [9]. This age cutoffs, initially set at 40 years [9], was thereafter lowered to 35 years [11], because a 100% concordance between imaging detection of an adrenal nodule and AVS results showing unilateral PA was found in 6 patients belonging to this younger cohort [10].

In this AVIS-2-Young study we could identify 472 PA patients aged 45 years or younger from a very large international cohort of PA patients seeking for surgical cure, and therefore submitted to AVS. Moreover, 252 of them were 40 years-old or younger, and 100 were 35 years-old or younger. Hence, in this large cohort the rate of young patients in these age groups corresponded to 29%, 15·5% and 6·1%, respectively. This implies that, overall, the aforementioned imaging criteria could have led to referring for surgery without performing AVS only less than one third of those aged 45 years of age or younger, about one sixth of those aged 40 years or less, and only a tiny proportion of those 35 years of age or younger.

Seeking for determining the diagnostic performance of the imaging criteria in these three categories of young patients, we found that the criterion of a unilateral nodule + contralateral normally appearing adrenal at imaging alongside the young age, would have allowed to identify the culprit side in 81·4% of the patients aged 45 years or younger, and in 84·1% and 92·3% among the patients aged 40 and 35 years.

However, by looking at these results from a different perspective, this criterion would have resulted into wrong assignment to treatment in 18·6%, 15·8% and 7·7% of the patients in the patients aged 45, 40 and 35 years of age or younger, respectively.

In the 35 years cohort one patient would have received inappropriate adrenalectomy because he had bilateral PA; one additional patient with unilateral right PA, who showed a left adrenal nodule, would have received a wrong adrenalectomy (Figure 2 and Table 2).

Moreover, in this age cohort of PA patients, there were 50·9% of the patients who had no nodules and 1·8% who had bilateral nodules on imaging. Of them, 19·6% of the total cohort, had unilateral PA, 4 on the right and 7 on the left side. Based on imaging these patients would have been assigned to life-long medical treatment and denied curative surgery (Table 1). However, 18 (32·2%). i.e. 62·0% of those with bilateral nodules or negative imaging, were confirmed as bilateral PA.

These results in patients younger than 35 years concerning the lack of identification of the culprit adrenal are fully consistent with those observed in larger series of PA belonging to all age groups: in the study by Lim et al [10] 39·4% of 127 surgically cured had inaccurate CT or MR findings; likewise, in the entire AVIS-2 cohort of PA patients 41% of the cases showed either bilateral nodules (7·1%) or negative imaging (33·9%); moreover, among those with a unilateral PA and 26% tested either negative or inconclusive at imaging. Noteworthy, the proportion of those with negative imaging at either CT and/or MR in this study was smaller than detected in a previous study by Williams et al who examined with CT only, using a minimum cut off of 8 mm, patients who underwent adrenalectomy [24].

It should be acknowledged that the algorithm originally proposed for patients younger than 35 years included only nodules between 10 and 20 mm in size [10], while in AVIS-2-Young, we used a lower cut off for nodules identification because current imaging technology allows an accurate identification of nodules down to a size of 5 mm (Supplemental results).

Of the two solitary unilateral nodules smaller than 10 mm identified in patients younger than 35 years (Figure 2), one was confirmed to have ipsilateral unilateral PA, while in the other the culprit adrenal was contralateral to the nodule side.

By applying the criteria proposed by Lim et al [10], i.e. considering only nodules between 10 mm and 20 mm in size to those ≤ 35 years of age, one out of 21 patients would have received inappropriate adrenalectomy. However, in the cohort of 61 patients with a unilateral nodule at imaging between 10 and 20 mm of size ≤ 40 years of age, 7 would have received inappropriate adrenalectomy and one wrong adrenalectomy. In the patients ≤ 45 years of age 15 would have undergone inappropriate and 2 wrong adrenalectomies.

The clinical decision making in PA patients younger than 35 years of age is not a trivial issue as it might appear.

In the study by Lim et al they comprised 4·5% of 133 patients referred to a single center [10] and in our multicenter international cohort they entailed 6·5% of 1625 patients. Moreover, with widening of the screening strategies to young patients, and by lowering the minimum size of nodules detected at imaging this cohort will increase steadily in the future.

By lowering the age cut off the diagnostic accuracy of the imaging criterion increased, at the price of a decreased applicability (Figure 11 supplemental material). We, therefore, undertook a formal analysis to identify the age cut off associated with the highest diagnostic accuracy in the cohort of patients aged 45 years or younger, which led to identify 38 years as the best age cut off.

Based on these results, we would like to propose that patients with age ≤ 38 years, a unilateral nodule > 10 mm in size at imaging and a contralateral normal appearing gland can be directly referred for surgery, as 94% of them would have been correctly classified while only 6% would have been sent to wrong or inappropriate surgery (Figure 4).

CONCLUSIONS

In summary, with the strength of the large size provided by an international multicenter registry, the results of the present study provided the following novel information that is relevant for the assignment to surgical or medical treatment of young patients diagnosed with PA. Negative or bilateral imaging findings occurred in 46·8% (123/263) of the patients aged 45 years or younger, who had unilateral PA. Therefore, denying AVS to these patients would preclude them the possibility of long-term surgical cure.

In patients aged 45 years or younger with an unequivocal diagnosis PA, who had an adrenal nodule ≥ 5 mm in size, and a contralateral normally appearing adrenal, non AVS-guided unilateral adrenalectomy can be offered if the patient is willing to accept a 2·9% chance of a wrong adrenalectomy and a 15·7% chance of performing an inappropriate adrenalectomy because of bilateral PA. The latter can, however, result into lowered blood pressure due to debulking of aldosterone-producing tissue.

In patients aged 40 years or younger with unequivocal PA, with the same imaging features non AVS-guided unilateral adrenalectomy can be offered if the patient is willing to accept a 3·7% chance of a wrong adrenalectomy and a 12·2% chance of performing an inappropriate adrenalectomy because of bilateral PA.

In patients aged 35 years or younger with unequivocal PA and the same findings at imaging, non AVS-guided unilateral adrenalectomy can be offered if the patient is willing to accept a 3·8% chance of a wrong adrenalectomy and a 3·8% chance of performing an inappropriate adrenalectomy because of bilateral PA.

The concordance with final diagnosis could be increased to almost 94% by applying an age cutoff of 38 years and the imaging criterion to nodules greater than 10 mm in size. However, even by these tighter criteria a 6% chance of performing a wrong or inappropriate adrenalectomy will remain.

FIGURES AND TABLES

Figure 1. Flow-chart of patients' selection for the AVIS-2 Young study.

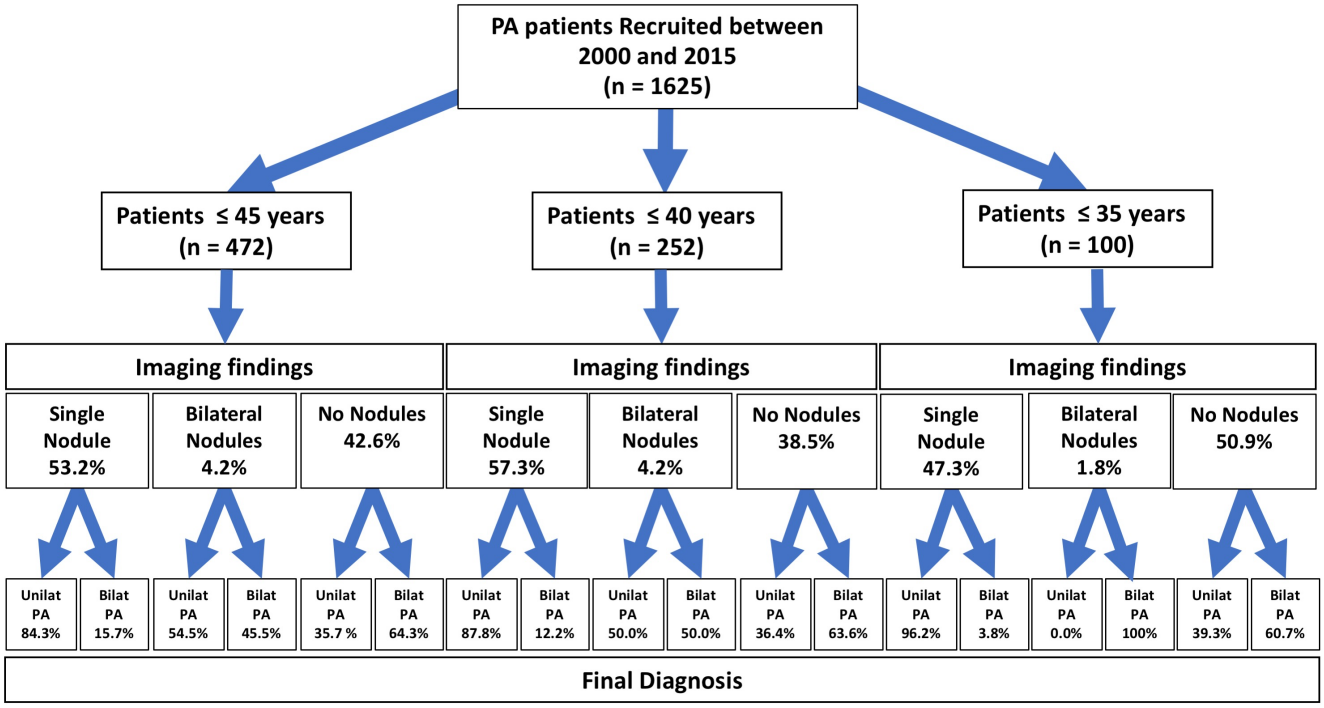

Figure 2. The scatter plot shows the distribution of age and unilateral nodules size, as identified by imaging in the patients 45 years-old or younger (140 ≤ 45 years; 82 ≤ 40 years; 26 ≤ 35 years) who showed a unilateral nodule at imaging and a contralateral normally appearing adrenal gland. The blue circles are the patients where the side of the nodule identified with imaging was concordant with final diagnosis, who would have undergone correct adrenalectomy based on imaging diagnosis; the yellow square are the patients with bilateral PA at final diagnosis, who would have undergone inappropriate adrenalectomy based on imaging diagnosis; the red triangles identify the patients with imaging nodule side contralateral to the side of unilateral PA at final diagnosis, who would have undergone wrong adrenalectomy based on imaging diagnosis; the dashed area shows the size range 10 – 20 mm suggested by Lim et al; the red line shows the calculated age cut off of 38 years old (cohort of 50 patients); grey square represents the calculated size range > 10 mm.

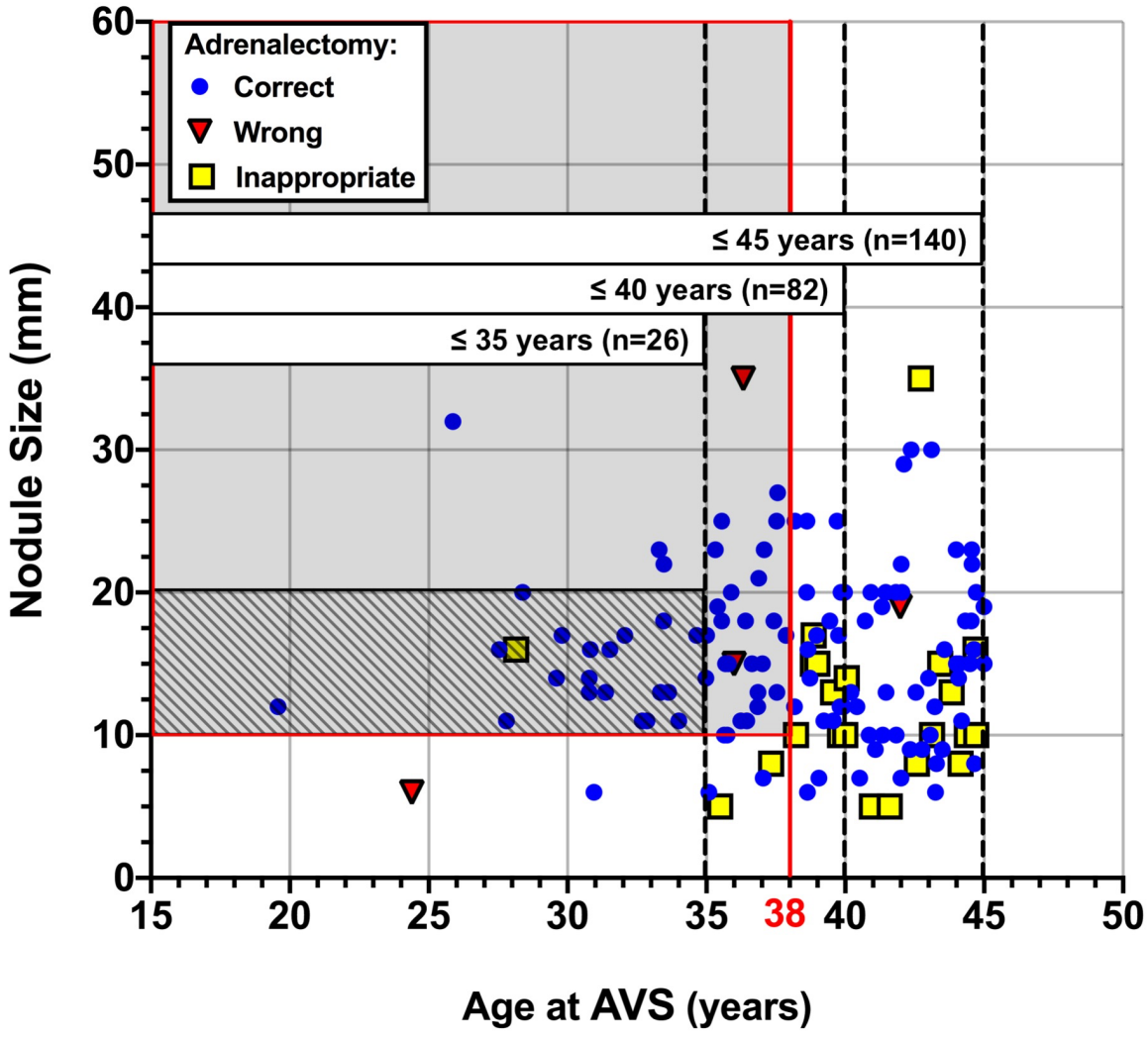

Figure 3. ROC curve analysis for the age effect on the identification of the culprit side in patients with a unilateral adrenal nodule at imaging alongside a normal appearing contralateral adrenal gland.

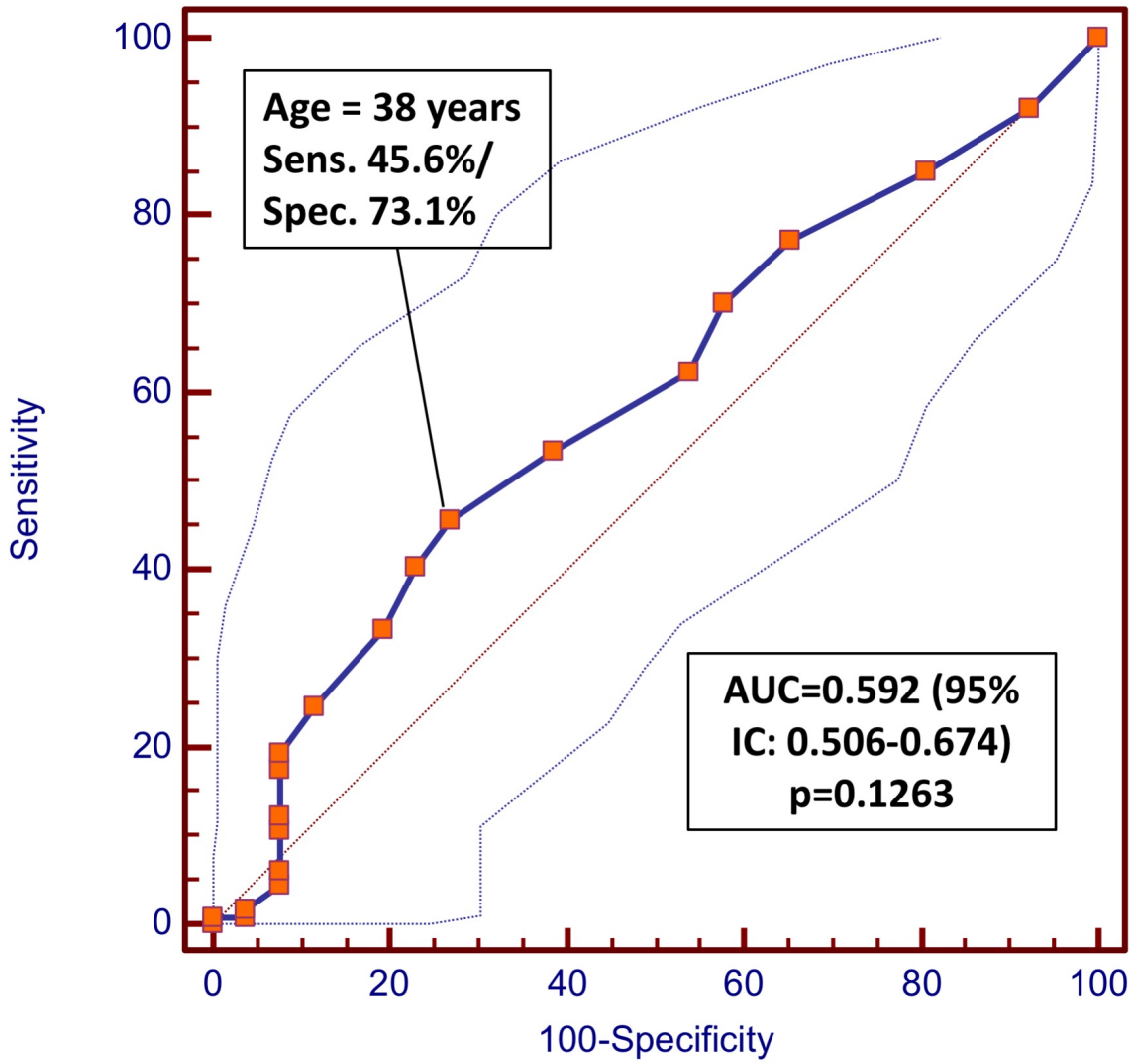

Figure 4. Algorithm proposal to identify patients that can be sent directly to surgery on the basis of imaging results. Adopting the identified cut off of ≤ 38 years of age and nodule size at imaging > 10 mm, the corrected identification of culprit adrenal occurs in 94% of cases with a 4 % chance of wrong adrenalectomy and 2% chance of inappropriate adrenalectomy.

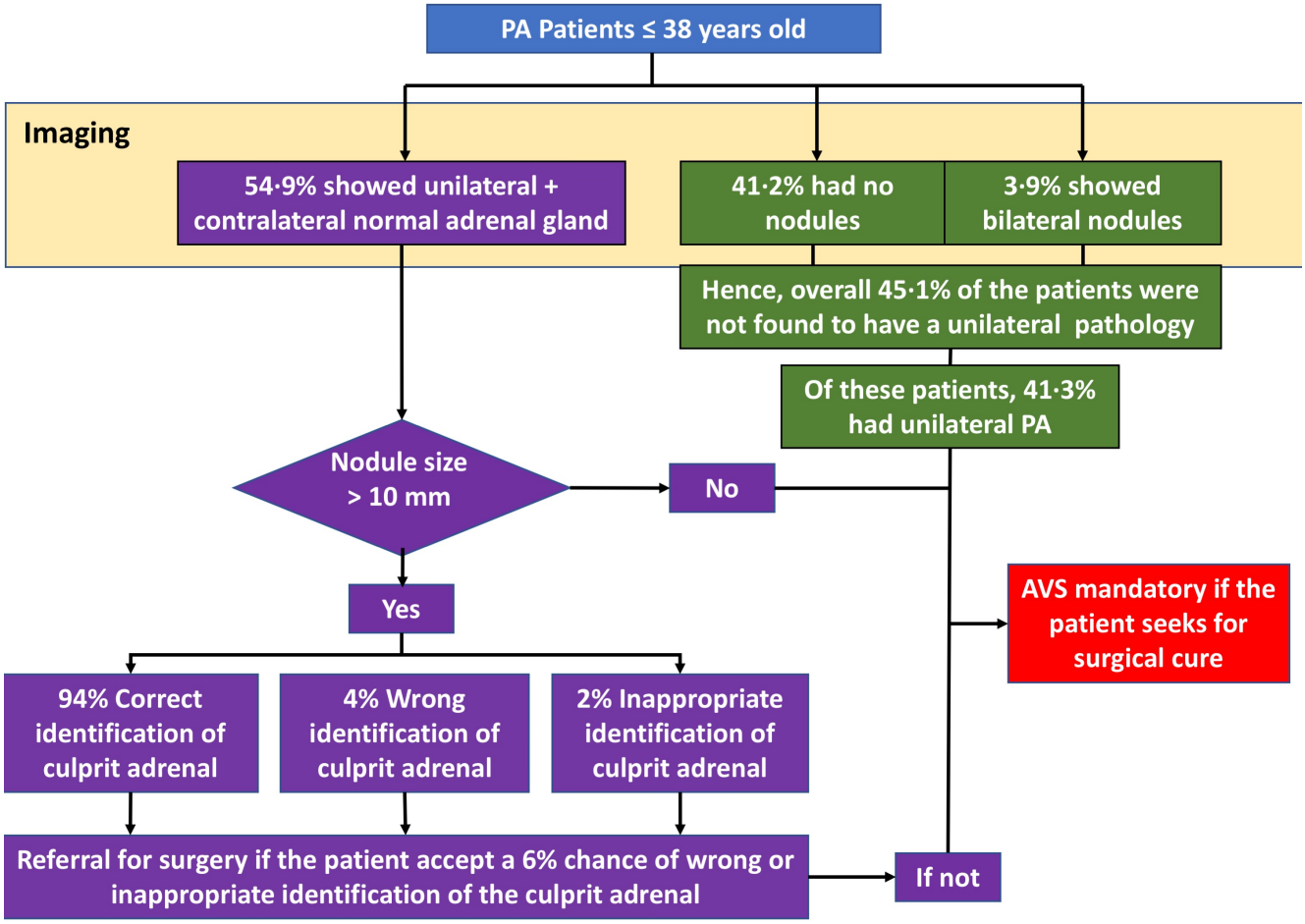

Table 1. Baseline demographic, clinical and biochemical features of patients younger ≤ than 45, 40 and 35 years old. M ± SD or median and IQ range. Abbreviations: PRA: plasma renin activity; PAC: plasma aldosterone concentration; ARR: aldosterone renin ratio. * = according to AHA 2018 criteria.

Variable	**Value in Patients ≤ 45 years (n = 472)**	**Value in Patients ≤ 40 years (n = 252)**	**Value in Patients ≤ 35 years (n = 100)**
Age (years)	38.2 ± 5.4	34.7 ± 4.8	30.1 ± 4.3
Sex (M/F), n (%)	225 (47.7%)/ 247 (52.3%)	94 (37.3%)/ 158 (62.7%)	26 (26.0%)/ 74 (74.0%)
Body Mass Index (Kg/m^2)	27.7 ± 5.5	26.8 ± 5.4	25.9 ± 5.0
Systolic BP (mmHg)	148.5 ± 19.6	147.4 ± 18.1	147.9 ± 17.7
Diastolic BP (mmHg)	93.4 ± 13.0	93.6 ± 12.8	93.4 ± 13.2
Heart rate (beats/min)	74.2 ± 13.6	74.3 ± 13.3	75.0 ± 12.7
Serum K+ (mmol/L)	3.5 ± 0.5	3.5 ± 0.5	3.5 ± 0.5
PRA (ng/mL/h)	0.30 (0.20-0.63)	0.30 (0.20-0.61)	0.24 (0.20-0.55)
PAC (ng/dL)	24.7 (16.4-38.5)	26.7 (17.0-40.0)	28.3 (17.3-41.2)
ARR (ng/dL)/(ng/mL/h)	67.6 (34.9-130.6)	72.0 (37.0-141.8)	87.5 (46.4-150.0)
Cases with Resistant Hypertension*	34/472 (7.2%)	16/252 (6.3%)	7/100 (7.0%)
Ethnicity (%): Caucasians Asians Africans Hispanics	 73.5% 20.1% 6.1% 0.3%	 67.8% 24.9% 6.8% 0.6%	 65.2% 27.3% 7.5% 0.0%
Patients with final diagnosis and imaging data available	263, 55.7%	143, 56.7%	55, 55.0%
Cases with a unilateral nodule and a contralateral normally appearing adrenal with final diagnosis available (n, %)	140, 29.7 %	82, 32.5 %	26, 26.0%

Table 2. The matrix table shows the concordance between imaging findings and final diagnosis of unilateral/bilateral PA in a) patients ≤ 45; b) patients ≤ 40; c) patients ≤ 35.

a) Findings at cross-sectional imaging in patients ≤ 45 years old	**Final Diagnosis n (%)**			
	Right Unilateral PA	**Left Unilateral PA**	**Bilateral PA**	**Total**
Right nodule, n (%)	41 (15.6%)	1 (0.4%)	3 (1.1%)	45 (17.1%)
Left nodule, n (%)	3 (1.1%)	73 (27.8%)	19 (7.2%)	95 (36.1%)
Bilateral nodules, n (%)	2 (0.8%)	4 (1.5%)	5 (1.9%)	11 (4.2%)
No nodules, n (%)	19 (7.2%)	21 (8.0%)	72 (27.4%)	112 (42.6%)
Total, n (%)	65 (24.7%)	99 (37.6%)	99 (37.6%)	263 (100.0)%

b) Findings at cross-sectional imaging in patients ≤ 40 years old	**Final Diagnosis n (%)**			
	Right Unilateral PA	**Left Unilateral PA**	**Bilateral PA**	**Total**
Right nodule, n (%)	22 (15.4%)	1 (0.7%)	1 (0.7%)	24 (16.8%)
Left nodule, n (%)	2 (1.4%)	47 (32.9%)	9 (6.3%)	58 (40.6%)
Bilateral nodules, n (%)	1 (0.7%)	2 (1.4%)	3 (2.1%)	6 (4.2%)
No nodules, n (%)	9 (6.3%)	11 (7.7%)	35 (24.5%)	55 (38.5%)
Total, n (%)	34 (23.8%)	61 (42.7%)	48 (33.6%)	143 (100.0)%

c) Findings at cross-sectional imaging in patients ≤ 35 years old	**Final Diagnosis n (%)**			
	Right Unilateral PA	**Left Unilateral PA**	**Bilateral PA**	**Total**
Right nodule, n (%)	5 (9.1%)	0 (0.0%)	0 (0.0%)	5 (9.1%)
Left nodule, n (%)	1 (1.8%)	19 (34.5%)	1 (1.8%)	21 (38.2%)
Bilateral nodules, n (%)	0 (0.0%)	0 (0.0%)	1 (1.8%)	1 (1.8%)
No nodules, n (%)	4 (7.3%)	7 (12.7%)	17 (30.9%)	28 (50.9%)
Total, n (%)	10 (18.2%)	26 (47.3%)	19 (34.5%)	55 (100.0)%

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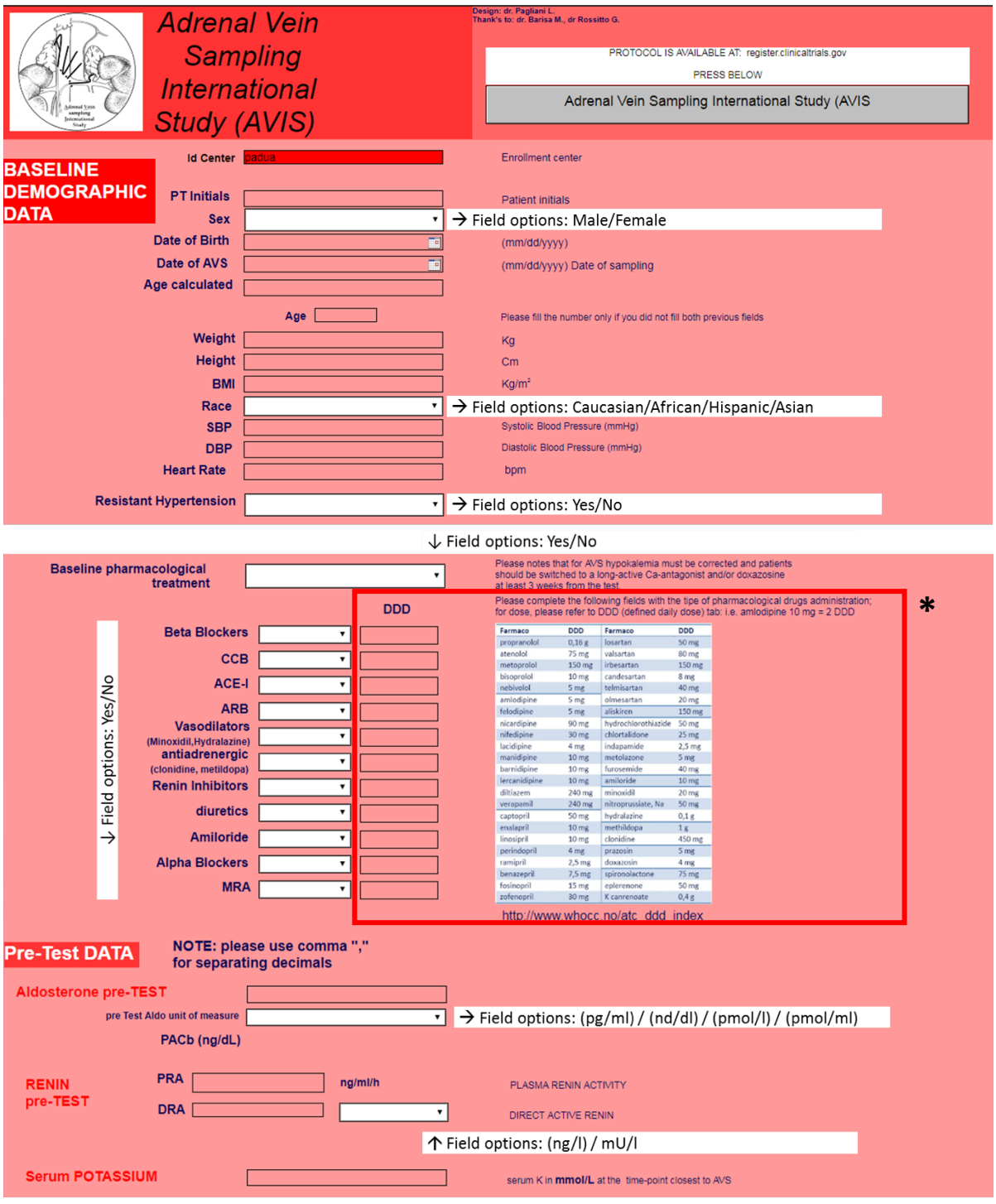
SUPPLEMENTARY DATA

Data collection form for AVIS2 (1)



* DDD was not prespecified at the beginning of the study; was later introduced but excluded from the current analysis because not available from all centers and/or all patients

Data collection form for AVIS2 (2)

BASELINE AVS DATA

NOTE: please use comma "," for separating decimals

ALDOSTERONE

Unit ALDO AVS baseline → Field options: (pg/ml) / (nd/dl) / (pmol/l) / (pmol/ml)

please note: pg/ml = ng/l

ALDOIVCb ALDOIVCb pg/ml — Aldosterone in inferior vena cava baseline

RightALDOb RightALDOb pg/ml — Aldosterone in right adrenal vein baseline

LeftALDOb LeftALDOb pg/ml — Aldosterone in left adrenal vein baseline

CORTISOL

Unit CORTISOL AVS baseline → Field options: (ng/dl) / (nmol/l) / (microg/dl) / (umol/l) / (ng/ml)

IVCCb CORTIVCb ng/ml — Cortisol in inferior vena cava baseline

RightCb RightCORTb ng/ml — Cortisol in right adrenal vein baseline

LeftCb LeftCORTb ng/ml — Cortisol in left adrenal vein baseline

STIMULATED AVS DATA

NOTE: please use comma "," for separating decimals

ALDOSTERONE

Unit ALDO AVS post stimulated → Field options: (pg/ml) / (nd/dl) / (pmol/l) / (pmol/ml)

ALDOIVCpost ALDOIVCpost pg/ml — Aldosterone in inferior vena cava post-stimulation

RightALDOpost RightALDOpost pg/ml — Aldosterone in right adrenal vein post-stimulation

LeftALDOpost LeftALDOpost pg/ml — Aldosterone in left adrenal vein post-stimulation

CORTISOL

Unit CORTISOL AVS post stimulated → Field options: (ng/dl) / (nmol/l) / (microg/dl) / (umol/l) / (ng/ml)

CortIVCpost CortIVCpost ng/ml — Cortisol in inferior vena cava post-stimulation

RightCortpost RightCortpost ng/ml — Cortisol in right adrenal vein post-stimulation

LeftCortpost LeftCortpost ng/ml — Cortisol in left adrenal vein post-stimulation

Rupture of adrenal vein → Field options: Yes/No

IMAGING DATA

↓ Field options: Yes/No

CT

CT right adrenal node — if yes indicate the maximum size of node in the field below

CT right adrenal node size mm

CT left adrenal node — if yes indicate the size of node in the field below

CT left adrenal node size mm

MR

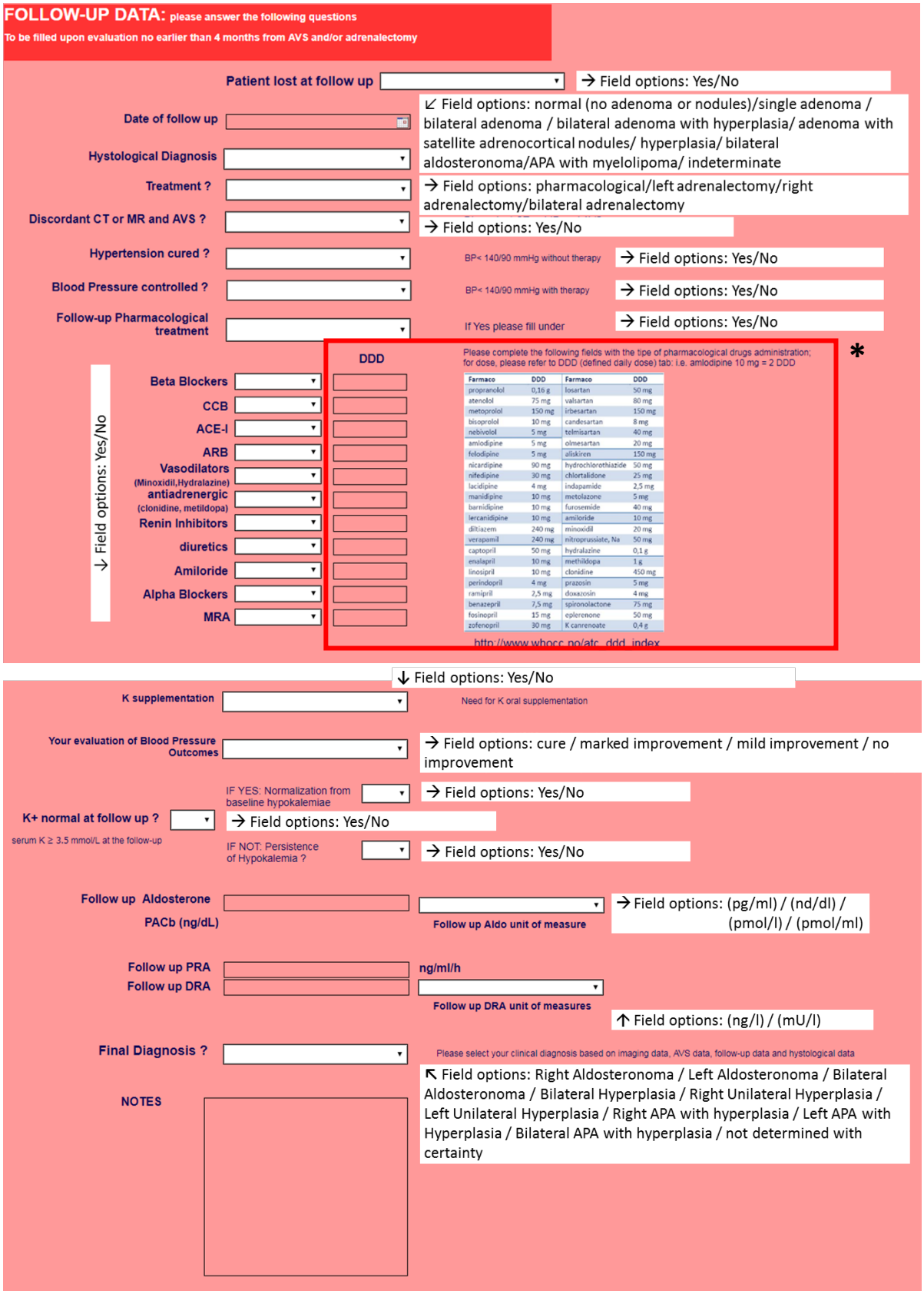
MR right adrenal node — if yes indicate the size of node in the field below

MR right adrenal node size mm

MR left adrenal node — if yes indicate the size of node in the field below

MR left adrenal node size mm

Data collection form for AVIS2 (3)



* DDD was not prespecified at the beginning of the study; was later introduced but excluded from the current analysis because not available from all centers and/or all patients

Summary List of the collected variables

- Demography (sex 1 =M 2=F, weight, BMI, race, etc.);
- AVS date (MM/DD/YYYY);
- Birth date (MM/DD/YYYY);
- Calculated age at AVS = AVS date (MM/DD/YYYY)- Birth date (MM/DD/YYYY);
- Systolic and diastolic blood pressure values at the time of AVS;
- Ongoing medical therapy at the time of AVS;
- Biochemical profile at baseline (sK^+, plasma aldosterone concentration (PAC); plasma renin activity (PRA).
- AVS protocol (bilaterally simultaneous/sequential; stimulated/unstimulated).
- PAC and plasma cortisol concentration (PCC) in each adrenal vein and in the inferior vena cava blood;
- Concordance/discordance between imaging and AVS results.
- Treatment modality: right/left/bilateral laparoscopic adrenalectomy; medical treatment.
- Blood pressure outcome at 6-months defined as reported in Supplemental Table 2.
- Persistence /correction of hypokalaemia at follow-up.
- Serum K^+, PAC and PRA at follow-up.
- Complications: adrenal vein rupture (appearance of persistent pain during or after catheterization, confirmed at imaging).
- Diagnosis (unilateral aldosterone-producing adenoma (APA); bilateral APA, unilateral adrenal hyperplasia; bilateral adrenal hyperplasia.

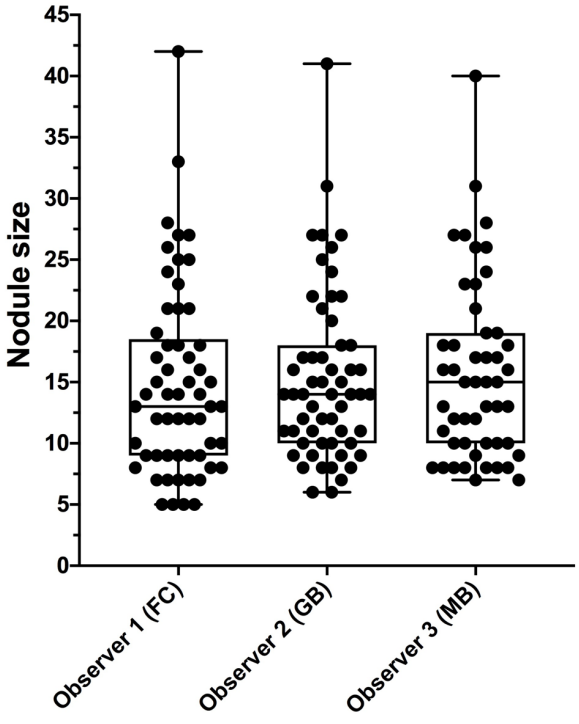
Conclusive diagnosis of unilateral PA required demonstration of biochemical cure at follow-up.

Pilot study on nodules' size at imaging

We enrolled 60 PA patients of the AVIS-2 examined in the same center with a 64 slice CT scanner (Somatom Sensation, Siemens Healthineers, Erlangen, Germany). CT protocol included unenhanced, arterial (10 seconds after the achievement of 100 HU within the abdominal aorta lumen) and venous phase (60 seconds after intravenous contrast injection) acquisitions after intravenous injection of 2 ml/kg of Iohexol 350 mg I/ml (Omnipaque, GE Healthcare, Milwaukee, USA) followed by a 50 ml saline flush. The slice thickness was 1.5 and 3 mm for all the acquisitions. Three radiologists experienced in adrenal imaging independently evaluated CT scans blinded to clinical data and to results of the other observers reporting the presence/absence of adrenal nodules and their maximum axial diameter. In case of absence of nodules, the measurement reported for the diameter was 0.

The distribution of nodules size measured by each observer are reported in Figure 1.

Figure 1. Distribution of the size of the nodules for the three observers



None of the observer was able to identify nodules below the size of 5 mm, indicating that this cut off is the resolution power of the CT for adrenal nodules detection. We compared the measurements recorded by observer 1 with those of observer 2 and the measurements of observer 1 to those of the observer 3 performing a Bland-Altman analysis (Figure 2 and 3). If one of the observers did not see any alteration of the adrenal gland and the other identified a measurable adrenal nodule the size reported in the analysis for the first observer was 0.

Figure 2. Bland-Altman analysis showing comparison between Observer 1 and 2

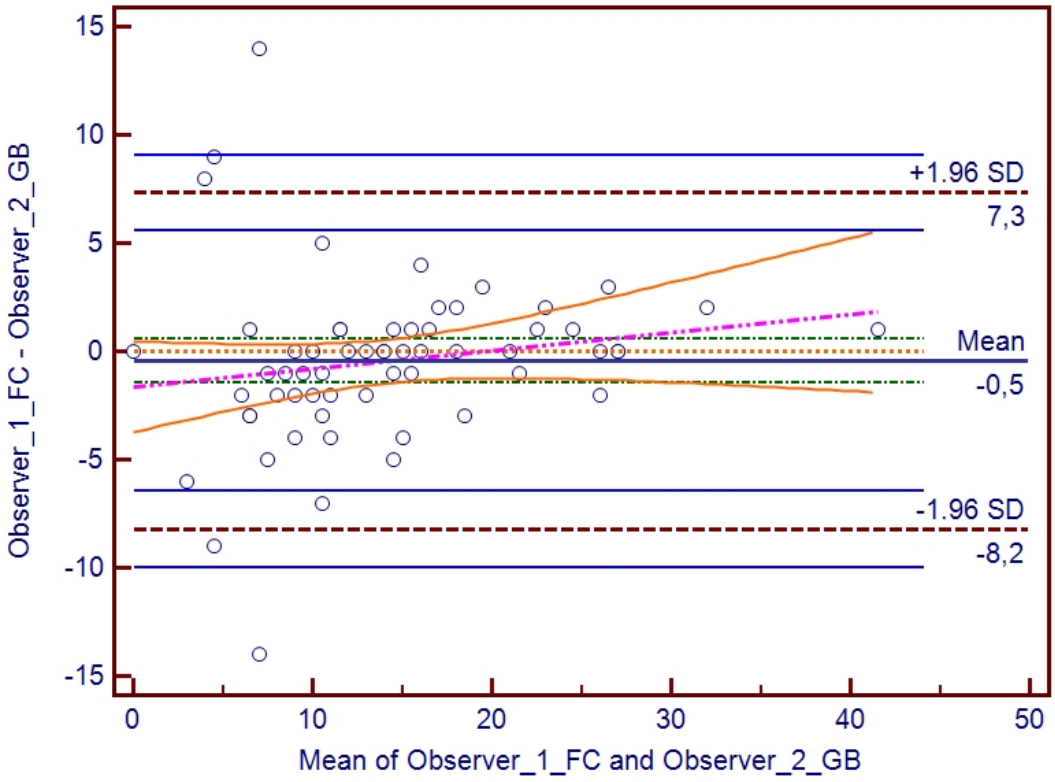
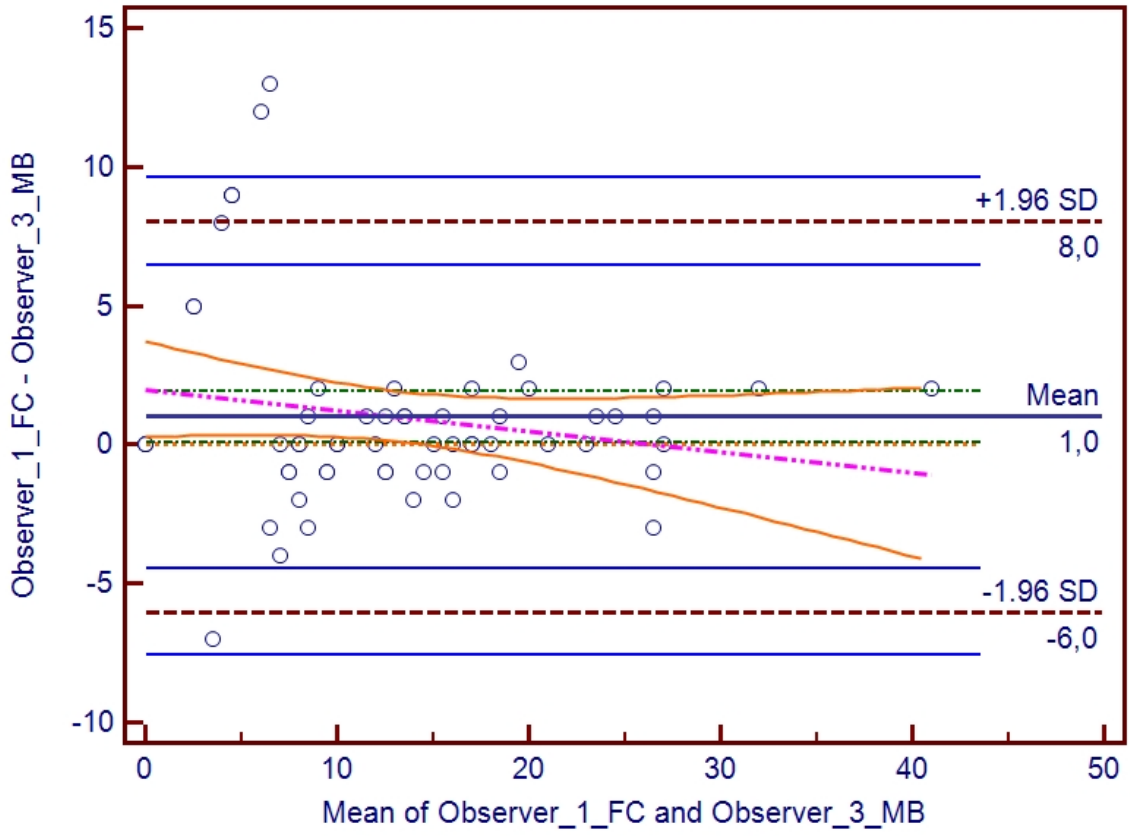


Figure 3. Bland-Altman analysis showing comparison between Observer 1 and 3



The Bland-Altman analysis showed that as the average size increases the differences between observers' measurements tend to get smaller and therefore the smaller are the nodules the wider are the discrepancies in the size of the nodules measured by the radiologists.

The Inter Class Coefficient (ICC) analysis showed a coefficient of 0.8764 (95% CI 0.8188-0.9194), revealing a good concordance among the three observers.

Starting from these evidences, we decided to exclude from the analysis of the study nodules smaller than 5 mm.

Table 1. Descriptives of nodules size in Patients ≤ 45 years old

			Statistic	Std. Error
***Node Size* ≤ 45 years old**	Mean		15,1990	0,51423
	95% Confidence Interval for Mean	Lower Bound	14,1850	
		Upper Bound	16,2130	
	5% Trimmed Mean		14,5345	
	Median		14,0000	
	Variance		53,150	
	Std. Deviation		7,29042	
	Minimum		5,00	
	Maximum		60,00	
	Range		55,00	
	Interquartile Range		7,50	
	Skewness		2,369	0,172
	Kurtosis		10,587	0,341

Figure 4. Histogram distribution of nodules' size in all Patients ≤ 45 years old with imaging data available

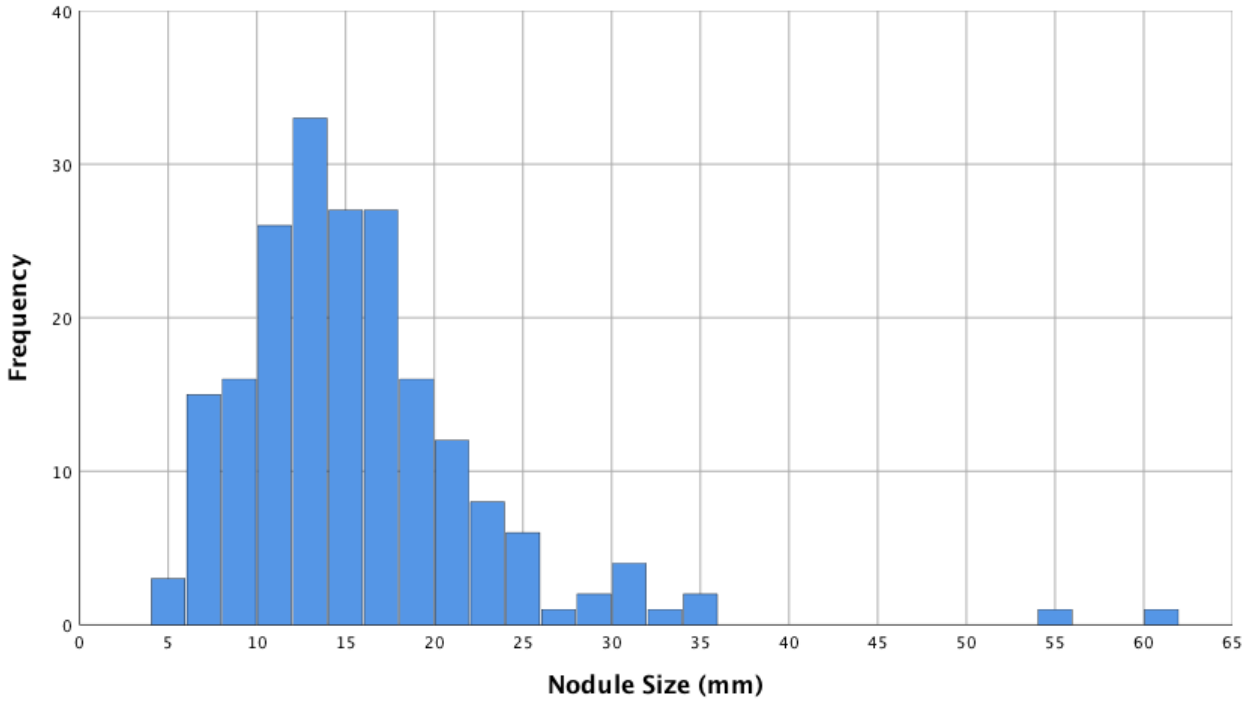


Table 2. Descriptives of nodules size in Patients ≤ 40 years old

			Statistic	Std. Error
***Node Size* ≤ 40 years old**	Mean		15,1990	0,51423
	95% Confidence Interval for Mean	Lower Bound	14,1850	
		Upper Bound	16,2130	
	5% Trimmed Mean		14,5345	
	Median		14,0000	
	Variance		53,150	
	Std. Deviation		7,29042	
	Minimum		5,00	
	Maximum		60,00	
	Range		55,00	
	Interquartile Range		7,50	
	Skewness		2,369	0,172
	Kurtosis		10,587	0,341

Figure 5. Histogram distribution of nodules' size in all Patients ≤ 40 years old with imaging data available

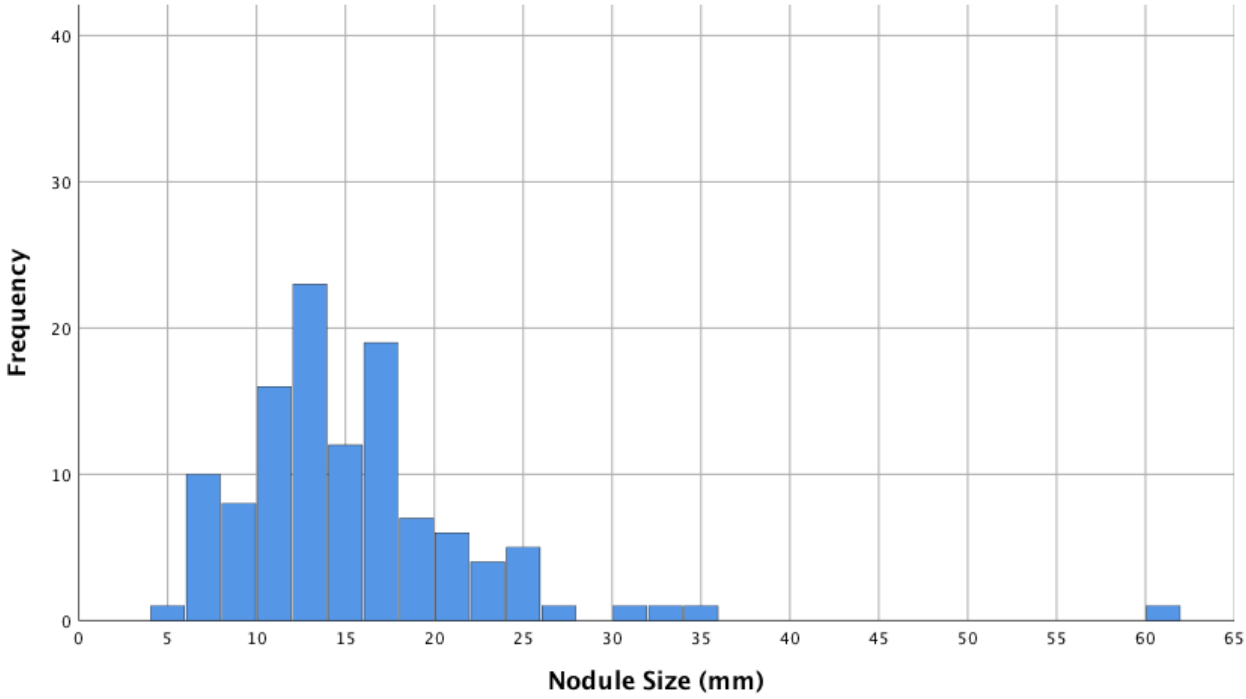


Table 3. Descriptives of nodules size in Patients ≤ 35 years old

			Statistic	Std. Error
***Node Size* ≤ 35 years old**	Mean		14,2571	0,88341
	95% Confidence Interval for Mean	Lower Bound	12,4618	
		Upper Bound	16,0524	
	5% Trimmed Mean		13,9444	
	Median		14,0000	
	Variance		27,314	
	Std. Deviation		5,22631	
	Minimum		6,00	
	Maximum		32,00	
	Range		26,00	
	Interquartile Range		6,00	
	Skewness		0,964	0,398
	Kurtosis		2,870	0,778

Figure 6. Histogram distribution of nodules' size in all Patients ≤ 35 years old with imaging data available

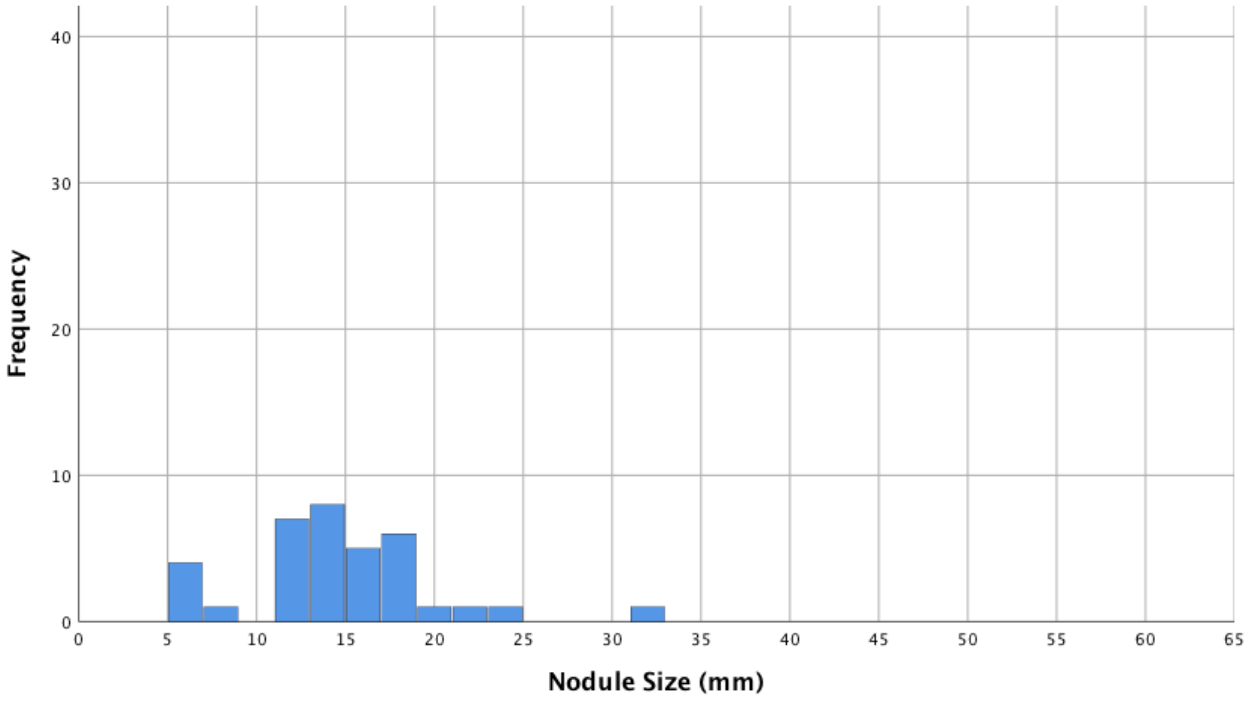


Figure 7. ROC curve analysis for the nodule size effect on the identification of the culprit side in patients ≤ 45 years old with unilateral nodule at imaging

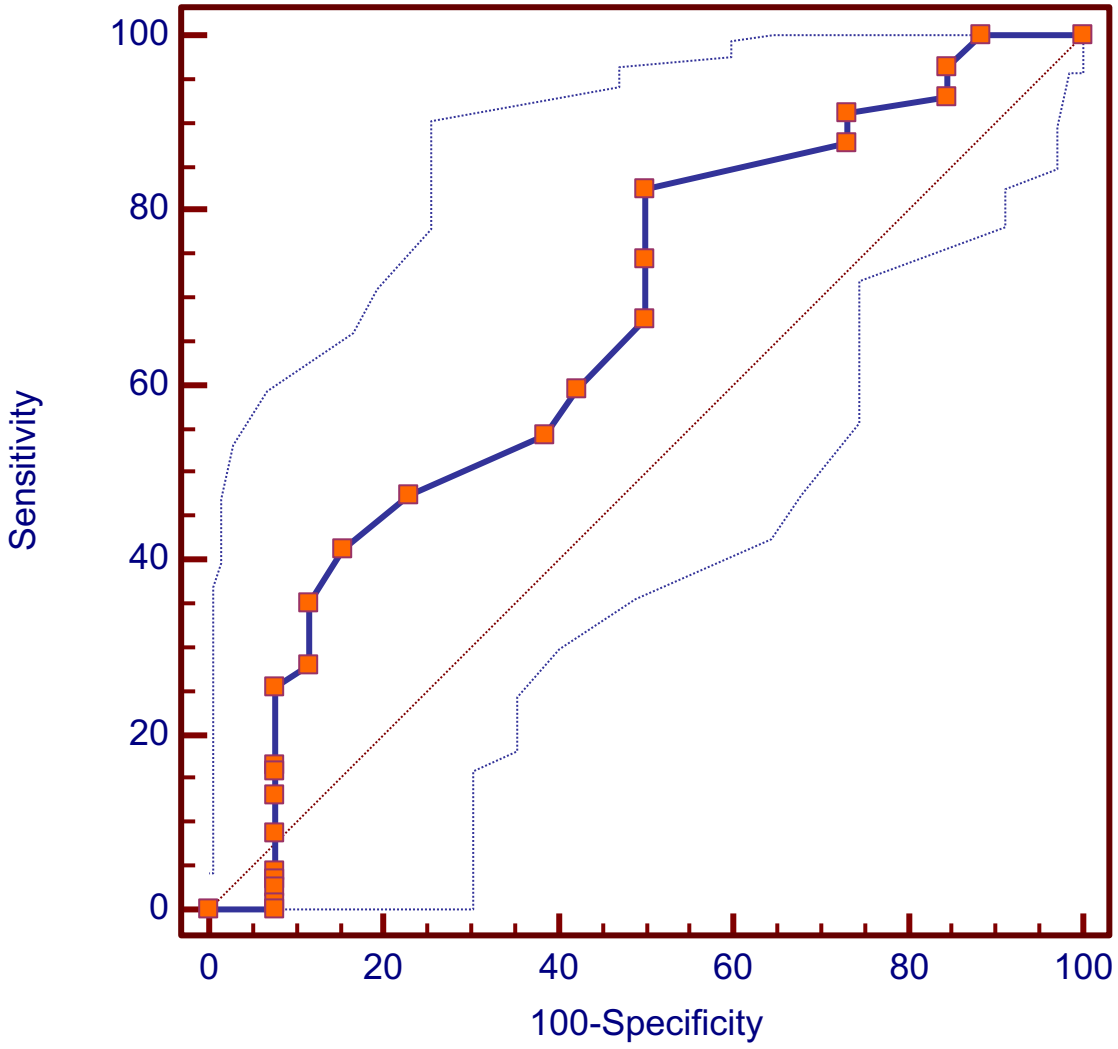


Table 7. ROC curve and Youden's index analyses for the nodule size effect on the identification of the culprit side in patients ≤ 45 years old with unilateral nodule at imaging

Variable	Nodule size Patients ≤ 45 years old
Classification variable	Concordance_with_Final_Diagnosis_45

Sample size	140
Positive group [a]	114 (81,43%)
Negative group [b]	26 (18,57%)

[a] Concordance_with_Final_Diagnosis_45 = 1
[b] Concordance_with_Final_Diagnosis_45 = 0

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0,664
Standard Error [a]	0,0625
95% Confidence interval [b]	0,579 to 0,742
z statistic	2,625
Significance level P (Area=0.5)	0,0087

[a] *DeLong et al., 1988*
[b] *Binomial exact*

Youden index

Youden index J	0,3246
95% Confidence interval [a]	0,1549 to 0,4872
Associated criterion	>10
95% Confidence interval [a]	>5,609141852 to >16
Sensitivity	82,46
Specificity	50,00

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Summary Table

Estimated specificity at fixed sensitivity			
Sensitivity	Specificity	95% CI [a]	Criterion
80,00	50,00	30,38 to 69,23	>10,311111111
90,00	26,92	9,23 to 51,54	>8,35
95,00	15,38	3,85 to 34,62	>6,425
97,50	14,28	3,85 to 30,77	>5,7125
99,00	12,63	3,85 to 31,43	>5,285
Estimated sensitivity at fixed specificity			
Specificity	Sensitivity	95% CI [a]	Criterion
80,00	44,91	23,16 to 57,54	>15,4
90,00	27,02	0,00 to 46,58	>18,4
95,00	0,00	0,00 to 28,80	>33,05
97,50	0,00	0,00 to 0,00	>34,025
99,00	0,00	0,00 to 0,00	>34,61

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
≥5	100,00	96,8 - 100,0	0,00	0,0 - 13,2	1,00	1,0 - 1,0		
>5	100,00	96,8 - 100,0	11,54	2,4 - 30,2	1,13	1,0 - 1,3	0,00	
>6	96,49	91,3 - 99,0	15,38	4,4 - 34,9	1,14	1,0 - 1,3	0,23	0,06 - 0,9
>7	92,98	86,6 - 96,9	15,38	4,4 - 34,9	1,10	0,9 - 1,3	0,46	0,1 - 1,4
>8	91,23	84,5 - 95,7	26,92	11,6 - 47,8	1,25	1,0 - 1,6	0,33	0,1 - 0,8
>9	87,72	80,3 - 93,1	26,92	11,6 - 47,8	1,20	0,9 - 1,5	0,46	0,2 - 1,0
>10	82,46	74,2 - 88,9	50,00	29,9 - 70,1	1,65	1,1 - 2,4	0,35	0,2 - 0,6
>11	74,56	65,6 - 82,3	50,00	29,9 - 70,1	1,49	1,0 - 2,2	0,51	0,3 - 0,8

>12	67,54	58,1 - 76,0	50,00	29,9 - 70,1	1,35	0,9 - 2,0	0,65	0,4 - 1,0
>13	59,65	50,1 - 68,7	57,69	36,9 - 76,6	1,41	0,9 - 2,3	0,70	0,5 - 1,0
>14	54,39	44,8 - 63,7	61,54	40,6 - 79,8	1,41	0,8 - 2,4	0,74	0,5 - 1,1
>15	47,37	37,9 - 56,9	76,92	56,4 - 91,0	2,05	1,0 - 4,3	0,68	0,5 - 0,9
>16	41,23	32,1 - 50,8	84,62	65,1 - 95,6	2,68	1,1 - 6,8	0,69	0,6 - 0,9
>17	35,09	26,4 - 44,6	88,46	69,8 - 97,6	3,04	1,0 - 9,1	0,73	0,6 - 0,9
>18	28,07	20,1 - 37,3	88,46	69,8 - 97,6	2,43	0,8 - 7,3	0,81	0,7 - 1,0
>19	25,44	17,7 - 34,4	92,31	74,9 - 99,1	3,31	0,8 - 13,0	0,81	0,7 - 0,9
>20	16,67	10,3 - 24,8	92,31	74,9 - 99,1	2,17	0,5 - 8,7	0,90	0,8 - 1,0
>21	15,79	9,6 - 23,8	92,31	74,9 - 99,1	2,05	0,5 - 8,3	0,91	0,8 - 1,0
>22	13,16	7,6 - 20,8	92,31	74,9 - 99,1	1,71	0,4 - 7,0	0,94	0,8 - 1,1
>23	8,77	4,3 - 15,5	92,31	74,9 - 99,1	1,14	0,3 - 4,9	0,99	0,9 - 1,1
>25	4,39	1,4 - 9,9	92,31	74,9 - 99,1	0,57	0,1 - 2,8	1,04	0,9 - 1,2
>27	3,51	1,0 - 8,7	92,31	74,9 - 99,1	0,46	0,09 - 2,4	1,05	0,9 - 1,2
>29	2,63	0,5 - 7,5	92,31	74,9 - 99,1	0,34	0,06 - 1,9	1,05	0,9 - 1,2
>30	0,88	0,02 - 4,8	92,31	74,9 - 99,1	0,11	0,01 - 1,2	1,07	1,0 - 1,2
>32	0,00	0,0 - 3,2	92,31	74,9 - 99,1	0,00		1,08	1,0 - 1,2
>35	0,00	0,0 - 3,2	100,00	86,8 - 100,0			1,00	1,0 - 1,0

Figure 8. ROC curve analysis for the nodule size effect on the identification of the culprit side in patients ≤ 40 years old with unilateral nodule at imaging

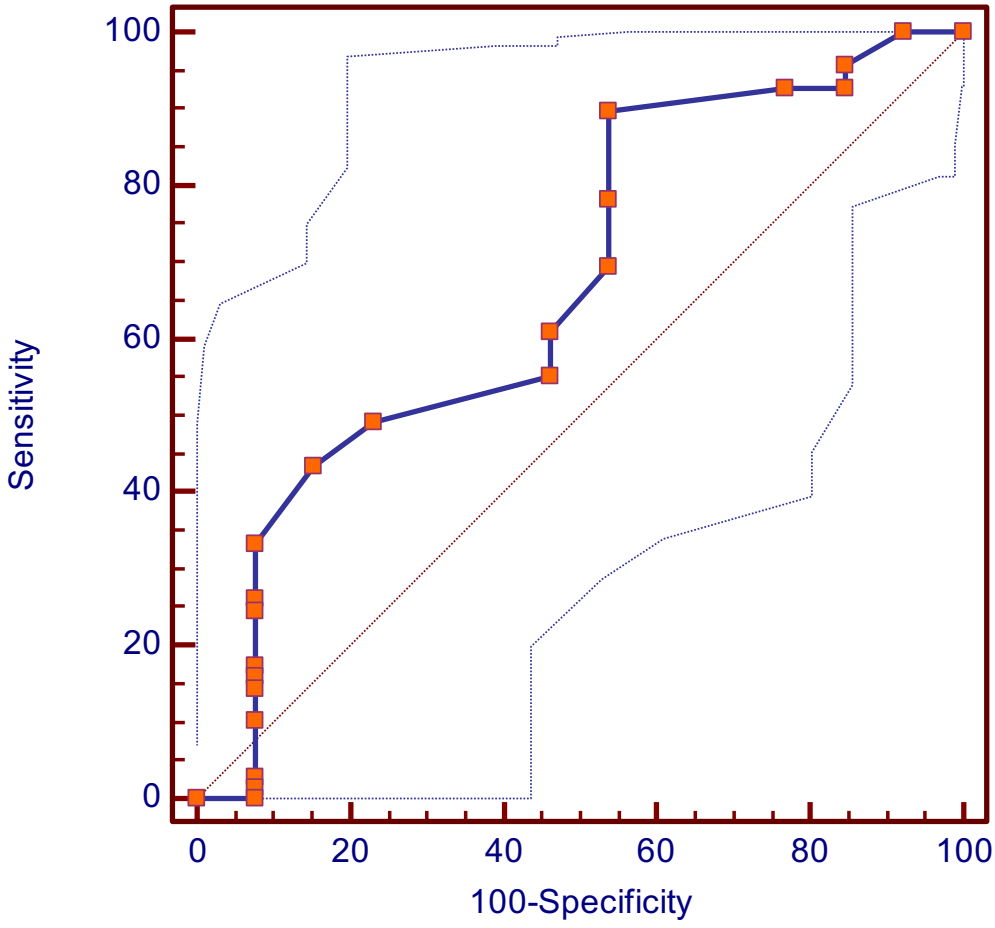


Table 8. ROC curve and Youden's index analyses for the nodule size effect on the identification of the culprit side in patients ≤ 40 years old with unilateral nodule at imaging

Variable	Nodule size Patients ≤ 40 years old
Classification variable	Concordance_with_Final_Diagnosis_40

Sample size	82
Positive group [a]	69 (84,15%)
Negative group [b]	13 (15,85%)

[a] Concordance_with_Final_Diagnosis_40 = 1
[b] Concordance_with_Final_Diagnosis_40 = 0

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0,670
Standard Error [a]	0,0889
95% Confidence interval [b]	0,557 to 0,770
z statistic	1,912
Significance level P (Area=0.5)	0,0558

[a] *DeLong et al., 1988*
[b] *Binomial exact*

Youden index

Youden index J	0,3601
95% Confidence interval [a]	0,1794 to 0,5429
Associated criterion	>10
95% Confidence interval [a]	>6 to >16
Sensitivity	89,86
Specificity	46,15

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Summary Table

Estimated specificity at fixed sensitivity			
Sensitivity	Specificity	95% CI [a]	Criterion
80,00	46,15	23,08 to 76,92	>10,85
90,00	45,00	7,69 to 77,89	>9,9
95,00	15,38	0,00 to 61,54	>6,225
97,50	12,12	0,00 to 34,11	>5,575
99,00	9,46	0,00 to 26,74	>5,23
Estimated sensitivity at fixed specificity			
Specificity	Sensitivity	95% CI [a]	Criterion
80,00	46,96	0,00 to 65,38	>15,4
90,00	36,38	0,00 to 53,55	>16,7
95,00	0,00	0,00 to 38,04	>33,05

97,50	0,00	0,00 to 0,00	>34,025
99,00	0,00	0,00 to 0,00	>34,61

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
≥5	100,00	94,8 - 100,0	0,00	0,0 - 24,7	1,00	1,0 - 1,0		
>5	100,00	94,8 - 100,0	7,69	0,2 - 36,0	1,08	0,9 - 1,3	0,00	
>6	95,65	87,8 - 99,1	15,38	1,9 - 45,4	1,13	0,9 - 1,4	0,28	0,05 - 1,5
>7	92,75	83,9 - 97,6	15,38	1,9 - 45,4	1,10	0,9 - 1,4	0,47	0,1 - 2,2
>8	92,75	83,9 - 97,6	23,08	5,0 - 53,8	1,21	0,9 - 1,6	0,31	0,09 - 1,2
>10	89,86	80,2 - 95,8	46,15	19,2 - 74,9	1,67	1,0 - 2,8	0,22	0,09 - 0,5
>11	78,26	66,7 - 87,3	46,15	19,2 - 74,9	1,45	0,9 - 2,4	0,47	0,2 - 1,0
>12	69,57	57,3 - 80,1	46,15	19,2 - 74,9	1,29	0,8 - 2,2	0,66	0,3 - 1,3
>13	60,87	48,4 - 72,4	53,85	25,1 - 80,8	1,32	0,7 - 2,4	0,73	0,4 - 1,3
>14	55,07	42,6 - 67,1	53,85	25,1 - 80,8	1,19	0,6 - 2,2	0,83	0,5 - 1,5
>15	49,28	37,0 - 61,6	76,92	46,2 - 95,0	2,14	0,8 - 5,9	0,66	0,5 - 1,0
>16	43,48	31,6 - 56,0	84,62	54,6 - 98,1	2,83	0,8 - 10,4	0,67	0,5 - 0,9
>17	33,33	22,4 - 45,7	92,31	64,0 - 99,8	4,33	0,6 - 29,3	0,72	0,6 - 0,9
>18	26,09	16,3 - 38,1	92,31	64,0 - 99,8	3,39	0,5 - 23,2	0,80	0,6 - 1,0
>19	24,64	15,1 - 36,5	92,31	64,0 - 99,8	3,20	0,5 - 22,0	0,82	0,7 - 1,0
>20	17,39	9,3 - 28,4	92,31	64,0 - 99,8	2,26	0,3 - 15,9	0,89	0,7 - 1,1
>21	15,94	8,2 - 26,7	92,31	64,0 - 99,8	2,07	0,3 - 14,7	0,91	0,8 - 1,1
>22	14,49	7,2 - 25,0	92,31	64,0 - 99,8	1,88	0,3 - 13,5	0,93	0,8 - 1,1
>23	10,14	4,2 - 19,8	92,31	64,0 - 99,8	1,32	0,2 - 9,8	0,97	0,8 - 1,2
>25	2,90	0,4 - 10,1	92,31	64,0 - 99,8	0,38	0,04 - 3,9	1,05	0,9 - 1,2
>27	1,45	0,04 - 7,8	92,31	64,0 - 99,8	0,19	0,01 - 2,8	1,07	0,9 - 1,3
>32	0,00	0,0 - 5,2	92,31	64,0 - 99,8	0,00		1,08	0,9 - 1,3
>35	0,00	0,0 - 5,2	100,00	75,3 - 100,0			1,00	1,0 - 1,0

Figure 9. ROC curve analysis for the nodule size effect on the identification of the culprit side in patients ≤ 35 years old with unilateral nodule at imaging

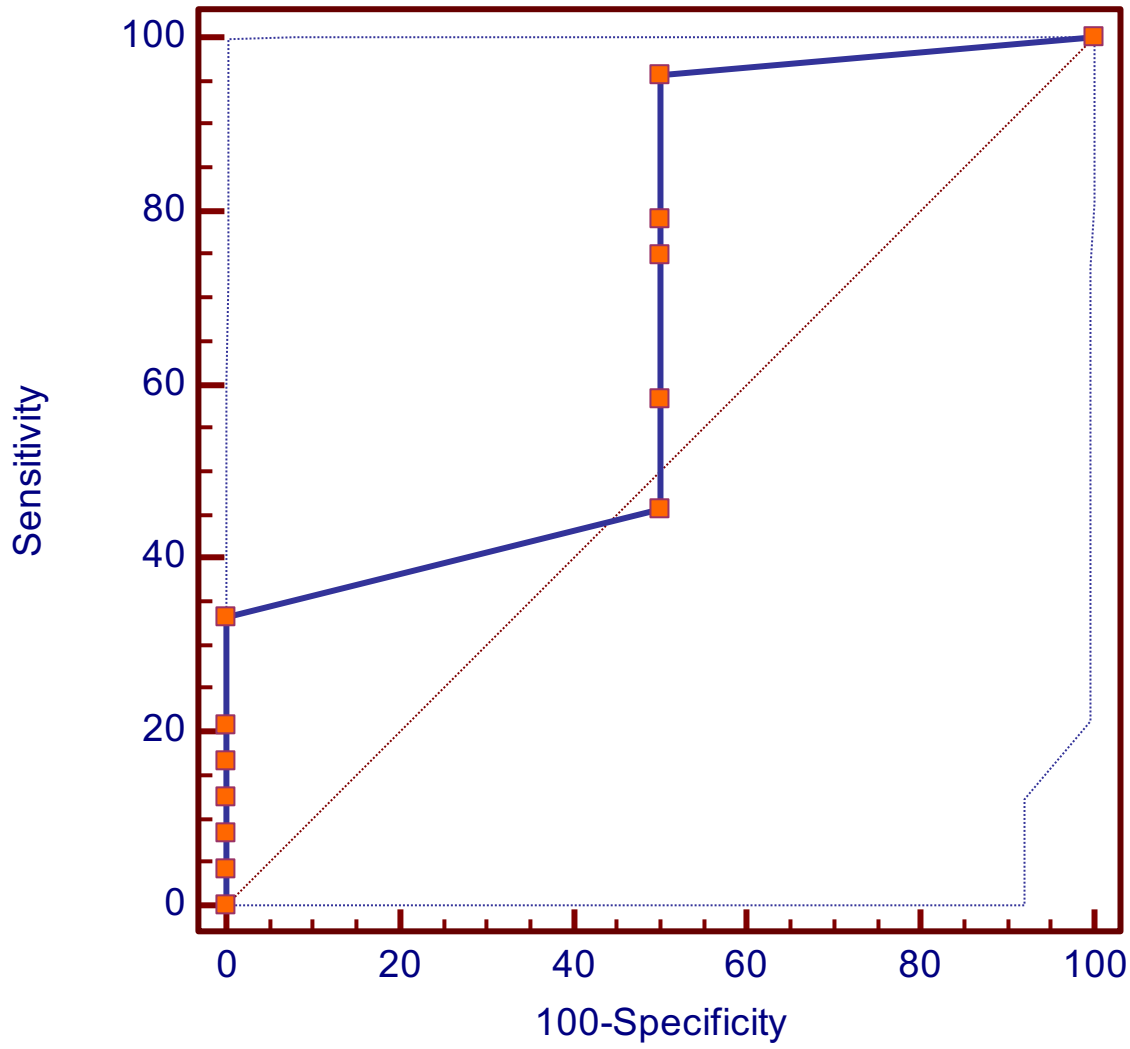


Table 9. ROC curve and Youden's index analyses for the nodule size effect on the identification of the culprit side in patients ≤ 35 years old with unilateral nodule at imaging

Variable	Nodule size Patients ≤ 35 years old
Classification variable	Concordance_with_Final_Diagnosis_35

Sample size	26
Positive group [a]	24 (92,31%)
Negative group [b]	2 (7,69%)

[a] Concordance_with_Final_Diagnosis_35 = 1
[b] Concordance_with_Final_Diagnosis_35 = 0

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0,688
Standard Error [a]	0,296
95% Confidence interval [b]	0,477 to 0,853
z statistic	0,633
Significance level P (Area=0.5)	0,5264

[a] *DeLong et al., 1988*
[b] *Binomial exact*

Youden index

Youden index J	0,4583
95% Confidence interval [a]	0,2917 to 0,5833
Associated criterion	>6
95% Confidence interval [a]	>6 to >14
Sensitivity	95,83
Specificity	50,00

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Summary Table

Estimated specificity at fixed sensitivity			
Sensitivity	Specificity	95% CI [a]	Criterion
80,00	50,00	0,00 to 100,00	>10,75
90,00	50,00	0,00 to 100,00	>7,75
95,00	50,00	0,00 to 100,00	>6,25
97,50	50,00	0,00 to 100,00	>6
99,00	50,00	0,00 to 100,00	>6
Estimated sensitivity at fixed specificity			
Specificity	Sensitivity	95% CI [a]	Criterion
80,00	38,33	9,17 to 76,67	>15,2
90,00	35,83	8,75 to 86,25	>15,6
95,00	34,58	8,54 to 91,04	>15,8
97,50	33,96	0,00 to 0,00	>15,9
99,00	33,58	0,00 to 0,00	>15,96

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
≥6	100,00	85,8 - 100,0	0,00	0,0 - 84,2	1,00	1,0 - 1,0		
>6	95,83	78,9 - 99,9	50,00	1,3 - 98,7	1,92	0,5 - 7,7	0,083	0,008 - 0,9
>11	79,17	57,8 - 92,9	50,00	1,3 - 98,7	1,58	0,4 - 6,4	0,42	0,08 - 2,0
>12	75,00	53,3 - 90,2	50,00	1,3 - 98,7	1,50	0,4 - 6,1	0,50	0,1 - 2,4
>13	58,33	36,6 - 77,9	50,00	1,3 - 98,7	1,17	0,3 - 4,9	0,83	0,2 - 3,6
>14	45,83	25,6 - 67,2	50,00	1,3 - 98,7	0,92	0,2 - 3,9	1,08	0,3 - 4,5
>16	33,33	15,6 - 55,3	100,00	15,8 - 100,0			0,67	0,5 - 0,9
>17	20,83	7,1 - 42,2	100,00	15,8 - 100,0			0,79	0,6 - 1,0
>18	16,67	4,7 - 37,4	100,00	15,8 - 100,0			0,83	0,7 - 1,0
>20	12,50	2,7 - 32,4	100,00	15,8 - 100,0			0,88	0,8 - 1,0
>22	8,33	1,0 - 27,0	100,00	15,8 - 100,0			0,92	0,8 - 1,0

>23	4,17	0,1 - 21,1	100,00	15,8 - 100,0			0,96	0,9 - 1,0
>32	0,00	0,0 - 14,2	100,00	15,8 - 100,0			1,00	1,0 - 1,0

Table 10. ROC curve and Youden's index analyses for the age effect on the identification of the culprit side in patients ≤ 45 years old with unilateral nodule at imaging

Variable	Age at AVS
Classification variable	Concordance with final diagnosis

Sample size	140
Positive group [a]	114 (81,43%)
Negative group [b]	26 (18,57%)

[a] Concordance_with_final_diagnosis = 1
[b] Concordance_with_final_diagnosis = 0

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0,592
Standard Error [a]	0,0604
95% Confidence interval [b]	0,506 to 0,674
z statistic	1,529
Significance level P (Area=0.5)	0,1263

[a] *DeLong et al., 1988*
[b] *Binomial exact*

Youden index

Youden index J	0,1869
95% Confidence interval [a]	0,07322 to 0,2982
Associated criterion	≤38
95% Confidence interval [a]	≤28 to ≤42
Sensitivity	45,61
Specificity	73,08

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Summary Table

Estimated specificity at fixed sensitivity			
Sensitivity	Specificity	95% CI [a]	Criterion
80,00	29,15	11,41 to 50,82	≤42,355555556
90,00	11,15	0,62 to 31,72	≤43,7
95,00	4,87	0,00 to 20,18	≤44,366666667
97,50	2,44	0,00 to 10,96	≤44,683333333
99,00	0,97	0,00 to 4,38	≤44,873333333

Estimated sensitivity at fixed specificity			
Specificity	Sensitivity	95% CI [a]	Criterion
80,00	34,74	3,73 to 52,28	≤36,2
90,00	22,46	0,37 to 43,58	≤34,6
95,00	2,54	0,00 to 26,81	≤26,6
97,50	0,88	0,00 to 0,00	≤22,6
99,00	0,88	0,00 to 0,00	≤21,04

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
<20	0,00	0,0 - 3,2	100,00	86,8 - 100,0			1,00	1,0 - 1,0
≤20	0,88	0,02 - 4,8	100,00	86,8 - 100,0			0,99	1,0 - 1,0
≤24	0,88	0,02 - 4,8	96,15	80,4 - 99,9	0,23	0,01 - 3,5	1,03	1,0 - 1,1
≤26	1,75	0,2 - 6,2	96,15	80,4 - 99,9	0,46	0,04 - 4,8	1,02	0,9 - 1,1
≤28	4,39	1,4 - 9,9	92,31	74,9 - 99,1	0,57	0,1 - 2,8	1,04	0,9 - 1,2
≤30	6,14	2,5 - 12,2	92,31	74,9 - 99,1	0,80	0,2 - 3,6	1,02	0,9 - 1,1
≤31	10,53	5,6 - 17,7	92,31	74,9 - 99,1	1,37	0,3 - 5,7	0,97	0,9 - 1,1
≤32	12,28	6,9 - 19,7	92,31	74,9 - 99,1	1,60	0,4 - 6,6	0,95	0,8 - 1,1
≤33	17,54	11,1 - 25,8	92,31	74,9 - 99,1	2,28	0,6 - 9,2	0,89	0,8 - 1,0
≤34	19,30	12,5 - 27,7	92,31	74,9 - 99,1	2,51	0,6 - 10,0	0,87	0,8 - 1,0
≤35	24,56	17,0 - 33,5	88,46	69,8 - 97,6	2,13	0,7 - 6,5	0,85	0,7 - 1,0
≤36	33,33	24,8 - 42,8	80,77	60,6 - 93,4	1,73	0,8 - 4,0	0,83	0,7 - 1,0
≤37	40,35	31,3 - 49,9	76,92	56,4 - 91,0	1,75	0,8 - 3,7	0,78	0,6 - 1,0
≤38	45,61	36,3 - 55,2	73,08	52,2 - 88,4	1,69	0,9 - 3,3	0,74	0,6 - 1,0
≤39	53,51	43,9 - 62,9	61,54	40,6 - 79,8	1,39	0,8 - 2,3	0,76	0,5 - 1,1
≤40	62,28	52,7 - 71,2	46,15	26,6 - 66,6	1,16	0,8 - 1,7	0,82	0,5 - 1,3
≤41	70,18	60,9 - 78,4	42,31	23,4 - 63,1	1,22	0,9 - 1,7	0,70	0,4 - 1,2
≤42	77,19	68,4 - 84,5	34,62	17,2 - 55,7	1,18	0,9 - 1,6	0,66	0,4 - 1,2
≤43	85,09	77,2 - 91,1	19,23	6,6 - 39,4	1,05	0,9 - 1,3	0,78	0,3 - 1,9
≤44	92,11	85,5 - 96,3	7,69	0,9 - 25,1	1,00	0,9 - 1,1	1,03	0,2 - 4,5
≤45	100,00	96,8 - 100,0	0,00	0,0 - 13,2	1,00	1,0 - 1,0		

Figure 10. ROC curve analysis for the nodule size effect on the identification of the culprit side in patients ≤ 38 years old

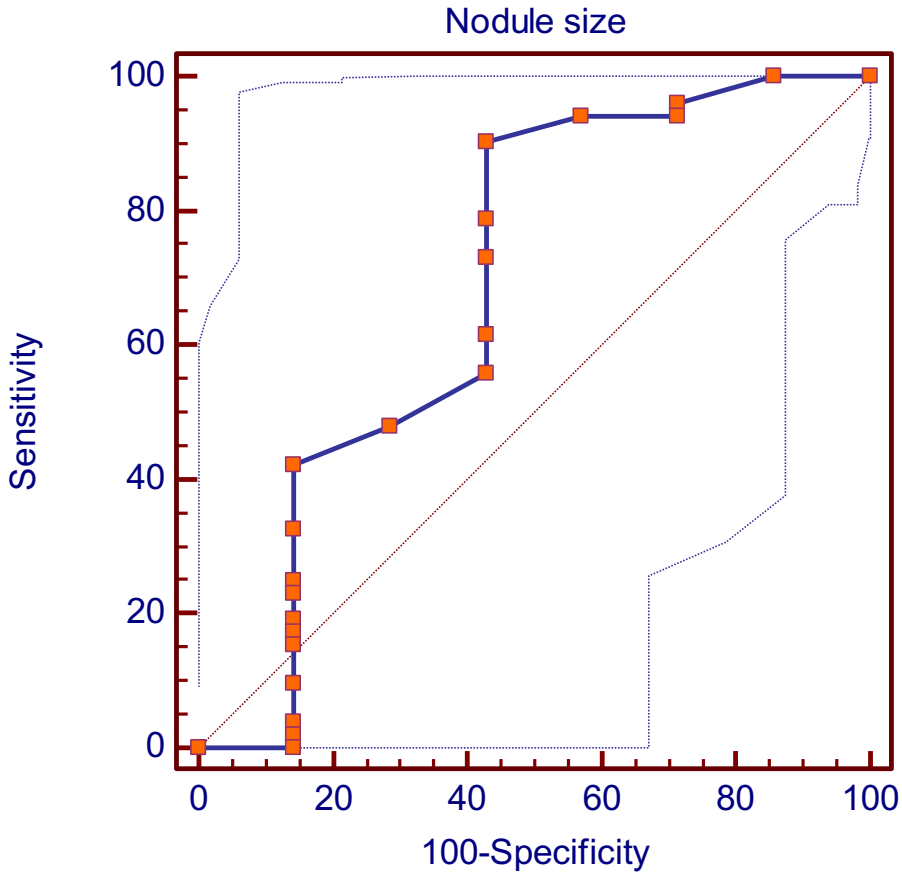


Table 11. ROC curve and Youden's index analyses for the nodule size effect on the identification of the culprit side in patients ≤ 38 years old with unilateral nodule at imaging

Variable	Nodule_size Nodule size
Classification variable	Concordance_with_Final_Diagnosis Concordance with Final Diagnosis

Sample size	59
Positive group [a]	52 (88,14%)
Negative group [b]	7 (11,86%)

[a] Concordance_with_Final_Diagnosis = 1
[b] Concordance_with_Final_Diagnosis = 0

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0,688
Standard Error [a]	0,135
95% Confidence interval [b]	0,554 to 0,802
z statistic	1,389
Significance level P (Area=0.5)	0,1647

[a] *Hanley & McNeil, 1982*
[b] *Binomial exact*

Youden index

Youden index J	0,4753
95% Confidence interval [a]	0,2676 to 0,7802
Associated criterion	>10
95% Confidence interval [a]	>8 to >32
Sensitivity	90,38
Specificity	57,14

[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Summary Table

Estimated specificity at fixed sensitivity			
Sensitivity	Specificity	95% CI [a]	Criterion
80,00	57,14	14,29 to 85,71	>10,9
90,00	57,14	14,29 to 100,00	>10,033333333
95,00	28,57	0,00 to 61,43	>6,6
97,50	23,57	0,00 to 57,14	>5,65
99,00	18,00	0,00 to 46,57	>5,26
Estimated sensitivity at fixed specificity			
Specificity	Sensitivity	95% CI [a]	Criterion
80,00	44,62	0,00 to 96,26	>15,6
90,00	0,00	0,00 to 43,35	>32,9
95,00	0,00	0,00 to 41,83	>33,95
97,50	0,00	0,00 to 0,00	>34,475
99,00	0,00	0,00 to 0,00	>34,79

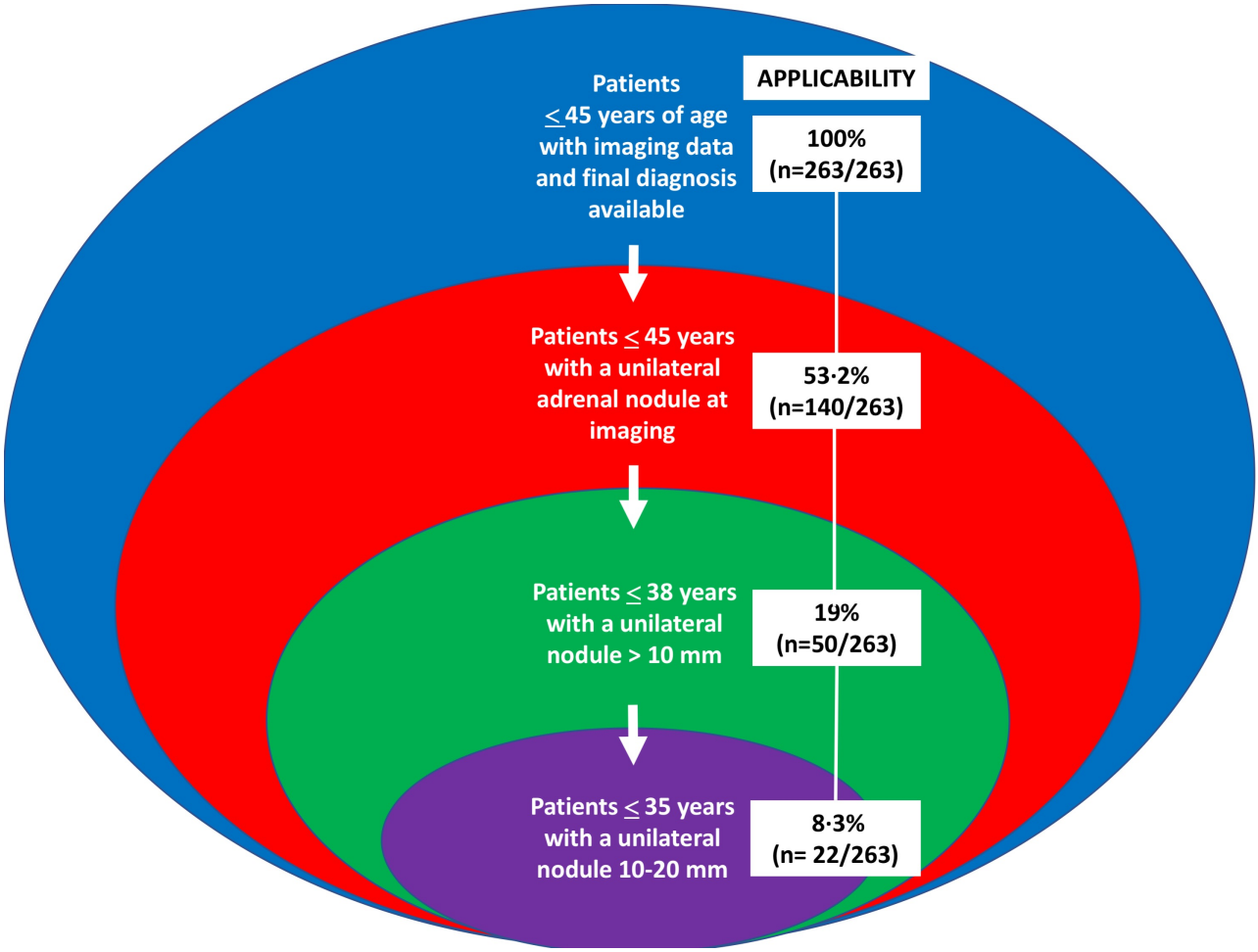
[a] *BC_a bootstrap confidence interval (1000 iterations; random number seed: 978).*

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
≥5	100,00	93,2 - 100,0	0,00	0,0 - 41,0	1,00	1,0 - 1,0		
>5	100,00	93,2 - 100,0	14,29	0,4 - 57,9	1,17	0,9 - 1,6	0,00	
>6	96,15	86,8 - 99,5	28,57	3,7 - 71,0	1,35	0,8 - 2,2	0,13	0,02 - 0,8
>7	94,23	84,1 - 98,8	28,57	3,7 - 71,0	1,32	0,8 - 2,1	0,20	0,04 - 1,0
>8	94,23	84,1 - 98,8	42,86	9,9 - 81,6	1,65	0,9 - 3,1	0,13	0,03 - 0,5
>10	90,38	79,0 - 96,8	57,14	18,4 - 90,1	2,11	0,9 - 5,0	0,17	0,06 - 0,5
>11	78,85	65,3 - 88,9	57,14	18,4 - 90,1	1,84	0,8 - 4,4	0,37	0,2 - 0,8
>12	73,08	59,0 - 84,4	57,14	18,4 - 90,1	1,71	0,7 - 4,1	0,47	0,2 - 1,0
>13	61,54	47,0 - 74,7	57,14	18,4 - 90,1	1,44	0,6 - 3,5	0,67	0,3 - 1,4
>14	55,77	41,3 - 69,5	57,14	18,4 - 90,1	1,30	0,5 - 3,2	0,77	0,4 - 1,6
>15	48,08	34,0 - 62,4	71,43	29,0 - 96,3	1,68	0,5 - 5,6	0,73	0,4 - 1,2
>16	42,31	28,7 - 56,8	85,71	42,1 - 99,6	2,96	0,5 - 18,7	0,67	0,5 - 1,0

>17	32,69	20,3 - 47,1	85,71	42,1 - 99,6	2,29	0,4 - 14,6	0,79	0,5 - 1,1
>18	25,00	14,0 - 38,9	85,71	42,1 - 99,6	1,75	0,3 - 11,4	0,88	0,6 - 1,2
>19	23,08	12,5 - 36,8	85,71	42,1 - 99,6	1,62	0,2 - 10,6	0,90	0,6 - 1,3
>20	19,23	9,6 - 32,5	85,71	42,1 - 99,6	1,35	0,2 - 9,0	0,94	0,7 - 1,3
>21	17,31	8,2 - 30,3	85,71	42,1 - 99,6	1,21	0,2 - 8,2	0,96	0,7 - 1,3
>22	15,38	6,9 - 28,1	85,71	42,1 - 99,6	1,08	0,2 - 7,4	0,99	0,7 - 1,4
>23	9,62	3,2 - 21,0	85,71	42,1 - 99,6	0,67	0,09 - 5,0	1,05	0,8 - 1,4
>25	3,85	0,5 - 13,2	85,71	42,1 - 99,6	0,27	0,03 - 2,6	1,12	0,8 - 1,5
>27	1,92	0,05 - 10,3	85,71	42,1 - 99,6	0,13	0,009 - 1,9	1,14	0,8 - 1,6
>32	0,00	0,0 - 6,8	85,71	42,1 - 99,6	0,00		1,17	0,9 - 1,6
>35	0,00	0,0 - 6,8	100,00	59,0 - 100,0			1,00	1,0 - 1,0

Figure 11. Applicability of age and imaging criteria in the AVIS-2-Young cohort



Chapter 4

Summary and Perspectives

SUMMARY

In **Chapter 1** a brief introduction describing arterial hypertension and its harmful cardiovascular complications is provided. I focused on secondary forms of hypertension and, in particular, on PA that is the most common form of secondary hypertension and, therefore, should always be screened in the work up of the hypertensive patients since the unilateral forms of aldosterone hypersecretion can be surgically treated with targeted adrenalectomy. Current guidelines suggest a second level imaging, such as CT or MR, to rule out malignant adrenal tumors and to depict the adrenal veins anatomy in order to help interventional radiologists in performing AVS. This last test is considered the key to distinguish between unilateral and bilateral form of aldosterone hypersecretion and, therefore, to address PA patients toward curative adrenalectomy or medical therapy with mineralocorticoid receptor antagonists. Anyway, since it is not widely available, AVS represents a bottle-neck in the work-flow of PA patients and therefore many different study groups tried to elaborate alternative strategies for PA subtyping. Only few, mainly monocentric, studies and a meta-analysis evaluated the accuracy of imaging "tout-court" in PA subtypes identification and therefore we planned an evaluation of imaging performance in the large setting of PA patients form the AVIS-2 study gathered from 19 centers world-wide.

In **Chapter 2**, the AVIS-2-IM study, we assessed the accuracy of imaging in detection and subtyping of PA in a large cohort of patients, using as reference standard a conclusive diagnosis of unilateral PA based on histopathology after adrenalectomy and biochemical cure at follow-up. All nodules with a size $\geq$ 5 mm were considered as a positive imaging result. Of the whole cohort of 1311 PA patients with imaging available, 41 % showed either bilateral (7.1%) or no nodules (33.9%) at imaging. Corresponding rates in those with unilateral surgically cured PA were 20.1% and 5.5%, respectively. Therefore, imaging did not identify unilateral nodules in 41% of the patients and did not detect the culprit adrenal in 25.6% of the cases with a final diagnosis of unilateral PA. Moreover, in 4.7% of patients with a final diagnosis of unilateral PA imaging detected a unilateral adrenal nodule contralateral to the lateralization at final diagnosis. Our data testify the low sensitivity of imaging for

detecting nodules in PA patients and do not support the use of CT and/or MR for identification of PA and its subtypes.

In **Chapter 3**, the AVIS-2-Young study, the accuracy of imaging in detection of unilateral PA was tested in a large cohort of PA patients ≤45 years-old. We started from the hypothesis, endorsed by the Endocrine Society guidelines, that AVS could be skipped when referring for surgery young PA patients with a unilateral adrenal nodule and a contralateral normally appearing gland at imaging.

The gold standard to evaluate imaging accuracy was the biochemical cure of PA after unilateral adrenalectomy. Out of the 1625 AVIS-2 patients those ≤45, 40 and 35 years-old were 29.0%, 15.5% and 6.1%, respectively. In the same age cohorts, imaging identified a unilateral adrenal nodule in 53.2%, 57.3% and 47.3% while no nodules were detected in 42.6%, 38.5%, and 50.9% of patients, respectively, and bilateral nodules in 4.2%, 4.2% and 1.8%. In the cohorts of patients that showed a unilateral nodule, imaging would have addressed to a correct adrenalectomy 81.4%, 84.1%, and 92.3% of the patients ≤45, 40 and 35 years-old. We identified 38 years as the best age cut-off for unilateral PA identification at the Youden's index analysis and, by restricting the nodule size to >10 mm, the likelihood of performing a correct adrenalectomy based on imaging results improved to 94.0%. However, bilateral or no nodules were detected at imaging in more than 1/3 of the patients with a final diagnosis of unilateral PA in all age cohorts. Therefore, the referral for surgery of PA patients ≤45, 40 and 35 years-old on the basis of a unilateral nodule at imaging carried a likelihood of wrong/inappropriate surgery in 18.6%, 15.8% and 7.7%, respectively. This error rate can be minimized to 6.0% by selecting patients ≤38 years with a nodule >10 mm.

PERSPECTIVES

This thesis demonstrates that imaging, although pivotal in adrenal veins detection for AVS as underlined by the guidelines, plays a limited role in subtyping and identification of PA. In young patients the accuracy of imaging for the detection on unilateral PA forms is higher and, therefore, in

case of not availability of AVS it could be offered to the patients for surgery referral, being aware that, even if small, a likelihood of performing an inappropriate of wrong adrenalectomy exists.

These results clearly raise a need for more accurate imaging techniques in PA. One of the possible solutions could be the development of specific radiotracer that label specifically to the aldosterone procuring clusters or APA, allowing to identify the lateralization of aldosterone hypersecretion.

Some authors (Bongarzone S et al and O'Shea PM et al) [1,2] have already started to evaluate this kind of approach and in future PET/CT or PET/MRI could be able even to substitute AVS in subtyping of PA.

Another possible approach could be the application of the radiomics and texture analysis of CT and MR images in the study of the adrenal glands of PA patients. The extrapolation of multiple parameters derived from the different pixel intensities inside the adrenal glands volume could be able to give more precise information about the micro-structure of the tissue and possibly to be related to the presence of lipid and cholesterol rich micro-adenomas. Indeed, both in a study by Akai H et al [3] and in the preliminary results observed by our research group this new radiological tool was promising and showed a good accuracy in the PA subtypes identification.

These new applications are intriguing and, if successful, will expand the possibilities of PA identification and treatment that nowadays are limited by the low availability of AVS.

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