Diagnosis of primary aldosteronism in the hypertension specialist centers in Italy. A national Survey.

Running title: diagnosis of primary aldosteronism among hypertension specialist centers

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Abstract

Primary aldosteronism (PA) is the most common endocrine cause of resistant hypertension. Individuals with PA are at increased cardiovascular risk, and an appropriate management and treatment would ideally reduce such risk. Screening and diagnosis of PA requires specific diagnostic test, it is considered as a time- and cost-consuming and, as a result, it is underperformed in clinical practice. An online survey reviewing available diagnostic procedures, laboratory testing and clinical protocols for screening and confirmation of PA diagnosis was conducted among clinical lead of Excellence centers of the Italian Hypertension Society.

A total of 102 questionnaires were sent and 62 centers participated to the survey. The assessment of the plasma renin (plasma renin activity/direct renin concentration) and plasma aldosterone concentration (PAC) were available among all centers. Captopril challenge test (CCT) and saline infusion test (SIT) were available in the 60% and 61% of centers, respectively. Fludrocortisone suppression test was available in the 32% of the units. Adrenal vein sampling was accessible at the 32% of the centers. We found discrepancies in cut-off levels of aldosterone-to-renin ratio (ARR), and PAC after SIT. Other discrepancies involved the duration of the wash-out period before ARR testing, and dosage of captopril administered during CCT.

In conclusion, although all centers are sufficiently equipped to perform PA screening, often patients should be referred to other centers to confirm the diagnosis of PA. A greater uniformity across centers to define precise cut-offs for screening and confirmatory testing for the diagnosis of PA would be of utility.

Summary table

What is known about topic

1. Screening and diagnosis of primary aldosteronism is usually underperformed by general practitioners, because of excessive time consumption, technical difficulties, and potential harm related to some procedures.
2. The evaluation of primary aldosteronism performed among Hypertension Reference Centers would ideally ensure an optimal allocation of resources, in view of the fact that most of the centers are equipped with dedicated medical staff and appropriate diagnostic techniques.

What this study adds

1. The present research demonstrated that, among Italian Excellence and Reference Centers, there is large availability of screening and confirmation test for the diagnosis of primary aldosteronism.
2. However, we found a rather heterogeneous behavior across Centers in terms of methodologies, protocols and cut-off values related to diagnostic work-up for PA, suggesting that a greater uniformity across centers to confirm/exclude PA is highly desirable.
3. Finally, some technically-demanding and costly procedures, such as lateralization procedures requiring adrenal venous sampling and genetic testing, are available in only a small minority of the centers, suggesting the need to create a national network to facilitate the access to these procedures.

Introduction

Arterial hypertension, defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg, is a major public health concern [1]. Despite increased awareness, the estimated rate of annual cardiovascular (CV) and all-cause deaths associated with arterial hypertension is on the rise [2]. This finding could be, in part, attributable to the increased burden of resistant hypertension (RH), defined as the failure of anti-hypertensive drug treatment with at least 3 drugs to obtain adequate BP control [3]. RH is frequently sustained by the presence of secondary causes, which require specific diagnostic testing and management.

Primary aldosteronism (PA), defined as the autonomous overproduction of aldosterone, inappropriate for sodium status, is the most common endocrine cause of arterial hypertension. Recent studies have described the true prevalence of PA to vary between 5% and 10% of all cases of arterial hypertension [4-6], with even higher rates of prevalence among subjects with RH [7]. As compared with individuals with essential hypertension, subjects with PA display higher rates of target organ damage [8-12] and CV events for similar BP values [13-17]. Therefore, the prompt diagnosis of PA and the identification of its subtypes is of outmost importance, not only to address affected patients to the appropriate management, but also to potentially revert this risk excess [15-17].

The Endocrine Society Clinical Practice Guideline recommends case detection of PA in certain patient subgroups with: BP >150/100 mmHg or RH, hypertension and spontaneous or diuretic-induced hypokalemia, hypertension and adrenal incidentaloma, hypertension and obstructive sleep apnoea, hypertension and a family history of early onset hypertension or cerebrovascular accident at a young age, and all hypertensive first-degree relatives of patients with PA [18]. These would cover nearly 50% of all hypertensive subjects. However, screening and diagnosing PA, in particular in the general practitioner setting, is often considered as time-consuming, cumbersome, and in some cases potentially harmful (e.g. due to wash-out of some anti-hypertensive drugs, salt-loading protocols and invasive

examinations). As a result, screening and diagnosis of PA by general practitioners is often largely underperformed [19].

The Italian Hypertension Society (Società Italiana dell'Ipertensione Arteriosa, SIIA) promotes a network of Italian Excellence and Reference Centers for the diagnosis and treatment of arterial hypertension, where hypertensive patients are usually referred by general practitioners for a second-level assessment. This network ensures an optimal allocation of health resources for an accurate diagnosis of PA. In fact, most of the centers do have availability of dedicated medical staff, advanced diagnostic techniques and the expertise to perform a comprehensive assessment of secondary forms of hypertension [20].

The current article presents the results of a National survey conducted among SIIA Italian Hypertension Centers, with the aim of reviewing the current available diagnostic procedures, laboratory testing and clinical protocols for screening and diagnosis of PA.

Methods

The survey was conducted between August and November 2016. An online questionnaire, drafted by the Young Investigator Group of the SIIA under the supervision of the SIIA Executive Committee, was sent to the clinical lead of Reference and Excellence centers of the SIIA. Those who gave explicit consent to participate, received a link with the electronic form, and were invited to fill the online questionnaire anonymously. The questionnaire was easy to fill in and the average time to complete it was around 15 minutes. The questionnaire included 12 items exploring the availability and accessibility of technologies, methodologies and related procedures, usually adopted to screen and confirm the diagnosis of PA in hypertensive outpatients (first wave). Non-responders were invited to participate two more times with repeated online invitations. Those who did not respond to any of the three invitations were excluded from the study. Reasons for not responding were not assessed.

In brief: the questionnaire included three questions to understand the list of laboratory exams, functional test and instrumental settings available within each center. Question 4 and 5 evaluated some aspects related to the methodology for PA screening. Two questions investigated the diagnostic cut-off values adopted by each center for the aldosterone-to-renin ratio (ARR) and plasma aldosterone levels after intravenous saline infusion test (SIT). Question 8 explored the drug dosage commonly used in captopril challenge test (CCT). Question 9 investigated the instrumental diagnostic for the evaluation of the morphology of the adrenal gland. Finally, questions 10 to 12 were related to the screening for obstructive sleep apnea syndrome (OSAS) in subjects with PA, the use of ABPM as a tool for the diagnosis of secondary hypertension, and the accessibility of each single center to genetic testing facilities. To better elucidate some aspects of the questionnaire, responders were subsequently invited to provide further details by filling a further list of brief questions (second wave) related to: details of drug withdrawal before screening test for PA, indications to fludrocortisone suppression test (FST) and dexamethasone suppression test, body position during SIT, availability at the center and success rate of adrenal vein sampling (AVS).

Results

The questionnaire was sent to 102 Hypertension Centers, and a total of 62 fully-filled questionnaires were collected, both including the first and second wave, giving an overall response rate of 61%. Responders were well distributed among the three Italian macro-regions (North n=30, 48%; Centre n=15, 24%, and South n=17, 28%).

Table 1 reported a list of the main diagnostic test and procedures for PA screening and diagnosis, and the number of excellence and reference centers where each test is available, divided by macro-regions. The assessment of the plasma renin activity (PRA) or direct renin concentration (DRC) plus plasma aldosterone concentration (PAC) was available among all centers (100%). Second-level functional tests to confirm/exclude the suspect of PA are

available in the 82% of the units (at least one available test in 51 out of 62 centers). Specifically, CCT and SIT are technically available at the 60% and 61% of centers, respectively, whereas the fludrocortisone suppression test (FST) is available at the 32% of the units. This test is routinely performed only in the 20% of these units (n=4), whereas in the majority of the cases (55%) the test is performed only in the presence of contrasting results from other confirmatory tests. In five units, although technically available, the test is usually not performed.

Dedicated staff and diagnostic resources to perform selective catheterization of adrenal veins (AVS) were available at 20 centers (32%, North=10, Center=6, South=4). However, only in half of the cases (n=10) the test is performed at the center, whereas in the remaining cases (n=10), although the procedure is technically available, the patients is usually referred to another center. Interestingly, in 5 centers (8%), the surgical treatment of lateralized PA is based only upon laboratory and imaging results. The overall approximate success rate in those centers where the procedure is usually performed (n=10) is >80% in 8 out of 10 centers, 60-70% in 1 center, and below 60% in the remaining center.

We found that in more than half of the centers (N=34, 55%), ARR was calculated by considering PRA, whereas in 27 units (45%) through the assessment of direct renin concentration (DRC). The evaluation of 24-h sodium and potassium urinary concentrations was systematically requested to all subjects screened for PA only by 27 centers (44%) whilst in the majority of the units (56%, n=35), this exam was performed only in selected cases. The discontinuation from drugs interfering with the renin-angiotensin-aldosterone system (e.g. β-blockers, ACE-inhibitors, angiotensin receptor blockers, diuretics, with the exception of alpha-blockers and calcium channel blockers) before plasma renin assay is variably performed among units, being always performed in rather 2/3 of the centers (n=40, 65%); in other cases, drugs with minor effects on the RAA system are maintained and results are interpreted by taking into account the effects of concomitant treatment on PRA/DRC and PAC levels (n=18, 29%). In 6% of the cases (n=4) the wash-out of interfering drugs is

usually not performed. The average duration of wash-out period is rather variable among units, being ≥15 days for 44 centers (71%), 3-4 days for 23% of the units (n=14).

The cut-off value of ARR suggesting the presence of PA was set at 30 (with PAC in ng/dL and PRA in ng/mL/h) / 2.7 (with PAC in ng/dL and DRC in mU/L) in 56% of the centers (n=35), at 40/4.9 in the 40% (n=25), and at 20/2.4 in only 4% of centers (n=2, Figure 1). The diagnostic cut-off level of serum aldosterone adopted to confirm the diagnosis of PA after SIT also showed heterogeneity among centers: it was 10 ng/dL in 47% of centers, 7.5 ng/dL in 24% of centers, and 5 ng/dL in 29% of the centers (Figure 2). Another discrepancy between centers is related to body position during SIT test: in 15 centers (39%) it is performed in seated position, whereas in 23 centers (61%) in supine position. Also drug dosage administered for CCT was relatively variable among centers: 50 mg was the dosage adopted in the 77% of the centers, while the remaining 23% performed the test after administering 25 mg.

With regards to imaging testing, CT-scan was the preferred test for 97% of the centers, whereas the remaining 3% considered magnetic resonance (MR) as the first imaging test to be performed after the confirmation of PA diagnosis. In subjects with PA, in the presence of adrenal nodule evaluated by imaging test, a dexamethasone overnight suppression test is always performed among 39% of the centers, performed only in the presence of large adrenal nodules (>1 cm) in 28% of the centers, and not routinely performed at the 33% of the centers unless a specific clinical suspicious of hypercortisolism is present.

The evaluation of characteristics of circadian BP profile through automated blood pressure monitoring (ABPM) was requested by the vast majority of centers (90%). Conversely, screening for features of obstructive sleep apnea syndrome (OSAS) through validated questionnaires (e.g. Epworth Sleepiness Scale) in subjects screened for PA was habitually performed only by 29% of the centers. Genetic testing for familial forms of PA (long PCR for the chimeric *CYP11B1/CYP11B2* gene and sequencing of the *KCNJ5* gene) [18] are available in a small minority of the centres (27%).

Discussion

The current article presents state-of-the-art results of laboratory and instrumental procedures and resources to perform a diagnosis of PA across Italian Hypertension Excellence and Reference centers endorsed by the SIIA. The two main findings of this survey are that there is large availability, among Italian centers, of laboratory and instrumental resources for screening and confirmation test for the diagnosis of PA. Conversely, some selected and cost-demanding procedures, which usually require dedicated staff and specific facilities, are available only in a limited proportion of the centers. The second evidence of the present survey is a rather heterogeneous behavior across centers in terms of methodologies and protocols related to diagnostic work-up for PA.

According to guidelines, the diagnosis of PA is a three step process, comprising screening test, confirmatory testing and subtype diagnosis. Each of these steps could be variably affected by sub-optimal sensitivities and specificities, depending on a number of factors such as the characteristics of the population and the choice of cut-off points. A missed diagnosis of PA would result in an inappropriate exposure to increased CV risk, since PA is associated with a worse CV prognosis as compared to essential hypertension [Monticone S., 2018]. Moreover, insufficient detection and treatment of lateralized PA may be associated with a residual increased risk for cardiovascular events and mortality [Hundemer GL, 2018]. Additionally, several studies pointed towards a reduced quality of life (QOL) in patients affected by PA compared with the general population [Veelema MS, JCEM 2018; Ahmed AH, JCEM 2011]. Specific PA treatment has been shown to improve QOL, with the unilateral adrenalectomy being more effective that MR antagonists [Veelema MS, JCEM 2018; Ahmed AH, JCEM 2011], underscoring once again the importance of diagnosing unilateral PA.

The most reliable means to screen for PA is the ARR, which can be calculated using both the PRA and DRC in the denominator [18]. PRA has been traditionally measured by Radio-Immuno-Assay , however DRC measurement with chemiluminescence assays (currently adopted in the 45% of the centers), which are fully automatized and do not produce radioactive waste, is progressively replacing the traditional PRA in the evaluation of patients

affected by arterial hypertension. Several studies showed that PRA and DRC display an overall good correlation, that becomes weaker for PRA values < 1 ng/mL/h, (Burrello J., 2016; Dorrian C.A., 2010). The ES guideline [*Funder JW, 2016*] proposes a conversion factor of PRA (ng/mL/h) to DRC (mU/L) of 8.2. Applying this factor, an ARR of 30 (calculated with PRA measured in ng/mL/h) corresponds to an AARR (aldosterone to active renin ratio) of 3.7 (calculated with the DRC measured in mU/L). However, three independent studies showed that the optimal sensitivity and specificity for the AARR are reached with significantly lower cut-offs (Burrello J., 2016; Rossi G.P., 2016; Manolopoulou J., 2015). According to these data, the use of a conversion factor between PRA and DRC should be discouraged and distinct cut-offs should be adopted for the ARR and the AARR. Several factors, including age, gender, time of day, serum K^+ levels and, most importantly, antihypertensive medications, may affect ARR and should be taken into account [Funder JW, 2016]. According to ES guideline, it is mandatory to withdraw the most interfering medications, including K^+ sparing and K^+ wasting diuretics while the ARR can be confidently interpreted under the relatively noninterfering medications. Significant heterogeneity is expected for this step, reflecting clinicians' preferences, the severity of hypertension and patients' comorbidities. The practice of drug discontinuation (with the exception of alpha-blockers and calcium channel blockers), despite potentially associated with side effects [*Fischer E, Rev Endocr Metab Disorder 2011*], carries the lowest risk of false positive or false negative results and it is therefore the most frequently adopted strategy across the SIIA centers.

Notably, the guidelines do not establish a precise cut-off for the ARR, which ideally should be tailored by each center according to the type of assay used and the Na^+ intake of the population. The choice of a low cut-off to define a positive screening test (e.g. 20 with PAC in ng/dL and PRA in ng/mL/h or 2.4 with PAC in ng/dL and direct renin concentration in mU/L), as it is performed at the 4% of the centres, if on one hand maximizes sensitivity, on the other hand results in a high rate of false positives, thereby reducing the specificity and increasing time and costs associated with the performance of a confirmatory test.

Giving the high rate of false positive results of the ARR (57% in the PATO study), a confirmatory testing should always be performed to avoid patients with low renin essential hypertension to undergo costly and invasive diagnostic procedures such as adrenal CT scanning and AVS [18]. According to some authors [22], the FST is regarded as the gold-standard test to definitively confirm or exclude PA diagnosis. However, it is costly and time consuming, and often requires the patient to be hospitalized for four days. For these reasons nowadays the SIT and the CCT, which represent valid alternatives to the cumbersome FST [23], are the most widely used ones. As expected, our results follow this trend, with the FST being available only in approximatively 1/3 of the centres. Surprisingly, also the possibility of performing a relatively simple confirmatory test is available only in 60% of the units in the case of SIT, and in 61% of the units in the case of CCT, indicating that a significant proportion of patients has to be referred to another centre. There is not an optimal protocol to perform the CCT test and two different doses (25 mg or 50 mg) of captopril can be administered. According to this survey, 50 mg is more frequently used dosage (in 77% of centres), but there is not enough evidence to prefer one protocol over the other. According to historical pharmacological studies, the main pharmacokinetic parameters after the administration of 25 mg or 50 mg of captopril were not significantly different (except for the area-under-the-curve standardized in relation to 1 mg of the dose) [24]: it is therefore conceivable that the administration of 25 mg or 50 mg of captopril will not significantly affect the performance of the test.

As for the screening test, also for the SIT and the CCT test there is not general agreement on the best cut-off to define complete aldosterone suppression and definitively exclude PA diagnosis. While post SIT infusion PAC >10 ng/dL are generally deemed to be diagnostic of PA and a concentration <5 ng/dL indicative of a normal aldosterone suppression, values between 5 and 10 ng/dL represent a grey zone. We observed a wide heterogeneity across centres with respect to the cut-off chosen to define normal suppression after SIT: the answers were in fact almost equally distributed among the three options (5 ng/dL, 7.5 ng/dL and 10 ng/dL). Choosing a cut-off of 10 ng/dL maximizes the specificity and reduces the number of patients that have to be addresses to lateralization procedures; however, in a

recent study it has been shown that 29% of the patients with a post SIT PAC <5 ng/dL had a lateralized aldosterone production and were successfully cured by unilateral adrenalectomy [25]. The results of this study, methodologically sound and using strict criteria to define lateralized AVS, challenge the performance and the validity of SIT in definitively confirming or excluding PA diagnosis [25]. Therefore, some authors claim that prospective studies are warranted to establish if AVS indication should be extended to all patients with a positive screening test, in order to offer curative surgery to a greater number of PA patients (Cornu E., Hypertension 2016).

After confirming the diagnosis, subtype testing should be performed in all PA patients who are candidate and desire surgical treatment by unilateral adrenalectomy. Subtype diagnosis comprises adrenal CT scanning (to rule out an aldosterone producing adrenal carcinoma) and the AVS to distinguish between unilateral and bilateral disease [18]. Despite significant advances in the optimization of the AVS procedure, with several issues having been addresed (Monticone S., Lancet DE 2015), it remains a poorly standardized procedure across centers (Kempers; Rossi). Different studies showed that adrenal imaging alone is not sensitive neither specific enough to define the source of aldosterone overproduction [26], notwithstanding the controversial SPARTACUS trial failed in demonstrating a superiority of AVS-based treatment over adrenal CT-scanning (*Dekkers T., Lancet DE 2016; Beuschlein F, HMR 2017)* in intensity of antihypertensive medication or clinical benefit. However, it must be acknowledged that the study was underpowered and the selected selection criteria did not allow to generalize the results to the overall PA population (Beuschlein F, HMR 2017).

Whereas all hypertension centres should be encouraged to set up their own protocol and perform one or more confirmatory tests (which are safe and very often uncomplicated procedures) the "centralization" of AVS performance in few referral centers may be supported by the fact that an expert and dedicated radiologist is a key factor for increasing the successful cannulation of adrenal veins [Buffolo F., IJMS 2017] and a higher rate of adrenal vein rupture was observed in centres where a low number of procedures is performed [27]; by contrast this complication is rarely observed in centres with long

experience and high number of procedure per year [28]. In our cohort good AVS performance is achieved in the majority of the hypertension units; in centers with a low success rate, ACTH(1-24) infusion and measurement of serum cortisol during the procedure should be considered as useful strategies to improve successful cannulation of adrenal veins [Buffolo F., IJMS 2017].

A centralized approach would also be effective to improve the diagnosis of genetic forms of PA, such as familial hyperaldosteronism [29,30]. In fact, we observed that genetic testing is available only in a small minority of the hypertension specialist centers, suggesting that genetic forms of hyperaldosteronism could be currently underdiagnosed.

We acknowledge that our results should be viewed in the lights of some limitations. Even whether the online survey could be conceived as a faster way of collecting data and increasing the response rate as compared to paper-and-pencil methods, some inherent disadvantages such as the absence of an interviewer, possible cooperator problems and potential dishonesty could negatively impact on results. In our survey we proposed anonymity to responders to reduce part of these limitations. As a consequence, those who did not respond to the survey could not be better characterized.

In conclusion, a greater uniformity across centres to confirm/exclude PA is highly desirable, in order to guarantee the consistency of a diagnosis across the country. Creating a national consensus to define precise cut-offs for screening and confirmatory testing would be of great utility, in particular for those centres with low experience in the clinical management of patients with PA. However, this still could be largely hampered by the great heterogeneity of the assays used for aldosterone measurement, as mentioned previously. The promotion a national network between Italian hypertension specialist centers endorsed by the SIIA, in order to improve the awareness and spread the knowledge on PA screening and diagnosis, would be desirable also to facilitate the access to technically-demanding or costly procedures.

Conflict of interest

None of the authors has financial or other conflicts of interest that might have biased the work.

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Figure legends

Figure 1: cut-off value of aldosterone-to-renin ratio adopted by centers, suggesting the presence of PA. Reported values are related to ARR calculated from plasma aldosterone

concentration (ng/mL) and plasma renin activity (ng/mL/h)/ plasma aldosterone concentration (ng/mL) and direct renin concentration (mU/L)

Figure 2: cut-off value of aldosterone-to-renin ratio adopted by centers, suggesting the presence of PA. Reported values are calculated from plasma renin activity, and correspond to 2.4, 3.7 and 4.9 ng/dL/mU/L if direct renin concentration is assessed

Tables

Table 1: number of centers (and %) with availability of the related test and procedure for screening and diagnosis of PA, divided by macro-regions

	Total (n=62)	North (n=30)	Centre (n=15)	South (n=17)
Plasma renin activity	50 (81)	22 (73)	13 (87)	15 (88)
Direct renin concentration	35 (56)	18 (60)	9 (60)	8 (47)
Plasma aldosterone	62 (100)	30 (100)	15 (100)	17 (100)
Urinary aldosterone	46 (74)	23 (77)	11 (73)	12 (71)
Saline loading test	37 (60)	18 (60)	9 (60)	10 (59)
Captopril challenge test	38 (61)	19 (63)	10 (67)	9 (53)
Fludrocortisone suppression test	20 (32)	11 (37)	4 (27)	5 (29)
Adrenal vein sampling	20 (32)	10 (33)	6 (40)	4 (27)