

**BLOOD SCIENCES
DEPARTMENT OF CLINICAL BIOCHEMISTRY**

Title of Document: *Investigative protocol for primary hyperaldosteronism*

Q Pulse Reference N^o: BS/CB/DCB/EN/13

Authoriser: Peter Beresford

Version N^o: 5

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Investigative protocol for Primary Hyperaldosteronism

Introduction

Primary hyperaldosteronism (PA) results from the renin independent over production of aldosterone. The most common causes are adrenal adenoma and unilateral or bilateral hyperplasia. Other subtypes include glucocorticoid suppressible aldosteronism and aldosterone producing adrenocortical carcinoma.

Indication

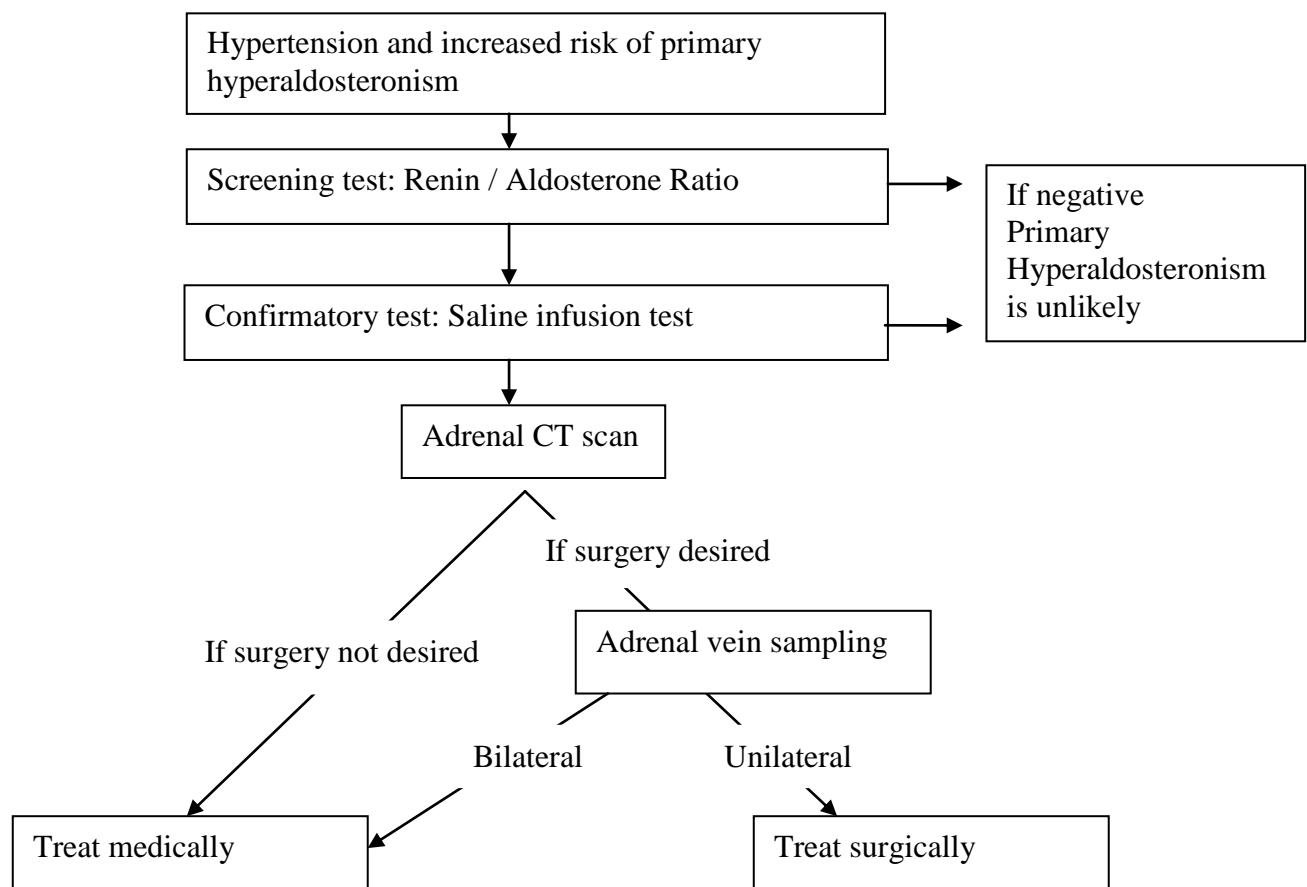
Recent studies suggest that more than 10% of hypertensive patients have primary hyperaldosteronism. Screening for PA is advised in the following circumstances:

- Resistant hypertension
- Hypertension and family history of early onset (<40yrs) CVE, PA or hypertension.
- Spontaneous or diuretic induced hypokalaemia
- Hypertension and an incidentaloma
- Hypertension onset at a young age

First degree relatives of patients with confirmed primary hyperaldosteronism should also be screened.

It is important to note that a significant proportion of patients with primary hyperaldosteronism are normokalaemic.

Investigation pathway



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1. Screening: Plasma Aldosterone/Renin activity ratio

Introduction

In PA, aldosterone should be raised and renin suppressed. Morning plasma aldosterone and plasma renin activity ratio is the most reliable screening test although it is not always easy to interpret. While the ratio is fairly robust when measured in patients on antihypertensives, false positives or negatives may occur. Ideally a patient should be off all hypertensive medication but this may be impractical. The physiological effects of common antihypertensive agents and biological factors are tabulated below.

Preparation

1. If safe to do so, withdraw medications that markedly affect results for at least 4 weeks:
 - a. Spironolactone, eplerone, amiloride, and triamterene
 - b. Potassium-wasting diuretics
 - c. Liquorice
2. If hypertension can be controlled with alternative medications or a previous aldosterone/renin screen has been non diagnostic then withdraw other medications which may affect result for at least 2 weeks:
 - a. Beta blockers, central alpha blockers, NSAIDs
 - b. ACE inhibitors, angiotensin receptor blockers, calcium antagonists
3. If alternative antihypertensive medications are required verapamil, hydralazine, doxazosin and prazosin may be used.
4. Give potassium replacement (Slow K/Sando-K) sufficient to raise serum potassium into the reference range (3.3-4.8 mmol/l). Low potassium will suppress aldosterone secretion and make interpretation unreliable.
5. Encourage salt intake

Procedure

1. Samples should be taken in the morning, after the patient has been up for at least 2 hours.
2. The patient should remain seated 5-15 minutes prior to venepuncture.
3. Take Renin and Aldosterone (5mls, EDTA purple top tube) and transport to the lab within 60 minutes. The sample must NOT be transported on ice as this activates conversion of the renin precursor to active renin.

Interpretation

A high plasma aldosterone and suppressed plasma renin activity is suggestive of primary hyperaldosteronism. Samples are sent to Viapath at King's College, London. The ranges they quote are as follows;

Plasma Renin Activity (units : mU/L) Supine: 5.4 – 30 Erect: 5.4 - 60	Aldosterone (units : pmol/L) Supine: 100-450 Erect: 100 – 800	Aldosterone/Renin Ratio <80 - Conn's unlikely 80-200 - Conn's not excluded >200 - Conn's likely
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If the ratio is positive advise a repeat to confirm an abnormal ratio before proceeding with further investigations.

Factors to take into account when interpreting results

Biological factors	Effect on Aldosterone	Effect on Renin	Effect on Ratio
Hypokalaemia	↓	↑	↓
Low salt intake	↑	↑	↑
Salt Loading	↓	↓	↓
Age	↓	↓	↓
Renal impairment	↔	↓	↑
Malignant Hypertension or renovascular disease	↑	↑	↓
Pregnancy	↑	↑	↓
Luteal phase	↑	↔	↑
Spironolactone	Variable effect	↑	
BBlockers	↓	↓	↑
NSAID's	↓	↓	↑
ACEi or ARBs Diuretics Ca Antagonists	↓	↑	↓

2. Confirmation of primary hyperaldosteronism

The principle underlying these tests is that an increase in intravascular volume should decrease renin release and subsequent aldosterone production in patients without primary aldosteronism. Both European and American Endocrine society clinical guidelines suggest the confirmatory test may be selected from a choice of four: oral salt loading test, saline infusion test, fludrocortisone suppression test and captopril challenge test. The most commonly used are either saline infusion or salt loading tests.

Although the guidelines suggest a confirmatory test is always necessary various editorials argue that if the patient is <40years old, with hypokalaemic hypertension and has an

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unequivocal aldosterone/renin ratio and unilateral adrenal nodule then further biochemical confirmation is not required.

Preparation

All of the investigation strategies require adequate salt intake for 4 days prior to the test, potassium within the normal range and the withdrawal of the following medications:

Drugs	Stop
Spironolactone	6 weeks
BBlockers	2 weeks
ACEi, ARBs	2 weeks
Diuretics	2 weeks
Nsaids	2 weeks
Calcium antagonists	Day of test

Patients should be given a home BP monitor so that if BP>150/100mm/Hg alternative antihypertensive medications such as doxazosin should be used.

A) Saline Infusion test

Procedure

Patient should be recumbent for 1 hour before and during the test.

Give the patient 2 litres of 0.9% saline I.V. over 4 hours starting at 08:00-09:30am.

Take Renin, Aldosterone and potassium at baseline and after 4 hours.

BP and pulse should be monitored hourly.

Interpretation

Post-infusion plasma aldosterone levels <171pmol/l rules out PA as a cause. The specificity and sensitivity of the immunoassay is such that >217pmol/L makes PA highly likely to be the cause.

Concerns

This test should not be performed in patients with severe uncontrolled hypertension, renal disease, cardiac failure, cardiac arrhythmia and severe hypokalaemia

B) Oral Salt Loading

Procedure

Patients should increase their salt intake to > 200mmol (6g) a day for 4 days. Slow sodium contains 10mmol.

Adequate potassium supplementation is required.

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On day 3-4 24 hour urinary sodium and aldosterone is requested. Before requesting urine aldosterone please discuss with the laboratory, this test is available from UCL. An acceptable alternative is to send serum aldosterone.

Interpretation

Primary hyperaldosteronism is unlikely if Aldosterone is lower than 10mcg/24hours (27.7nmol/l), urinary Na >200mmol/l and in the absence of renal disease. Elevated urine Aldosterone makes the diagnosis likely.

Concerns

This test should not be performed in severe uncontrolled hypertension, renal disease, cardiac failure, cardiac arrhythmia or severe hypokalaemia.

Urinary Aldosterone should be performed by HPLC-MS to provide adequate test performance.

C) Fludrocortisone suppression test

Procedure

1. Admission to hospital
2. Give 0.1mg fludrocortisone 6-hrly (06.00, 12.00, 18.00, 24.00) for 4 days.
3. Give supplemental salt 30 mmol t.d.s with meals (Slow Sodium, 10 mmol tablet).
4. Check U&E daily
5. Give oral potassium supplements with a dose that will maintain K around 4.0mmol/l.
6. Day 4 (post fludrocortisone):
Plasma cortisol at 07:00 and 10:00 am
Plasma aldosterone at 10.00 with patient seated

Interpretation

Suppression of aldosterone to <140 pmol/l on day 4 excludes primary hyperaldosteronism. A Cortisol lower at 10am than at 7am excludes a confounding ACTH effect.

Concerns

Requires hospitalisation of patients for at least 4 days.

May be contra-indicated in severely hypertensive or elderly patients.

In favour of the FST is that it is less intrusive than saline or salt loading and therefore less likely to provoke non-renin dependent alterations of aldosterone levels.

D) Captopril Challenge test

Procedure

Patients receive 25-50mg of captopril orally after sitting or standing for at least 1 hour. Blood samples are drawn for measurement of PRA, Aldosterone and cortisol at 0, 1 or 2 hours after the challenge with the patient remaining seated during this period.

Interpretation

Plasma Aldosterone is normally suppressed by captopril (>30%). In patients with primary hyperaldosteronism it remains elevated and renin remains suppressed.

Concerns

There are reports of substantial number of false negative or equivocal results.

3. Differentiation of the different subtypes of primary hyperaldosteronism

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1. CT scan of the adrenal glands

CT scan of the adrenal glands should only be performed once biochemical hyperaldosteronism has been established. This is to avoid misdiagnosis of an adrenal incidentaloma (present in between 1 and 10% patients undergoing CT scanning). While an adrenal CT can identify an adrenal adenoma or bilateral adrenal hyperplasia it may be unhelpful, owing to the small size of many adenomas and the inability to distinguish functioning adenomas from incidentalomas.

2. Adrenal venous sampling

The Endocrine society guidelines suggest if surgery is desirable adrenal vein sampling should always be done in conjunction with a CT scan. This is the gold standard for sub-type classification. However, this is operator dependent and it should be noted that adrenal vein sampling does not fulfil the usual criteria for a gold standard test. Catheterisation is only successful 40-70% of the time, the results are not always reproducible, there are safety considerations (1-2% complication rate), there is a lack of standardisation of protocols (whether ACTH is used and cut offs for interpretation) and cost. Where undertaken it should be by an experienced radiologist who performs a large number.

Simultaneous measurement of aldosterone:cortisol ratios in each adrenal vein and a peripheral vein will show whether one or both adrenals are producing excess aldosterone. This will therefore discriminate between adrenal adenoma and bilateral adrenal hyperplasia. See Protocol DS/CB/DCB/EN/22

3. Posture Studies

Historically this was used to differentiate bilateral hyperplasia from aldosterone producing adenomas. The premise was that with standing aldosterone increased when due to bilateral hyperplasia but decreased with adenomas or familial hyperaldosteronism type 2. However, it has been noted that about 30% of patients with adenomas have an increase therefore few authorities recommend this test now.

It has an ancillary role if CT scan shows an adenoma and adrenal vein sampling is unsuccessful.

See appendix for protocol.

4. Genetic testing for Glucocorticoid responsive hyperaldosteronism

Glucocorticoid responsive hyperaldosteronism (GRA) is responsible for less than 1% Primary Hyperaldosteronism cases. The presentation can be highly variable from some patients being normotensive to others presenting with early onset refractory hypertension. It should be considered if the patient is <20 years old, has a strong family history of primary hyperaldosteronism, hypertension or cerebrovascular disease before the age 40 years.

GRA is an autosomal dominant disorder. It is caused by a mutation arising as a result of cross-over of the genes encoding steroid 11 β hydroxylase and aldosterone synthase which results in a chimeric gene that has aldosterone synthase activity but is regulated by ACTH

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rather than angiotensin II. Mutation analysis is available for this disorder and has replaced the need for dexamethasone suppression testing.

Treatment

1. Surgery

If the patient is willing to have surgery and has a unilateral adenoma then laparoscopic adrenalectomy is the preferred option.

- Preoperatively

It is important that hypertension and hypokalaemia are controlled.

- Post operatively

Aldosterone and renin should be measured shortly after surgery to assess response.

Day 1 post op potassium supplements should be withdrawn and spironolactone discontinued. Antihypertensive medication will require reviewing.

High salt intake should be recommended to avoid hypoaldosterone induced hyperkalaemia

Hypertension is cured in about 50% of patients.

2. Medical management

This is recommended for those who do not undergo surgery. In the UK Spironolactone is first line although eplerone is a more specific mineralocorticoid receptor antagonist so causes less side effects. Amiloride which inhibits sodium channels in the distal tubule may also be used.

3. GRA

GRA requires glucocorticoid to suppress pituitary ACTH secretion. The recommendation is to use a synthetic steroid that is longer acting than hydrocortisone such as dexamethasone or prednisolone. Examples of doses required are dexamethasone 0.125-0.25mg daily or prednisolone 2.5-5mg daily. Ideally the steroid should be taken at midnight to suppress morning ACTH. Plasma renin and aldosterone may be helpful in determining dose.

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4. Coded interpretative comments for Renin/Aldosterone measurement

Below are draft coded comments which can be added to reports which come from Viapath for Renin/Aldosterone ratio's conducted as part of screening for Primary Hyperaldosteronism.

Scenario	Code	Coded comment
Comment to go on all reports (given that advice about interfering medications is crucial to all aldosterone/renin interpretations) to be placed in the "report comment" line on Winpath		Note: the only anti-hypertensives that have minimal effect on aldosterone and renin are the alpha blockers, e.g. doxazosin. ACEi and ARB, CCB and diuretics may increase renin and decrease aldosterone to variable extent. B-blockers and NSAIDs often suppress renin alone from usual baseline, leading to false positive aldosterone/renin ratios.
A/R ratio >200 = Conn's likely	@arr1	Raised aldosterone/renin ratio with low renin. Provided the patient has an inappropriate kaliuria and is NOT on interfering medications: results are suggestive of conns syndrome. Suggest repeat plasma aldosterone/renin ratio to confirm, after discontinuing any interfering drugs (see below) for 2 weeks.
A/R ratio 80-200 = Conn's indeterminate	@arr2	Borderline aldosterone/renin ratio. Advise review possible interfering drugs (see below), electrolyte/volume status (note marked hypokalaemia may impair aldosterone production) and evidence of renovascular pathology. Repeat recommended after removing interfering medication for 2 weeks and potassium replacement if required.
A/R ratio <80 = Conn's unlikely	@arr3	Normal aldosterone/renin ratio. These results are not suggestive of conns syndrome, provided the patient is not in receipt of interfering medications (e.g. spironolactone or high dose ACE inhibitor).
Raised Renin making A/R ratio very low	@arr4	Markedly raised renin with low A/R ratio, not consistent with conn's syndrome. Other causes to consider include abnormal electrolyte and volume status, adrenal dysfunction, interfering drugs (e.g. ACEi/NSAID/diuretics) and renovascular pathology (eg. Renal artery stenosis). Please phone if you wish to discuss.
Renin measurement for mineralocorticoid status (whether result is high or low or normal)	@arr5	Limited evidence supports the use of renin levels as a marker of mineralocorticoid replacement. Any evidence that does exist suggests that renin in the upper half of the reference range or just above is acceptable.

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Appendix 1: Historical tests

Dexamethasone suppression test

Procedure

- 1) Day 1. Take a baseline sample (5mls EDTA purple top bottle sent to lab within 60 mins) between 8 and 10am for measurement of plasma aldosterone and plasma rennin activity.
- 2) Then give dexamethasone, 0.5 mg, every 6 hours for 5 days.
- 3) Day 4 and 5 collect early morning blood samples (as above), for the measurement of plasma aldosterone and renin.

Interpretation

Plasma aldosterone levels should suppress from high baseline levels (with very low or suppressed renin) to very low levels (less than 150 pmol/L) in patients with this condition.

Posture test

Procedure

- a.) Discontinue potassium supplements for 24 hours prior to testing.
- b.) The patient must be admitted overnight to ensure strict overnight recumbency. After waking, the patient must remain lying down until after the initial fasting blood sample has been taken.
- c.) At 8.00am take 5 mls blood into a single EDTA tube (purple top). This sample should be taken to the laboratory for separation and freezing within 60 minutes. The patient must not sit up until this sample has been taken. Request aldosterone, plasma renin activity and cortisol.
- d.) After the patient has been out of bed for 30 mins and before breakfast take a further EDTA tube (purple top) sample as in c. above. Request plasma renin activity. The patient can then have breakfast and remain ambulant.
- e.) At 12.00 noon take blood as in c. above and request aldosterone and cortisol. The test is then complete.

Interpretation

Primary hyperaldosteronism is indicated by an elevated aldosterone value at 08.00 together with a suppressed plasma renin activity which shows little or no increase after 30 mins mobility. To aid the distinction between hyperaldosteronism due to adrenal adenoma and that due to bilateral adrenal hyperplasia, it is helpful to consider the plasma aldosterone

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concentrations at 08.00 and 12.00. If cortisol values at 08.00 and 12.00 show a decrease due to normal diurnal variation, an elevated aldosterone level at 08.00 decreasing by 50% or more at 12.00 is suggestive of, but not exclusive to, an adenoma.