Original Article

Prevalence of Primary Aldosteronism among Unselected Hypertensive Patients: A Prospective Study Based on the Use of an Aldosterone/Renin Ratio above 25 as a Screening Test

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Primary aldosteronism (PA) has been considered a rare cause of hypertension. The introduction of the aldosterone/renin ratio (ARR) as a screening test has led to an increase in the detection rate. The aim of this study was to evaluate the prevalence of PA among unselected hypertensive patients by using an ARR >25 as a screening test. We studied 3,000 consecutive unselected hypertensive patients. Blood samples for the determination of plasma renin activity (PRA), aldosterone (ALD) and electrolytes were drawn in the morning, and patients with an ARR >25 underwent intravenous saline infusion as a confirmatory test. Adrenal CT and a dexamethasone suppression test were performed in patients with confirmed PA. Patients with a positive dexamethasone test underwent genetic testing for glucocorticoid-remediable aldosteronism (GRA). Out of 3,000 hypertensives, 684 (22.8%) showed an ARR >25 and 177 of them (5.9% of the whole population) had a positive saline loading test. Only 44 of them (24.8%) were hypokalemic. CT was performed in all the patients with confirmed PA and 53 of them (29.9%) had a solitary adrenal macroadenoma, 112 (63.3%) had bilateral adrenal enlargement and 12 (6.8%) had normal appearing adrenal glands. Of 177 patients given dexamethasone to identify GRA, 8 (4.5%) showed aldosterone suppression but only one (0.1%) tested positive for the chimeric gene. In conclusion, our findings indicate that standardized application of an ARR >25 to unselected hypertensive patients, followed by i.v. saline loading as a confirmatory test, can result in the detection of a large number of patients with PA (5.9% of the studied population), most of whom are normokalemic. Bilateral adrenal hypertrophy represents the more common form of PA. (*Hypertens Res* 2007; 30: 111–117)

***Key Words*: hypertension, primary aldosteronism, epidemiology**

Introduction

Primary aldosteronism (PA) was first described in 1955 by Conn in a 34-year-old woman suffering from a form of resistant hypertension associated with hypokalemia, excessive aldosterone production and suppressed plasma renin activity (PRA), and caused by an aldosterone (ALD)-producing adrenal adenoma (*1*). Ten years later Conn recognized that normokalemic PA could masquerade as essential hypertension and predicted that PA could be a common cause of hypertension (*2*). This theory was at that time generally rejected by the medical community, who historically reserved exploration for PA to patients with hypokalemia or resistant

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hypertension, with consequent PA prevalence estimates ranging from 0.01% to 3% of hypertensive subjects (*3–5*). The frequency of detection began to change in 1981, when Hiramatsu *et al*. proposed the ratio of plasma aldosterone to renin levels (ARR) as a screening test for diagnosing PA (*6*). An increased ratio is believed to identify individuals who have an increased ALD for the measured renin, with or without low renin. The ARR has been advocated as a convenient and effective method to screen for PA, with some limitations concerning a possible reduction of its accuracy by various antihypertensive drugs, posture changes, time of day, potassium levels and renal insufficiency (*7–9*). An elevated ARR is not diagnostic by itself and PA must be confirmed by other tests, such as a saline infusion test, fludrocortisone suppression test, captopril test and oral salt loading test, which are able to demonstrate an inappropriate autonomous ALD production (*8*, *10–14*). The application of the ARR to a broader population of hypertensives has uncovered a higher prevalence of PA in both hypertensive clinic and primary care populations, with prevalence rates ranging from 3% to 32% and most patients being normokalemic (*15–21*). This wide variability in PA prevalence rates in the different studies probably reflects the different populations, methodology and diagnostic criteria, particularly the type of hypertensives examined (selected or unselected), the cut-off levels for which an ARR was considered positive, the type and cut-off level for the confirmatory tests and the technique used for adrenal imaging.

The major causes of PA are idiopathic bilateral adrenal hyperplasia (BAH) and unilateral ALD producing adenoma (APA). Minor causes include familial varieties and unilateral adrenal hyperplasia (*22*). Glucocorticoid-remediable aldosteronism (GRA) or familial aldosteronism type I is the only subtype of PA whose underlying genetic and molecular basis is clearly understood (*23–28*). The pathogenetic mechanism derives from an asymmetrical crossing-over with a nonreciprocal recombination between the 11β-hydroxylase (CYP11B1) and ALD synthase (CYP11B2) genes. The resulting chimeric gene has a 5′ regulatory element of CYP11B1 fused to 3′ coding sequences of CYP11B2. The preferential expression of this mutant form drives a transcription process under the control of adrenocorticotropic hormone (ACTH) instead of physiological angiotensin (Ang) II stimulus (*23–27*). Subtype detection of PA, which requires one or more tests including adrenal CT, adrenal venous sampling (AVS) and genetic blood testing, is of clinical relevance since patients with APA are potentially curable with unilateral adrenalectomy while the other forms are treated medically.

With this historical background, the present prospective study was undertaken in order to: 1) evaluate the prevalence of PA among unselected hypertensive patients by using an ARR >25 as a screening test; 2) assess the validity of basal ARR and intravenous saline suppression test as standard criteria for the detection of patients with PA; and 3) identify among patients with PA those affected by GRA. Most previous studies were limited by the patient selection process, which basically included all patients referred to the Hypertension Unit. Since the prevalence of PA among referred hypertensive patients does not necessarily reflect the prevalence among unselected hypertensive individuals, in order to avoid selection bias in the present study only hypertensive patients sent consecutively by family doctors were considered.

Methods

The study population included 3,000 consecutive hypertensive patients, 1,427 males and 1,573 females, aged 25 to 70 years, referred by general practitioners to our Hypertension Center between June 1999 and October 2002. To avoid any patient selection bias, we asked the general practitioners cooperating with the study to refer any treated or untreated hypertensive patients they examined during the first 2 days of the week (Monday and Tuesday) for the entire duration of the study. Hypertension was defined by blood pressure (BP) values >140/90 mmHg on at least three separate occasions or by use of antihypertensive drugs. Patients with confounding conditions such as congestive heart failure, cirrhosis, renal failure, diabetes, or obesity (BMI >30 kg/m^2) or who had other secondary causes of hypertension such as Cushing's syndrome, pheochromocytoma or renal artery stenosis were excluded from the study. The study protocol was approved by the local Ethical Committee and informed consent was obtained from each participant before entering the study.

Every referred patient was screened prospectively for PA by ARR. ALD antagonists were discontinued at least 6 weeks before the investigation; angiotensin converting enzyme (ACE) inhibitors and Ang II receptor blockers were discontinued 4 weeks before; β-blockers, dihydropyridine-type calcium antagonists and clonidine were progressively reduced and withdrawn 2 weeks before. α1-Blockers and slow-release forms of verapamil, which have a lesser effect on the ARR (*29*, *30*) were withdrawn 1 week before. Medications that might interfere with the renin-ALD axis, such as steroids, sex hormones, liquorice or non-steroidal anti-inflammatory drugs were also withheld for at least 3 weeks. Subjects with hypokalemia, which can cause false-negative ratios (*29*), were given adequate oral KCl supplements before the study. Since we wanted to examine a population of unselected patients that was as representative as possible of the general population dealt with in the clinical practice, all subjects were asked to follow their usual diet, without any attempt to control sodium intake or modify lifestyle.

Patients presented between 8:00 AM and 9:00 AM in the morning, after an overnight fast. Since obtaining the ARR while the patient is in the upright position has been reported to enhance the predictability of the ratio (*8*), blood samples for PRA, ALD, sodium, potassium and creatinine were obtained in patients who had been standing for 2 h. Plasma samples for PRA and ALD determinations were collected in chilled anticoagulated glass tubes, immediately centrifuged

and frozen at −20°C. Each sample was assayed in duplicate and the averages were used in the analysis. PRA was determined by using an immunoradiometric assay kit. Intra- and inter-assay coefficients of variation were 5.7% and 8.5%, respectively. In our laboratory normal reference values for PRA were 0.51–2.64 ng/ml/h in the supine position and 0.98–4.18 ng/ml/h in the upright position; the lower limit of detection of the PRA assay was 0.2 ng/ml/h. Plasma ALD was measured by radioimmunoassay with a commercially available kit. Intra- and inter-assay coefficients of variation were 5.3% and 8.4%, respectively. In our laboratory normal reference values for ALD were 1.2–5 ng/dl in the supine position and 7–35 ng/dl in the upright position.

An ARR greater than 25 ng/dl per ng/ml/h was considered suggestive of PA. We arbitrarily chose this cut-off value based on the 95th percentile ARR derived from our healthy normotensive volunteers, with an estimated sensitivity and specificity of 97.8% and 93.1%, respectively. Patients with an ARR greater than 25 underwent further diagnostic testing. In this case, prior to testing the patients underwent controlled salt intake (80–100 mmol/day sodium and 60–80 mmol/day potassium) in addition to pharmacological wash-out. The confirmatory test was an i.v. saline load (2 l of 0.9% NaCl infused over 4 h with the patient supine) (*8*, *10*, *12*). The test was considered positive if the post-test ALD levels were greater than 7.5 ng/dl (*18*). All patients with biochemically confirmed PA underwent a high resolution, thin-section CT scan of the adrenal gland and a dexamethasone suppression test, which is considered a useful screen for GRA (*24*, *31*, *32*). The adrenal CT scan was judged compatible with hyperplasia when any adrenal area thicker than 10 mm was detected. Diagnosis of APA was considered appropriate if the scan showed a unilateral solitary adrenal macroadenoma (>1 cm), provided that the contralateral adrenal gland was morphologically normal. For the dexamethasone suppression test, supine plasma ALD and cortisol were measured under baseline conditions and after 4 days of dexamethasone (2 mg/day, orally; 0.5 mg every 6 h); blood samples were taken on the fifth day, 2 h after the morning dose of dexamethasone. The test was considered positive if ALD was suppressed below 2 ng/dl. Plasma cortisol suppression was used as an index of the dexamethasone effect. As a high frequency of false-positive diagnoses has been documented (*24*, *31*, *32*), all patients with a positive dexamethasone suppression test underwent genetic testing of peripheral blood DNA for the presence of the hybrid 11β-hydroxylase/ALD synthase gene responsible for the GRA. Genomic DNA was prepared from EDTA-treated blood. The presence of the CYP11B1/CYP11B2 chimeric gene was studied using the long polymerase chain reaction (PCR) technique introduced by Jonsson *et al*. (*33*). Briefly, each DNA sample was subjected to two concurrent amplification reactions with sense primers specific for the 5′-untranslated regions of genes encoding ALD synthase or 11β-hydroxylase. The conditions used in both amplification reactions were a denaturation step at 95°C for 3 min, followed by 12 cycles of denaturation at 95°C for 1 min and annealing/extension at 68°C for 5 min. Following amplification, PCR products were loaded onto 0.7% agarose gel containing 1 mg/ml ethidium bromide. The gel was electrophoresed for 1 h at 60 V and photographed with UV transillumination. DNA from all subjects produced a 3.9-kb fragment when amplified with the pair of primers specific for the ALD synthase gene (CYP11B2). The CYP11B2 amplification for each individual served as a control for the integrity of each DNA sample. Only DNA from the GRA patient produced a 3.9-kb fragment when the sense primer for the 11β-hydroxylase gene and the antisense primer for intron E of the ALD synthase gene were used for amplification.

Table 1. Demographic Characteristic of All Hypertensive Patients Enrolled

Patients (*n*)	3,000
Untreated	51%
One drug	37%
Two drugs	8%
Three drugs	4%
SBP (mmHg)	158.9±8
DBP (mmHg)	99.6±7
Age (years)	50.7±6
Sex (female/male)	1,573/1,427
BMI (kg/m^2)	26.43±4.2
Serum K^+ (mEq/l)	4.2±0.33
Serum Na^+ (mEq/l)	141±2.5
Serum creatinine (mg/dl)	0.9±0.10
Duration hypertension (years)	7±2

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

Statistical Analysis

Data are expressed as the means±SD. The Student's *t*-test for paired measurements was used to establish statistically significant differences observed in subjects over the study period.

Results

The general characteristics of the whole study population are summarized in Table 1. Forty-nine percent of the patients were receiving pharmacological therapy for hypertension. A total of 684 (22.8%) hypertensive patients showed an ARR greater than 25. Confirmatory studies for PA were performed in 650 of the 684 patients who had a positive screening test (34 patients did not give their informed consent to continue the study). Of these, 177 (5.9% of the entire population) were diagnosed as having PA on the basis of the intravenous saline load test (ALD level >7.5 ng/dl). Their main clinical characteristics are shown in Table 2. In the remaining patients, the intravenous saline test did not confirm PA. All hypertensive

Table 2. Demographic and Biochemical Parameters of Patients with Essential Hypertension (EH) and with Confirmed Primary Aldosteronism (PA), Indicated as Means±SD

	PA	EH	*p* value
Patients (*n*)	177	2,823	
SBP (mmHg)	162±3	158±6	<0.01
DBP (mmHg)	101±3	99±4	n.s.
Age (years)	48±7	52±6	n.s.
Sex (female/male)	88/89	1,485/1,338	n.s.
BMI (kg/m^2)	26.2±1.9	26.5±1.8	n.s.
Serum K^+ (mEq/l)	3.8±0.3	4.3±0.23	<0.05
Serum creatinine (mg/dl)	0.9±0.09	0.9±0.11	n.s.
Serum ALD (ng/dl)	13.6±6.2	8.1±3.8	0.001
PRA (ng/ml/h)	0.29±0.18	0.78±0.18	<0.05
ALD/PRA ratio (ng/dl per ng/ml/h)	48.6±18.4	17.3±6.9	<0.0001
Duration hypertension (years)	9.3±3.6	8.1±2.4	n.s.

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; ALD, aldosterone; PRA, plasma renin activity; n.s., not significant.

patients with false-positive ARR were found to have essential hypertension. We calculated that by using an ARR cut-off value above 25 no one subject with PA remained without being diagnosed. If a cut-off value >40 had been used, a lower number of hypertensives (570) would have been screened positive for PA and one patient would not have been diagnosed. Further increasing the cut-off value above 65, only 450 patients would have tested positive but 4 patients would have been not diagnosed. In other terms, raising the cut-off value would have improved the specificity but would have worsened the sensitivity of the test.

Among the 177 hypertensives in whom PA was confirmed on the basis of the saline loading test, only 44 (24.8%) were hypokalemic.

Adrenal CT scans were performed in all 177 patients with biochemically confirmed PA, and 53 of them (29.9%) were considered to have APA, based on the finding of a solitary adrenal macroadenoma and a normal appearing contralateral adrenal gland. The CT scan showed bilateral enlargement in 112 of the 177 patients (63.3%) and was reported to be normal in the other 12 cases (6.8%). The main demographic and clinic characteristics of patients with solitary adrenal macroadenoma, bilateral enlargement and normal CT are shown in Table 3. Out of 53 patients with adrenal adenoma detected by CT, 52 underwent adrenal mass removal (1 patient refused the operation). In all patients radiological diagnosis of adenoma performed using CT was later confirmed by the histological analysis after surgical removal.

PRA and ALD levels returned to normal in 98% of cases, with average PRA of 0.83 ng/ml/h and ALD levels of 7.6 ng/dl.

Of the 177 patients given 4-day dexamethasone treatment in order to identify GRA, 8 (4.5%) showed a suppression of ALD (<2 ng/dl). In all patients, correct glucocorticoid intake was confirmed by the suppression of cortisol levels. Despite a positive dexamethasone test, only 1 patient (0.5% of patients with PA) tested positive for the chimeric gene of GRA.

Discussion

Although unanimity is still lacking among experts, the evidence from almost every continent suggests that PA affects 5–13% of patients with hypertension (*15*, *16*, *20*, *21*, *33*). In keeping with this estimate, in the present study the screening of all (not just hypokaliemic or resistant) hypertensive individuals by ARR testing with a cut-off level of 25 allowed us to detect a 5.9% prevalence of PA. This rate of diagnosis is higher than traditionally thought, especially when considering that the examined population consisted of non-selected hypertensives consecutively referred to our Hypertension Center by general practitioners, so that our data may apply to the general hypertensive population found in the clinical setting.

Although calculation of the ARR has been presented as a convenient and effective method to screen for PA (*7–9*), there is currently no consensus on the ideal cut-off value for ARR, whose variability in the different studies depends on the different assay techniques, laboratory conditions and influence of several variables on PRA and ALD levels (*34*). Hence the importance of locally validated criteria and standardized conditions of sampling for establishing the cut-off levels. In the present study we chose a cut-off value of 25 ng/dl per ng/ml/h based on the 95th percentile ARR derived from our healthy normotensive volunteers, with an estimated sensitivity and specificity of 97.8% and 93.1%, respectively. In addition, the evaluation was made under standardized conditions (pharmacological wash-out, sampling in the morning hours, upright position before testing). We estimated that by using a cut-off value above 25, no subject with PA remained undiagnosed. Raising the cut-off value above 40 the number of

Table 3. Main Demographic and Clinic Characteristics of Patients with Confirmed Primary Aldosteronism

	APA	BAH	Normal at CT
Number of patients (%)	53 (29.9%)	112 (63.3%)	12 (6.8%)
SBP (mmHg)	166±3	159±5	165±4
DBP (mmHg)	103±3	100±3	101±3
Age (years)	46±6	51±8	52±12
Sex (female/male)	28/25	55/57	5/7
BMI (kg/m^2)	26.3±2.1	26.1±1.9	26.4±4.2
Serum K^+ (mEq/l)	3.6±0.4	3.8±0.3	3.5±0.2
Serum creatinine (mg/dl)	0.9±0.1	0.9±0.09	0.9±0.11
Serum aldosterone (ng/dl)	14.7±5.2	13.5±6.3	14.6±8.1
PRA (ng/ml/h)	0.19±0.09	0.31±0.17	0.25±0.18
ARR (ng/dl per ng/ml/h)	66.4±26.6	44.1±16.2	45.2±17.3
Duration of hypertension (years)	7.1±2.4	9.4±3.5	10.5±4.1

APA, aldosterone producing adenoma; BAH, bilateral adrenal hyperplasia; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; PRA, plasma renin activity; ARR, aldosterone renin ratio.

patients screened positive would have been lower (570 *vs*. 684), but 1 patient would not have been diagnosed. Further increasing the ratio above 65, only 450 patients would have tested positive but 4 patients would have not been diagnosed. In other words, raising the cut-off value would have improved the specificity and the positive predictive value of the test but would have caused a loss in sensitivity, compromising its utility for screening purposes.

Most of our patients with PA (75.2%) had normal serum potassium levels, which confirms previous observations (*15*–*21*). This suggests that normokalemic PA constitutes the most common presentation of the disease and does not support the theory that diagnostic screening for PA should be avoided unless patients are hypokalemic. Respecting this criterion in the present study would have led to our missing 133 of 177 subjects, thereby denying such patients the chance of a cure or at least of tailored antihypertensive therapy.

Since ARR *per se* is not diagnostic, patients screened positive for PA with ARR must undergo confirmatory tests, which have in turn their own limitations in sensitivity and specificity and suffer from variations in protocols and cut-off values. In the present study we chose the intravenous saline loading test, which has the advantages of low cost, ease and practicability for both the patients and the clinicians, requiring only a brief outpatient visit (*8*, *10*, *12*). The level of normal ALD suppression by saline infusion has been commonly defined as 10 ng/ml (*12*, *16*, *21*), but a cut-off value of 5 ng/ml has also been recommended (*8*, *10*, *35*). To minimize the possibility of misdiagnosing PA as essential hypertension, in agreement with other authors (*18*) we employed the cut-off value of a post-saline ALD level of 7.5 ng/dl with saline loading.

We have observed a proportion of APA among our patients with PA (53/177, 29.9%) that was lower than that reported in some earlier reports, where unilateral adenoma was found in more than 50% of patients with PA (*36*). This inconsistency might be due in part to the fact that those studies used hypokalemia, which is more frequently associated with APA than with BAH, as a screening tool for PA. Besides, as we did not perform adrenal venous sampling (AVS), which is the most accurate tool for detecting unilateral APA (*34*, *37*, *38*), we may have overlooked several cases of APA. Another explanation for the lower proportion of APA might be the high sensitivity of the ARR as a screening test, which allows detection of even mild PA, that is more frequently associated with BAH than with APA (*14*). Indeed, the application of the ARR in all hypertensive patients has resulted in a decrease in the ratio of APA to BAH even in studies where adrenal venous sampling was performed in all patients with biochemically confirmed PA (*39*). On the other hand, the proportion of APA in our study was similar to that reported by other authors who studied the prevalence of PA among non-selected hypertensives using the ARR as a screening test, followed by acute saline loading test and adrenal CT scan (*18*).

In the present study BAH was confirmed to be the most frequent form of PA. Identification of hypertensive patients with BAH is a worthwhile aim, since specific medical treatment with ALD antagonists may be more effective than standard antihypertensive therapy in these subjects (*40*).

Our findings confirmed that a short-term dexamethasone course can be misleading in identifying GRA among patients with PA, as 8 of 177 cases (4.5%) tested by us showed ALD suppression, but only one (0.5%) tested positive for the chimeric gene of GRA. Indeed, a transient ACTH dependency of ALD secretion has been described in patients with either APA or BAH (*41*). This phenomenon might be explained by an increased expression of ACTH receptor messenger ribonucleic acid in adrenal tissues, as found in some patients with APA (*42*). The fact that hybrid gene testing was positive in only one case was not surprising, given the relative rarity of that subtype, which accounts for about 0.5–1% of PA (*18*, *21*, *23*, *32*). Definitive diagnosis of GRA can only be reached by

genetic testing.

Increasing evidence has accumulated showing that excessive levels of plasma ALD are able to promote cardiac and vascular hypertrophy, remodelling and fibrosis through non–BP-dependent means (*43–46*). The longer ALD excess is left untreated, the more severe and irreversible these cardiovascular changes become, with hypertension becoming more difficult to control and cardiovascular events more probable. Therefore early detection, diagnosis and management of PA is likely to result in beneficial effects extending beyond improved hypertension control. It has been argued that the use of wider screening strategies might result in significantly increased health expenditure resulting from the costs of performing ARR measurements, confirmatory tests, CT scan and eventual adrenal venous sampling in a greater number of hypertensives (*47*). However, it should be remembered that the great majority of patients who undergo ARR testing, which *per se* is relatively inexpensive, will test negative and therefore will be excluded from having to undergo the more expensive confirmatory tests, CT and AVS. Besides, the potential costs associated with diagnosis and treatment of complications and time-off from work arising from long-term exposure to increased ALD levels and less adequately controlled hypertension should be taken into consideration.

The results of this study indicate that, when performed carefully and with regard to factors that can complicate interpretation of the results, application of the ARR to all patients with hypertension, followed by intravenous saline load to confirm or exclude PA, can result in the detection of large number of patients with PA (5.9% of our unselected hypertensives), most of whom are normokalemic and therefore likely to be misdiagnosed as having essential hypertension unless subjected to a specific screening test. BAH may represent the more common form of PA, while GRA appears to be rare. It is important to underscore that the relevance of diagnosing PA is not just an issue of classification, since hypertension in the APA patients can be cured or significantly improved by unilateral adrenalectomy, while the determination of the underlying cause of the elevated blood pressure in BAH and GRA patients is important for targeted pharmacotherapy.

References

1. Conn JW: Primary aldosteronism, a new clinical syndrome. *J Lab Clin Med* 1955; **45**: 3–7.
2. Conn JW: The evolution of primary aldosteronism: 1954–1967. *Harvey Lect* 1968; **62**: 257–291.
3. Kaplan NM: Commentary on the incidence of primary aldosteronism. Current estimations based on objective data. *Arch Intern Med* 1969; **123**: 152–155.
4. Berglund G, Andersson O, Wilhemsen L: Prevalence of primary and secondary hypertension: studies in a random population. *Br Med J* 1976; **2**: 554–556.
5. Tucker R, Labarthe D: Frequency of surgical treatment for hypertension in adults at the Mayo Clinic from 1973 to 1975. *Mayo Clin Proc* 1977; **52**: 549–555.
6. Hiramatsu K, Yamada T, Yukimura Y, *et al*: A screening test to identify aldosterone-producing adenoma by measuring renin activity. Results in hypertensive patients. *Arch Intern Med* 1981; **141**: 1589–1593.
7. Moneva MH, Gomez-Sanchez CE: Establishing a diagnosis of primary aldosteronism. *Curr Opin Endocrinol Diabetes* 2001; **8**: 124–129.
8. Brown CA, Bouldin MJ, Blackston JW, Duddleston DN, Shepherd JM, Hicks GS: Hyperaldosteronism: the internist's hypertensive disease. *Am J Med Sci* 2002; **324**: 227–231.
9. Young WF: Minireview: primary aldosteronism—changing concepts in diagnosis and treatment. *Endocrinology* 2003; **144**: 2208–2213.
10. Kem DC, Weinberger MH, Mayes DM, Nugent CA: Saline suppression of plasma aldosterone in hypertension. *Arch Intern Med* 1971; **128**: 380–386.
11. Lyons DF, Kem DC, Brown RD, Hanson CS, Carollo ML: Single dose captopril as a diagnostic test for primary aldosteronism. *J Clin Endocrinol Metab* 1983; **57**: 892–896.
12. Holland O, Brown H, Kuhnert L: Further evaluation of saline infusion for the diagnosis of primary hyperaldosteronism. *Hypertension* 1984; **6**: 717–723.
13. Castro OL, Yu X, Kem DC: Diagnostic value of the post-captopril test in primary aldosteronism. *Hypertension* 2002; **39**: 935–938.
14. Young WF: Primary aldosteronism. Management issues. *Ann N Y Sci* 2002; **970**: 61–76.
15. Gordon RD, Stowasser M, Tunny TJ, Klemm SA, Rutherford JC: High incidence of primary aldosteronism in 199 patients referred with hypertension. *Clin Exp Pharmacol Physiol* 1994; **21**: 315–318.
16. Loh KC, Koay ES, Khaw MC, Emmanuel SC, Young WF Jr: Prevalence of primary aldosteronism among Asian hypertensive patients in Singapore. *J Clin Endocrinol Metab* 2000; **85**: 2854–2859.
17. Rayner BL, Opie LH, Davidson JS: Primary hyperaldosteronism: how common is it in patients with severe hypertension? *J Hypertens* 1999; **17** (Suppl 3): S177.
18. Rossi E, Regolisti G, Negro A, Sani C, Davoli S, Perazzoli F: High prevalence of primary aldosteronism using postcaptopril plasma aldosterone to renin ratio as a screening test among Italian hypertensives. *Am J Hypertens* 2002; **15**: 896–902.
19. Stowasser M, Gordon RD, Gunasekera TG, *et al*: High rate of detection of primary aldosteronism, including surgically treatable forms, after non-selective screening of hypertensive patients. *J Hypertens* 2003; **21**: 2149–2157.
20. Mosso L, Carvajal C, Gonzalez A, *et al*: Primary aldosteronism and hypertensive disease. *Hypertension* 2003; **42**: 161–165.
21. Mulatero P, Stowasser M, Loh KC, *et al*: Increased diagnosis of primary aldosteronism, including surgically correctable forms, in Centers from five Continents. *J Clin Endocrinol Metab* 2004; **89**: 1045–1050.
22. Matsubara M, Sato T, Nishimura T, *et al*: CYP11B2 polymorphisms and home blood pressure in a population-based cohort in Japanese: the Ohasama study. *Hypertens Res* 2004; **27**: 1–6.
23. Lifton RP: A chimaeric 11 beta-hydroxylase/aldosterone

synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature* 1992; **355**: 262–265.
24. Mulatero P, Morello F, Veglio F: Genetics of primary aldosteronism. *J Hypertens* 2004; **22**: 663–670.
25. Takeda Y: Genetic alterations in patients with primary aldosteronism. *Hypertens Res* 2001; **24**: 469–474.
26. Spoto B, Feurlo G, Gervasi A, Bresolin G, Zoccali C: Familial hyperaldosteronism. *G Ital Nefrol* 2004; **21**: 139–143.
27. Katayama Y, Takata N, Tamura T, *et al*: A case of primary aldosteronism due to unilateral adrenal hyperplasia. *Hypertens Res* 2005; **28**: 379–384.
28. Yokota K, Ogura T, Kishida M, *et al*: Japanese family with glucocorticoid-remediable aldosteronism diagnosed by long-polymerase chain reaction. *Hypertens Res* 2001; **24**: 589–594.
29. Stowasser M, Gordon RD, Rutherford JC, Nikwan NZ, Daunt N, Slater GJ: Diagnosis and management of primary aldosteronism. *J Renin Angiotensin Aldosterone Syst* 2001; **2**: 156–169.
30. Seifarth C, Trenkel S, Schobel H, Hahn EG, Hensen J: Influence of antihypertensive medication on aldosterone and renin concentration in the differential diagnosis of essential hypertension and primary aldosteronism. *Clin Endocrinol* (*Oxf*) 2002; **57**: 457–465.
31. Mulatero P, Veglio F, Pilon C, *et al*: Diagnosis of glucocorticoid-remediable aldosteronism in primary aldosteronism: aldosterone response to dexamethasone and long polymerase chain reaction for chimeric gene. *J Clin Endocrinol Metab* 1998; **83**: 2573–2575.
32. Mulatero P, Curnow KM, Aupetit-Faisant B, *et al*: Recombinant CYP11B genes encode enzymes that can catalyse conversion of 11-deoxycortisol to cortisol, 18-hydroxycortisol and 18-oxocortisol. *J Endocrinol Metab* 1998; **83**: 3996–4001.
33. Jonsson JR, Klemm SA, Tunny TJ, Stowasser M, Gordon RD: A new genetic test for familial hyperaldosteronism type I aids in the detection of curable hypertension. *Biochem Biophys Res Commun* 1995; **207**: 565–571.
34. Omura M, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T: Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan. *Hypertens Res* 2004; **27**: 193–202.
35. Tiu SC, Choi CH, Shek CC, *et al*: The use of aldosterone-renin ratio (ARR) as a diagnostic test for primary hyperaldosteronism and its test characteristics under different conditions of blood sampling. *J Clin Endocrinol Metab* 2005; **90**: 72–78.
36. Kaplan NM: Primary aldosteronism, in: Kaplan's Clinical Hypertension, 8th ed. Philadelphia, Lippincott Williams & Wilkins 2002, pp 455–479.
37. Magill SB: Adrenal vein sampling: an overview. *Endocrinologist* 2001; **11**: 357–363.
38. Yamahara K, Itoh H, Yamamoto A, *et al*: New diagnostic procedure for primary aldosteronism: adrenal venous sampling under adrenocorticotropic hormone and angiotensin II receptor blocker–application to a case of bilateral multiple adrenal microadenomas. *Hypertens Res* 2002; **25**: 145–152.
39. Stowasser M: How common is adrenal-based mineralcorticoid hypertension? *Curr Opin Endocrinol Diabetes* 2000; **7**: 143–150.
40. Lim PO, Jung RT, MacDonald TM: Raised aldosterone to renin ratio predicts antihypertensive efficacy of spironolactone: a prospective cohort follow-up study. *Br J Clin Pharmacol* 1999; **48**: 756–760.
41. Ganguly A, Chavarri M, Luetscher JA, Dowdy AJ: Transient fall and subsequent return of high aldosterone secretion during continued dexamethasone administration. *J Clin Endocrinol Metab* 1977; **44**: 775–779.
42. Reincke M, Beuschlein F, Latronico AC, Arlt W, Chrousos G, Allolio B: Expression of adrenocorticotrophic hormone receptor mRNA in human adrenocortical neoplasm: correlation with P450scc expression. *Clin Endocrinol* 1997; **46**: 619–626.
43. Rossi GP: Remodelling of left ventricle in primary aldosteronism due to Conn's adenoma. *Circulation* 1997; **95**: 1471–1478.
44. Duprez D, De Buyzere M, Rietzschel ER, Clement DL: Aldosterone and vascular damage. *Curr Hypertens Rep* 2000; **2**: 327–334.
45. Sato A, Saruta T: Aldosterone-induced organ damage: plasma aldosterone level and inappropriate salt status. *Hypertens Res* 2004; **27**: 303–310.
46. Iwashima Y, Horio T, Kuroda S, Takishita S, Kawano Y: Influence of plasma aldosterone on left ventricular geometry and diastolic function in treated essential hypertension. *Hypertens Res* 2002; **25**: 49–56.
47. Kaplan NM: The current epidemic of primary aldosteronism: causes and consequences. *J Hypertens* 2004; **22**: 863–869.