ENDOCRINE INVESTIGATION PROTOCOLS

PENNINE ACUTE TRUST.

Alphabetical test index.

Insulin stress test (IST)	15
Intersex screen	63
Long Synacthen test	14
Low dose dexamethasone suppression test (LDDST)	27
Lying and standing renin and aldosterone levels	41
Magnesium	56
Multiple endocrine neoplasia 1 (MEN1)	35
Multiple endocrine neoplasia 2 (MEN2)	35
Octreotide test dose – short-acting	69
Octreotide test dose - long acting	70
OGTT with GH levels	24
Osteoporosis screen	58
Overnight dexamethasone suppression test (ONDST)	26
Pagets screen	58
Parathyroid Hormone	56
Parathyroid infusion test	56
Pentolinium suppression test	44
Peripheral venous sampling of ectopic ACTH	31
Plasma catecholamines	43
Plasma renin activity	60
Precocious puberty screen	63
Prolactins (cannulated)	34
Renal stone tests	57
Renin and aldosterone assessment	41
Secretin test	49
Sex hormone priming prior to IST	16
Short synacthen test (SST)	13
SST with 17OH progesterone	13
Somatostatin tests	49
Steroid withdrawal protocol	17
Testosterone profile	52
Thyroid blood test	37
Thyroid imaging	37
Thyroid investigation protocol	38
Thyroptrophin releasing hormone test (TRH)	20
Urinary catecholamines	43
Urinary free cortisol (UFC)	25,75
Urinary 5HIAA	47
VIPoma test	49
Vitamin D	55
Water deprivation test	21

Sample Requirements for Endocrine Investigations

1. Blood Tests

Test	Abbreviation	Sample Type/ Preservative	On ice	Straight to lab in under 15min from sample collection
Adrenocorticotrophic hormone	ACTH	Plasma (Li heparin)	YES	YES
Aldosterone		Plasma(Li heparin)	NO	YES
Aldosterone/renin ratio		Plasma (Li heparin)	NO	YES
Androstenedione		Serum	NO	NO
Angiotensin converting enzyme	ACE	Serum	NO	NO
Antidiuretic hormone	ADH	Plasma (Li heparin)	YES	YES
Calcitonin		Plasma (Li heparin)	YES	YES
Cortisol		Plasma (Li heparin) at NMGH, serum at ROH	NO	NO
C-peptide		Plasma (Li heparin) at NMGH, serum at ROH	NO	YES
Dehydroepiandrosterone	DHEA	Serum	NO	NO
11-deoxycortisol		Serum	NO	NO
5α dihydrotestosterone	5α DHT	Serum	NO	NO
Follicle stimulating hormone	FSH	Plasma (Li heparin) at NMGH, serum at ROH	NO	NO
Free T3	FT3	Serum at ROH	NO	NO
Free T4	FT4	Plasma	NO	NO
Gastrin		Plasma (Li hep+Trasylol)	YES	YES
Glucagon		Plasma (Li hep+Trasylol)	YES	YES
Glucose	GLUC	Plasma (fluoride oxalate)	NO	NO
Growth hormone	GH	Serum	NO	NO
Gut hormones	GUT	Plasma (Li hep+Trasylol)	YES	YES
Human chorionic gonadotrophin	HCG	Serum	NO	NO
17αhydroxyprogesterone	17αOH-P	Serum or plasma (Li hep)	NO	NO
Insulin		Plasma (Li heparin) at NMGH, serum at ROH	NO	YES
Insulin-like growth factor	IGF	Serum	NO	YES
Insulin antibodies		Serum	YES	YES
Lutenising hormone	LH	Plasma (Li heparin) at NMGH, serum at ROH	NO	NO
Neurotensin		Plasma (Li hep+Trasylol)	YES	YES
Osmolality	OSMO	Plasma (Li heparin) at NMGH, serum at ROH	NO	NO
Pancreatic polypeptide	PP	Plasma (Li hep+Trasylol)	YES	YES
Parathyroid hormone	PTH	Plasma (EDTA)	YES	YES
Parathyroid hormone	PTHRP	Contact lab as special		

related peptide		tubes will have to be ordered		
Pituitary polypeptide α subunit	α Subunit	Serum	NO	NO
Progesterone		Serum	NO	NO
Prolactin	PRL	Plasma (Li heparin) at NMGH, serum at ROH	NO	NO
Renin		Plasma (Li heparin)	NO	YES
Sex hormone binding globulin	SHBG	Serum	NO	NO
Somatostatin	SST	Plasma (Li hep+Trasylol)	YES	YES
Testosterone	TEST	Serum	NO	NO
Total T3	TT3	Plasma (Li heparin) at NMGH, serum at ROH	NO	NO
Total T4	TT4	Serum at ROH, not done at NMGH	NO	NO
Thyroid stimulating hormone	TSH	Plasma (Li heparin) at NMGH, serum at ROH	NO	NO
Vasoactive intestinal polypeptide	VIP	Plasma (Li hep+Trasylol)	YES	YES
Vitamin D		Serum	NO	NO

NB Serum samples can be collected into plain tubes with no preservative or gel containing tubes

2. Urine Tests

Test	**Preservative required for urine collection**
Calcium	50ml M Sulphuric acid at NMGH***, 10ml 6M HCL at ROH***
Cortisol	No preservative
Creatinine clearance	No preservative
5 HIAA	50ml Glacial Acetic acid at NMGH*** 10ml 6M HCL at ROH***
Hydroxyproline (Second passed morning urine, i.e. not 24h collection)	No preservative
Oxalate	50ml M Sulphuric acid at NMGH*** 20ml 6M HCL at ROH***
Metadrenaline	50ml M Sulphuric acid at NMGH*** 20ml 6M HCL at ROH***
Protein	No preservative
Urate	No preservative

NB 24hour urine collection bottles can be obtained from Biochemistry Dept.
*** bottles contain strong corrosive acid which must not be discarded.

Index of Investigations.

SECTION 1-
INVESTIGATION OF HYPOGLYCAEMIA

Fasting Plasma Glucose

Purpose of test: Investigation of patients with suspected hypoglycaemia e.g. secondary to insulinoma. Hypoglycaemia is defined as a laboratory plasma blood of <2.2mmol/l.

Rationale: secretion of insulin by an insulinoma is inappropriate for the level of blood glucose and does not suppress following exogenous insulin infusion

Protocol: Take fasting samples for glucose and insulin on 3 separate occasions. Save plasma samples for insulin measurement, which should be measured **ONLY** if hypoglycaemic.

Interpretation: Three normal fasting glucose reading will effectively exclude >90% of insulinomas. If equivocal or history strongly suggestive of insulinoma then proceed to a 3 day fast. Hypoglycaemia associated with hyperinsulinaemia is suggestive of an insulinoma or facticious administration of insulin or OHA (detectable in urine)

72 hour (prolonged) fast.

Purpose of test: investigation of patients with suspected hypoglycaemia e.g. secondary to insulinoma

Rationale: secretion of insulin by an insulinoma is inappropriate for the level of blood glucose and does not suppress following exogenous insulin infusion

Protocol: Advise laboratory that test is underway. Admit patient and give water or non-caloric drinks only for 72hrs. Exercise is encouraged. Capillary glucose taken four hourly or when symptoms of hypoglycaemia occur. When symptomatic, take blood for glucose, C-peptide and insulin together with a blood sample for sulphonylureas. Record when patient leaves the ward. If no symptoms have occurred at the end of 72hours, exercise the patient for 15 – 30 minutes, then take tests as above. Feed the patient.

Interpretation: Hypoglycaemia associated with hyperinsulinaemia is suggestive of an insulinoma or facticious administration of insulin or OHA (detectable in urine). 30% of patients with an insulinoma will become hypo within 12 hors, 80% within 24 hours and 100% at 72 hours. If still in doubt perform a C-peptide suppression test

C - peptide suppression test

Purpose of test: investigation of patients with suspected hypoglycaemia secondary to insulinoma

Rationale: C-peptide secretion should be suppressed in the presence of exogenous insulin administration. Presence of C-peptide suggests autonomous secretion of insulin i.e. an insulinoma.

Protocol: Iv cannula inserted.
0.1 U/kg of actrapid is infused over 60mins.
Take bloods at 1/2 hourly intervals for glucose, insulin and C - peptide up to 150 mins.

Interpretation: Normally, C-peptide concentrations suppress to undetectable levels during insulin infusion. In those with an insulinoma, C-peptide levels do not suppress.

5 hour OGTT

Purpose of Test: To detect reactive or post-prandial hypoglycaemia.

Rationale: Normally, the amount of insulin secreted in response to a glucose challenge will match requirements exactly. In some, the insulin secretion is delayed, leading to hypoglycaemia.

Patient preparation: Fasting from 10pm night before test. Do not perform in patients with DM

Protocol: 75g of anhydrous glucose (Polycal or similar) in 300mls of cold water given orally over 5 mins.
Glucose taken every 30mins or when symptomatic
The latter are processed only if hypoglycaemia detected.

Interpretation: Hypoglycaemia together with hyperinsulinaemia at any time throughout the test is diagnostic of the condition

SECTION 2 –
INVESTIGATION OF ENDOCRINE DEFICIENCIES

TESTS FOR HYPOCORTISOLAEMIA

Short synacthen test
Long synacthen test
Insulin stress test
Sex hormone priming prior to IST
Glucagon test

1. Short Synacthen Test (SST)

Purpose of test: assessment of adrenocortical function, diagnosis of Addison's disease, diagnosis of congenital adrenal hyperplasia (CAH) when performed with 17-OH progesterone levels, diagnosis of 11B OH'ase deficiency when performed with 11 deoxycortisol.

Rationale: Synacthen is a synthetic preparation of the biologically active N-terminal first 24 amino acids of ACTH. It stimulates cortisol release from the adrenals

Patient preparation: It can be performed at any time of day but more information is gleaned by starting the test at 9am. Patient can be fed or fasting. If the patient is taking hydrocortisone, stop this the night before test and recommence after the test has been completed. If taking prednisolone or cortisone, transfer to dexamethasone two weeks prior to test. If assessing for CAH in women, perform test in follicular phase.

Protocol: insert IV cannula and wait for 10 mins.
0 min blood for basal cortisol and ACTH Give Synacthen 250 mcg IM. Bloods at 30 min and 60 min for cortisol

If CAH is suspected take additional samples at all time points for 17-OH progesterone

Normal response: basal cortisol > 130 nmol/l (but not diagnostic)
Increment should be > 200 nmol/l
Absolute minimum level of cortisol should be > 550 nmol/l

A high ACTH level at baseline is suggestive of primary adrenal failure. 9 am17-OH progesterone levels >15nmol/l together with hypocortisolaemia are suggestive of CAH; levels greater then 45nmol/l suggest homozygous disease, those between 15-45 nmol/l suggest heterozygous disease

2. Long synacthen test (LST)

Purpose of test: to assess the adrenal cortisol response to prolonged stimulation; to help differentiate between primary and secondary adrenal insufficiency.

Rationale: patients with primary adrenal failure do not respond to synacthen, but those with secondary adrenal failure (i.e. after treatment with exogenous steroids) may have a delayed response

Patient preparation: If the patient is taking hydrocortisone, stop this the night before test and recommence after the test has been completed. If taking prednisolone or cortisone, transfer to dexamethasone two weeks prior to test. Patient needs to be admitted for 2 days.

Protocol: insert IV cannula and flush regularly
0 min blood for plasma cortisol and ACTH
give Synacthen depot 1 mg IM
bloods at 1, 4, 6, 8, 24 and 48 hours for cortisol

Interpretation: In primary adrenal failure, cortisol < 200 nmol/l for the entire 48 hours. In secondary adrenal failure, cortisol levels remain low for the first 4 - 6 hours and then rise slowly usually to > 600nmol/l

NB Further injections of Synacthen (1 mg) may be given every 24 hours for 3 days when plasma cortisol level on day 4 should be > 650 nmol/l. This is the definitive test. Stop hydrocortisone dose on the evening before and restart on the morning of day 4

3. Insulin stress test (IST) / Insulin tolerance test (ITT)

Purpose of test To assess pituitary ACTH and GH responses to stress

Rationale hypoglycaemia induces secretion of ACTH (&cortisol) and GH

Doctor preparation: A doctor must remain with the patient for the entire test. Inform laboratory that test is underway.

Patient preparation Contra-indicated if patient has EPILEPSY or IHD
Patient fasting from midnight and is admitted for the day
Must ensure normal U and E's, ECG, consent form signed, IV 50mls of 50% dextrose, glucagon & IV hydrocortisone is at the bedside to treat the hypoglycaemia

Protocol measure weight, lie patient down, BP cuff on, IV cannula
At start of test (0 min), take blood for glucose, cortisol & GH
Give ACTRAPID 0.1 U / kg sc (or 0.3 U/kg in acromegaly or diabetes). Perform blood tests as detailed in table below and use to document blood results

Time	BP	HR	Capillary glucose	Glucose	Cortisol	GH
0						
15 mins						
30 mins						
45 mins						
60 mins						
1hr 15 mins						
1hr 30 mins						
1 hr 45mins						
2 hr						

If hypo not achieved within 45 mins, give a repeat dose of insulin and continue sampling for 1 hour after hypoglycaemic episode (usually 20-40 minutes into the test). Keep patient in bed for a further 2h and give them a good meal

Hypoglycaemia When capillary glucose<4mmol/l/l and patient is symptomatic, take blood for laboratory glucose, GH and cortisol levels. Give oral glucose if possible or IV if not possible. If slow to respond, give Hydrocortisone 100mg IV stat. Ensure glucose greater then 5mmol/l prior to discharge.

Interpretation: Patient must be hypoglycaemic for interpretation of the test. Cortisol should rise 200nmol/l above baseline and to at least 550nmol/l. GH should rise to at least 16 mU/l

4. Sex hormone priming prior to IST for investigation of short stature in prepubertal subjects

Girls: Stilboestrol 1 mg bd for 2 days, with IST on Day 3

Boys: Same as above **OR**
Sustanon 100 mg on alternate days for 3 doses
IST one day after the last dose

NB Oestrogen is a better priming agent in both sexes

5. Glucagon test

Purpose of test: to assess GH and ACTH reserve when IST contraindicated

Rationale: glucagon induces a rise in blood glucose and during the subsequent fall, both ACTH (hence cortisol) & GH are released
It is less reliable than IST as 20% of people with normal GH/ACTH reserves fail to respond. It is unreliable in people with diabetes.

Patient preparation: fasting from midnight
warn patients they will experience nausea at the end of the test
insert IV cannula and allow patient to rest on bed for 30 min

Protocol: Start test at 09:00am
monitor pulse and blood pressure every 30 min
0 min blood for GH and cortisol
give glucagon 1 mg sc (1.5 mg if weight > 95 kg)
bloods for GH and cortisol at 90, 120, 150, 180, 210 & 240 min

Normal response: GH should rise within 2-3hours to at least 16 mU/l. Cortisol should reach a maximum of > 550 nmol/l .

6. Steroid withdrawal protocol

Purpose of test assessment of the pituitary / adrenal axis after suppression by exogenous steroids

Rationale Adrenal function is assessed (with LST) before the full axis and if adequate, proceed to evaluate the ACTH response to stress (with IST)

Patient preparation stop steroids from Day 1. Do as an inpatient only

Protocol

Warn chemical pathology and discuss cortisol measurements and ability to return test results on time with them

Day 1 no steroids given
blood for cortisol & electrolytes at 0900h (t=0)
give synacthen depot 1 mg intramuscularly (IM)
blood for cortisol at t=6h and t=8h
check lying / standing BP twice daily

Day 2 blood for cortisol at 0900h

Day 3 blood for cortisol at 0900h
get cortisol results at 1700h
if any results > 550 nmol / l proceed to DAY 4
if all cortisols < 550 nmol / l discharge patient on hydrocortisone, as evidence of adrenal insufficiency irrespective of pituitary function

Day 4 Insulin stress test with glucose and cortisol measurements. Only measure growth hormone if required

Discharge the patient on their usual steroid therapy with a blue steroid card, pending results.

TESTS OF GH DEFICIENCY

In patients with multiple pituitary endocrine deficiencies, suspected GH deficiency can be diagnosed by a low age and sex matched IF-1 concentration together with inadequate GH levels following one of the following stimulation tests. In patients with suspected isolated GH deficiency, two of the following stimulation tests need to be performed.

Insulin stress test
Glucagon test
Clonidine test
Arginine test

1. Insulin stress test.

As detailed under tests of ACTH deficiency

2. Glucagon test

As detailed under tests of ACTH deficiency

3. Clonidine test

Purpose of test: to assess GH reserve when IST is contraindicated

Rationale: clonidine stimulates GH release in normal subjects. This is not seen in hypopituitarism or other forms of GH deficiency. **BUT** the response is variable even in normals and interpretation is difficult. Only use in patients where it is essential to assess GH status but IST is impossible (epilepsy, severe ischaemic heart disease etc). Glucagon or arginine test is preferred to this one.

Patient preparation: fasting from midnight
insert IV cannula and allow patient to rest for 30 min before test

Protocol: monitor pulse & BP every 15 min throughout
blood at 0 min for GH
give clonidine 25 - 50 mcg IV
bloods for GH at 30, 60, 90,120 &150 min
patient to remain in bed for 2h after the test and continue to monitor BP every 30 min for this period

Normal response: GH should rise to values > 16 mU/l. Failure to rise to >9mU/l is suggestive of severe GHD

NB SYSTOLIC BLOOD PRESSURE FALLS BY 20 - 30 mm Hg IN ALMOST ALL SUBJECTS

4. Arginine test

Purpose of test: to assess GH reserve when IST is contraindicated

Rationale: arginine stimulates GH secretion in normal subjects.
The response is impaired in hypopituitarism and other forms of GH deficiency. The response is variable and the same precautions apply as in the use of the clonidine test (see p 13)

Patient preparation: fasting from midnight
insert IV cannulae into both forearms and allow patient to rest for 60 min before the test

Protocol: Monitor pulse and blood pressure every 15 min throughout
09:00 am sample (t=0 min) blood for GH
infuse 30g arginine in 100 ml 0.9% saline over 30 min
bloods at 30, 60, 90 and 120 min for GH

Interpretation: GH should rise to at least 16 mU/l. Failure to reach a GH level of >9 is suggestive of severe GHD

C. TESTS FOR TSH DEFICIENCY.

Most cases of hypothyroidism can be diagnosed by an elevated TSH with a low T4 concentration. However, in hypopituitary patients the TSH assay is obviously of no value in assessing hypothyroidism and free thyroxine (T4) should be measured. The TRH test is of no value in secondary hypothyroidism**.**

1. Thyrotrophin releasing hormone test (TRH test)

This test was originally used to assess patients with equivocal thyrotoxicosis. The sensitive TSH assay has made this test redundant. Sometimes a technetium uptake scan can help in such patients. The TRH test is a poor differentiating test.

D. TESTS OF HYPOGONADISM.

Most cases of hypogonadism can be assessed by basal measurements of gonadotrophins. The LHRH test previously used to diagnose hypogonadotrophic hypogonadism has since been shown to be a differentiating poor test, with no benefit. The RCOG plus the guidelines issued from the RCP do not recommend its use.

E. AVP DEFICIENCY

1. 24 hour Urine volumes

Purpose of study: To confirm excess urine production one cause of which may be an inability to concentrate urine ie. Diabetes insipidus. DI is unlikely in the absence of polyuria in the patient with an intact thirst centre.

Test: Three 24 hour urinary collections are performed.

Interpretation: Urinary volumes <3l/24hrs make DI unlikely, but still possible

2. Water deprivation test

Purpose of study: To differentiate primary polydipsia, cranial diabetes insipidus (DI) and nephrogenic DI

Rationale: ADH secretion is stimulated by hypovolaemia and hypertonicity. Failure to maintain normal urine and plasma osmolarity when dehydrated suggests DI. Failure to correct the osmolarity with exogenous DDAVP suggests a nephrogenic problem, whereas correction following exogenous DDAVP suggests AVP deficiency (Cranial DI)

Preparation: Inform laboratory that test is being undertaken. Exclude adrenocortical or thyroid deficiency. Stop DDAVP 8h beforehand in patients already on treatment, but continue with anterior pituitary hormone replacement. Fluid and food are allowed until 8:00 on morning of test

Protocol: 0800h insert IV cannula, weigh patient, patient empties bladder, measure urine volume and send for osmolality (ask for result of this to be telephoned back as the test can be terminated if urine osmolality >750 before fluid deprivation commences). NO FLUIDS FOR 8 HOURS (WATCH CAREFULLY). Measure BP, P, weight, urine and plasma osmolarity, urine volume hourly for 8 hrs. Use the chart to document results

Stop test if :
Urinary osmolality ever greater than 800
Plasma osmolarity >350 -give DDAVP 2mcg im and fluids
Weight loss >3% of baseline value -check plasma osmo
Urine output exceeds 5 litres in absence of weight loss, as suggests surreptitious drinking

	weight	BP	U&Es	Plasma Osmolarity	Urine Osmolarity	Urine volume
8:00						
8:30						
9:30						
10:30						
11:30						
12:30						
13:30						
14:30						
15:30						
16:30						
	If urine osmolarity remains< 750, give desmopressin 2mcg im. Give free fluids from now onwards.					
17:30						
18:30						
19:30						
20:30						

Interpretation: Urine osmolality > 750 mmol/kg excludes DI, especially if the plasma osmolality remains < 295 mmol/kg

Hypotonic urine after dehydration followed by urine osmolality > 650 mmol/kg after exogenous DDAVP suggests cranial DI

Hypotonic urine after dehydration with NO response to DDAVP suggests nephrogenic DI

Submaximal urine concentration (500 - 700 mmol/kg) with no response to DDAVP suggests compulsive drinking or partial nephrogenic DI

SECTION 3 –
INVESTIGATION OF ENDOCRINE EXCESS

Tests for Acromegaly

1. Prolonged OGTT with GH measurements.

Purpose of the test: To confirm the diagnosis of acromegaly (excess GH secretion)

Rationale of test: A glucose load usually suppresses GH to <2mU/l, but this is not the case in subjects with acromegaly. In fact, in 20% a paradoxical rise may be seen

Patient preparation: Fast from 10pm

Protocol: iv cannula inserted, rest for 20mins
75g oral glucose load in 200mls water over 5 mins
bloods for GH and glucose at times 0, 30, 60, 90, 120, 150, 180.

Interpretation: GH levels should suppress to <2mU/l

2. GH day curve

purpose: to assess mean GH secretion either for the diagnosis of acromegaly or to assess cure of acromegaly.

Rationale: GH fluctuate throughout the day and are suppressed by glucose (ie meals). A meal GH level estimates average GH secretion.

Patient preparation: Fast from 10pm. Take medications as normal

Protocol: Iv cannula inserted, rest for 20mins
Take GH and IGF-1 at 8:00, then have breakfast at 9:00 and lunch at 12:30
Take GH samples at 11am, 1pm, 3pm, 4:30pm

Interpretation: GH should be undetectable in at least 2 samples. Average GH levels of <5mU/l suggests cure of acromegaly and also make the diagnosis of acromegaly unlikely

Tests for Hypercortisolaemia (Cushing's syndrome)

Tests confirming the diagnosis of Cushing's Syndrome include:

24 hour Urinary free cortisol (UFC)
Loss of diurnal variation
Overnight dexamethasone suppression test (O/N DST)
Low dose dexamethasone suppression test

Note: Oestrogens (COCP or HRT) increase CBG and thus may overestimate cortisol levels; it is recommended that they are stopped 6 weeks prior to testing.

1. 24 hour Urinary free cortisol (UFC)

Purpose of the test: To screen for Cushing's syndrome (excess cortisol secretion)

Rationale of test: Cortisol secretion and hence excretion is increased in patients with Cushing's syndrome

Patient preparation: Can be performed fasting or fed. Ensure patient is not taking steroids in any form (including creams and nasal sprays). Interpretation may be difficult if performed at a time of intercurrent illness, when cortisol secretion would physiologically increase anyway.

Protocol: A 24hour urine collection is performed in a PLAIN bottle and should be performed at least three times.

Interpretation: UFC should be less than 400 nmol/24 hr. If greater levels are confirmed on 2 occasions then the patient should undergo further investigation. The sensitivity and specificity of this test are fairly low and hence 3 UFCs are required to exclude Cushing's syndrome. If all 3 UFCs are normal then it is extremely unlikely that the patient has Cushings syndrome. Conversely, if the cortisol level on one sample is four times the upper limit of normal then Cushing's syndrome is highly probable. False positive results seen in patients with pseudocushings, alcoholism and PCOS

Sensitivity When 3 UFC are performed:
3.3% false positive rate, 5.6% false negative rate

2. Diurnal variation in cortisol.

Rational: Plasma ACTH levels usually rise at 3-4am and peak between 8-9 am, a pattern which is mirrored by plasma cortisol levels. Cortisol levels then fall progressively to <50 nmol/l at midnight

Patient preparation: admit patient. Do not warn them of the timing of blood tests as this will increase anxiety and may increase cortisol levels. Patient eats, drinks and takes tablets as necessary. Ensure patient has not taking any form of steroids for 2 weeks prior to test. Ensure patient goes to bed before 10:30pm

Protocol: Blood taken for cortisol at midnight (record if pt awake or not) and 15minutes after awakening the following day. Do not allow the patient to sleep past 8:30 am as the sample needs to be done before 9am.

Interpretation: a single sleeping midnight cortisol of greater than 50nmol/l is the single most sensitive indicator of Cushings syndrome (97% of normals will have a cortisol<50nmol/l. Highest cortisol level is normally at 9am.

3. Overnight dexamethasone suppression test.

Purpose of test: To screen for Cushing's Syndrome (oversecretion of cortisol)

Rationale of test: Exogenous steroids would normally suppress the patients own production of ACTH and hence cortisol levels would be expected to be low

Patient preparation: nil, performed as an outpatient. The timing of the tablet and blood test is important, so patient must be co-operative

Protocol: 1mg of dexamethasone taken at 23:00 with a blood test for cortisol levels at 8:00am the following morning

Interpretation: suppression of 8:00am cortisol levels to less than 100nmol/l is inconsistent with Cushing's syndrome. Some patients with Cushing's will suppress their cortisol levels to some degree.

Specificity: The test is 87% specific for Cushing's syndrome (i.e. not disease).

4. Low dose dexamethasone suppression test (LD DST).

Rational: Dexamethasone usually inhibits ACTH and cortisol production. In Cushing's syndrome there is a loss of this inhibition and failure of cortisol levels to suppress.

Patient Preparation: ensure patient is not taking any steroids or drugs which increase the metabolism of dexamethasone (ie carbamazepine, phenytoin, phenobarbitone, rifampicin). Ideally these drugs should be stopped prior to the test. Similarly drugs increasing corticosteroid binding globulin (CBG) ie oral oestrogens, will give falsely elevated cortisol levels. The oestrogens must be stopped for 6 weeks before cortisol levels can reliably be measured.

Protocol: 0.5mg of dexamethasone given exactly six hourly for 2 days. The timing is essential- tablets must be 6hrs apart. Consecutive 24hr urine free cortisol assessments are started at 9am of each day. See day investigation sheet at end of section.

Interpretation: Plasma cortisol concentrations of >50nmol/l are suggestive of Cushing's syndrome. This with pseudo-Cushing's should also suppress. Urine cortisol levels should suppress to less than 50nmol/l

Sensitivity: Close to 100%

Specificity: 97%

Consider, as appropriate, these additional investigations:
Other pituitary hormone function tests, testosterone, U&Es, bone X-ray and bone mineral density measurement, visual field assessment with MRI of the pituitary, ultrasound scan of ovaries in women.

Tests for the differential diagnosis of Cushing's Syndrome.

Include:
- Plasma ACTH levels
- Plasma potassium levels
- Plasma testosterone levels
- High dose dexamethasone suppression test
- Corticotrophin releasing hormone test (CRH)
- Basal inferior petrosal sinus sampling (BIPSS)

1. ACTH levels.

Rationale: Basal ACTH levels are lowest in Cushing's syndrome (adrenal disease), high in Cushing's disease (pituitary disease) and highest in ectopic ACTH production (associated with malignancy). Note ACTH levels may also be raised in Addison's disease.

Pt preparation: None required

Protocol: A paired random ACTH (in special tubes available from path lab) and cortisol level is taken at 9:00am.

Interpretation: There are no ranges given that will help differentiate the cause of excess cortisol production as considerable overlap exists; however, extremely high levels would suggest that the cortisol production is ACTH driven, whilst low/normal levels would suggest differently.

2. Plasma potassium levels

A hypokalaemic alkalosis is commonly seen in ectopic ACTH production, but it should be noted that this is also observed in 10% of patients with pituitary driven disease.

3. Plasma testosterone levels.

Virilisation of females with high testosterone levels (greater than 5nmol/l) are strongly suggestive of an adrenal tumour.

4. High Dose DST.

Rationale: Corticotroph adenomas of the pituitary retain some responsiveness to the suppressive effects of dexamethasone, unlike ectopic ACTH secreting tumours, which do not.

Preparation: as above re: medication. Patient must be able to take the dexamethasone exactly 6 hourly.

Protocol: Plasma cortisol levels are taken (baseline level). 2mg of Dexamethasone is given exactly six hourly for 2 days and the cortisol level is rechecked.

Interpretation: Suppression of cortisol levels by greater than 50% occurs in 75% of patients with Cushing's disease or alcohol- induced Cushings, 10-15% of patients with ectopic ACTH and 0-6% of patients with adrenal tumours. Alcohol-related pseudo-Cushing's resolves biochemically when the alcohol is removed. The 9:00 cortisol after the 48hr test is considered to be the most sensitive means of differentiating between Cushing's disease and ectopic production.

5. CRH (Corticotrophin releasing hormone) test

Rationale: Pit.dependent cushings show a normal or increased response of cortisol and ACTH to CRH

Pt preparation: fast from midnight.

Protocol: Iv cannula, rest for 30mins.
Take a basal cortisol at –15 and 0 mins.
Give CRH 100mcg iv
Take samples for cortisol and ACTH at 15, 30, 45, 60, 90 and 120 mins. Samples should be taken on ice and sent immediately to the laboratory as soon as each timed collection is taken.

Interpretation: An 3 fold increase in cortisol and ACTH is suggestive of pituitary driven disease. The ACTH response is more reliable if the cortisol level >1000nmol/l

6. Bilateral inferior petrosal sinus sampling (BIPSS) with CRF.

At present this test is performed at MRI but is mentioned here for completion.

Rational: The pituitary effluent drains into the petrosal sinus, thus a gradient in the ACTH from here compared to one taken from a peripheral site indicates a central source of ACTH. In ectopic ACTH, pituitary ACTH should be suppressed and therefore no gradient in ACTH levels will exist between central and peripheral levels.

Preparation: Patient must be hypercortisolaemic when testing. Heparinisation is necessary.

Protocol: Intravenous catheter is inserted in the radiology department. Two catheters are sited in the left and right IPS and a third catheter is placed peripherally. Two baseline samples are taken 5 minutes apart for ACTH, cortisol and prolactin.1ug/Kg body weight ovine CRH is given iv over one minute.
Samples for ACTH, cortisol and prolactin are collected simultaneously from the three sampling sites (RIPS, LIPS and P) 2, 5 and 10mins post CRF.

Samples must be collected into plastic lithium heparin tubes - see section re: sample handling. Care must be taken to prevent haemolysis since visible haemolysis invalidates the ACTH measurement (lower results may be obtained). Samples must be kept on ice and transferred to the lab immediately

Interpretation: A basal central to peripheral ACTH gradient of more than 2 to 1 is indicative of Cushing's disease (95% specific, 100% sensitive). A stimulated gradient of 3:1 is 100% specific and 100% sensitive.

To achieve the sensitivity described above it was essential to use the highest values obtained for the IPS/P, which is why samples are taken from both the left and right sinus. If the IPS/P ratio was calculated using the ACTH level in the sinus with the lower ACTH concentration, the sensitivity of the post stimulation ratio reduces to 86%.

This test is not good at lateralising the side of pituitary in which the tumour may be sited. In patients with Cushing's disease, a difference of > 1.5 fold between the two sinuses correctly predicted the location of the micro-adenoma in only 68% of the 104 patients during basal sampling and in 71% of 105 patients after CRH administration. The positioning of the cannula is important; if actually in the jugular then the test is not good at lateralising the pituitary tumour. The prolactin may be a useful guide as to how far from the pituitary the cannula is sited.

7. Peripheral venous sampling for sources of Ectopic ACTH

At present this test is performed at MRI but is measured here for completion

Indications: To localize ectopic ACTH production. Should be performed after a fine cut CT thorax

Preparation: As for IPS sampling

Protocol: 14-16g catheter inserted peripherally. Sampling sites include:
Adrenal veins,
Hepatic veins
High IVC
Azygos vein and hemiazygous vein
Right atrium
Right and left innominate and thymic vein
Bilateral jugular, superior and middle thyroid vein

Interpretation: No gold standard ratios have been set. A sharp increase across a certain vein may be useful, but even in the Barts series, this technique found such an increase in only 6/16 patients and in only 4 of these, was there any evidence for a tumour. CT and tumour markers were more successful.

Hypercortisolaemia protocol sheet.

Day 1		Admit, CT Adrenal, do basal pituitary function tests if not already performed (Testosterone, LH and FSH, TSH and T4, Prolactin, GH, IGF-1)
Day 2	8:00	Void bladder into the toilet. Start 24hr UFC at 9am.
	16:00	Blood for Cortisol and ACTH (special bottles from lab on ice sent immediately to lab)
Day	8:00	Send UFC to laboratory
	8:30	blood for Cortisol and ACTH
	9.00	Start low dose of dexamethasone suppression test (0.5 mg dexamethasone six hourly for 2 days).
Day 4	9.00 am	Continue to give LOW dose dexamethasone (0.5 mg six hourly)
Day 5	8.30 am	Blood for Cortisol only
	9.00 am	Start high dose Dexamethosone suppression test (2mg dexamethasone six hourly for 2 days
Day 6	9.00 am	Continue to give HIGH dose dexamethasone (2 mg six hourly)
Day 7	8.30 am	Blood for Cortisol only The investigative protocol is now ENDED

At the end of this admission the patient should be sent home to allow time for the results to be returned from the laboratory. The biochemist in charge of the endocrine laboratory will ONLY arrange for the ACTH levels to be measured if the cortisol level does not suppress in response to low dose dexamethasone.

Hb	PCV	WCC	
Na	K	Creat	Glucose
LH	FSH	Testo	DHEAS
PRL	T4	TSH	

		RESULTS		
	Date and time	UFC (nmol/24 hr)	Plasma cortisol (nmol)	ACTH (IU/l)
24 hr UFC				
24 hr UFC				
Diurnal rhythm	24.00			
	09.00			
Low dose dex suppression				
High dose dex suppression				

TESTS FOR HYPERPROLACTINAEMIA.

Include basal prolactin level and test to exclude secondary causes of hyperprolactinaemia.

Cannulated prolactin levels

Preparation: None required but ensure that secondary causes of hyperprolactinaemia have been excluded, especially hypothyroidism and drugs

Protocol: As prolactin increases with stress, patient should be cannulated and then allowed to rest for 30mins prior to sampling for prolactin levels. Prolactin is secreted in a pulsatile manner, so three samples taken 30 mins apart may be required to confirm the diagnosis of pathological hyperprolactinaemia. The laboratory should be asked to exclude macroprolactinaemia.

Interpretation:

Prolactin level (mU/l)	**Cause**
<1000	Non-tumourous aetiology Pseudoprolactinaemia macroprolactinaemia
1000-4000	Microprolactinoma Pseudoprolactinoma Non-tumourous aetiology macroprolactinaemia
4000-8000	Microprolactinoma Macroprolactinoma Pseudoprolactinaemia
8000	Macroprolactinoma

TESTS FOR MULTIPLE ENDOCRINE NEOPLASIA (MEN)

Purpose of test: To detect subclinical cases of MEN in relatives of sufferers

Rationale: Syndromes of MEN show a strong familial tendency and screening is worthwhile in ALL first-degree relatives. Consider referral to Dr. Fiona Lalloo, clinical Geneticist at St. Mary's Hospital
There are 2 main types outlined below

MEN 1 combinations of pituitary, pancreatic and parathyroid tumours
screening tests
- fasting glucose
- serum calcium
- gut hormone screen

these should be performed in **ALL** asymptomatic first degree relatives every 3 years up to the age of 40

MEN 11a combination of medullary thyroid carcinoma and phaeochromocytoma (occasionally parathyroid adenoma also)
screening tests
- blood pressure
- serum calcium
- 24h urine catecholamines
- plasma calcitonin
- plasma CEA

these should be performed in **ALL** asymptomatic first-degree relatives every year up to the age of 65. Genetic screening is available

MEN 11B MEN 11b is similar to 11a but is associated with mucosal neuromatoma, marfanoid habitus and proximal myopathy. Screening tests are as for MEN 11a

FMTC familial medullary cell thyroid carcinoma

GENETIC SCREEN 80% of MENIIa cases and 50% of FMTC cases arise as a result of mutations altering codon 634 in exon 11 of the RET oncogene. A further 17% of MENIIa cases carry mutations, which alter one of the four conserved Cys codons in exon 10.

93% MENIIb and 38% of sporadic MTC tumours are due to a point mutation altering codon 918 in exon 16 of the RET oncogene on chromosome 10q11.2

genetic screening can be preformed using one of the following
1. 3ml of blood in EDTA
2. mouthwash sample
3. A few shavings of tissue which has been fixed in paraffin

SECTION 4
INVESTIGATION OF THYROID DISEASE

TESTS FOR THYROTOXICOSIS

Tests include: Basal blood test
Imaging of the thyroid
FNA

1. Basal blood test.

Include FBC, ESR, TSH, fT4 (fT3 when available), Thyroid autoantibodies.

The third generation TSH assay precludes the need for a TRH test. A undetectable TSH is suggestive but not diagnostic of hyperthyroidism, as a low concentration may also be found in the elderly, those on glucocorticoids, beta-blockers, in depression, non-thyroidal illness, nodular goitre or in those receiving thyroxine or amiodarone. However, a detectable level of TSH effectively excludes hyperthyroidism except in the rare case of thyroid hormone resistance.

Serum T3 and T4 concentrations are elevated in 98% of cases of hyperthyroidism. In T3 thyrotoxicosis the T4 is normal but the T3 is raised. This form of hyperthyroidism is most commonly found in those with nodular goitres, in those who have relapsed following surgery and in areas of relative iodine deficiency. Thus if a free T4 is normal and the TSH suppressed ask for a free T3.

Autoantibodies to peroxidase (T microsomal) are present in 90% with autoimmune thyrotoxicosis and 10% of normals. Very high T microsomal and thyroglobulin antibodies suggest Hashimotos thyrotoxicosis.

The ESR and wbc count may be raised in acute thyroiditis or in non-thyroidal illness.

2. Imaging.

A radioiodine uptake scan (I^{131} or technetium) is not required if the diagnosis is plainly Graves or radioactive iodine is planned whatever the cause. One wants to avoid a year of thionamide treatment if the diagnosis is a multinodular goitre or single toxic adenoma.

Uptake is reduced in postpartum thyroiditis, de Quervains thyroiditis, thyrotoxicosis factitia and iodine induced thyrotoxicosis.

Ultrasound of the thyroid is generally to investigate neck masses and goitres, not Thyrotoxicosis, although an experienced ultrasonographer using Doppler may detect a hypervascular gland, suggestive of Graves' disease

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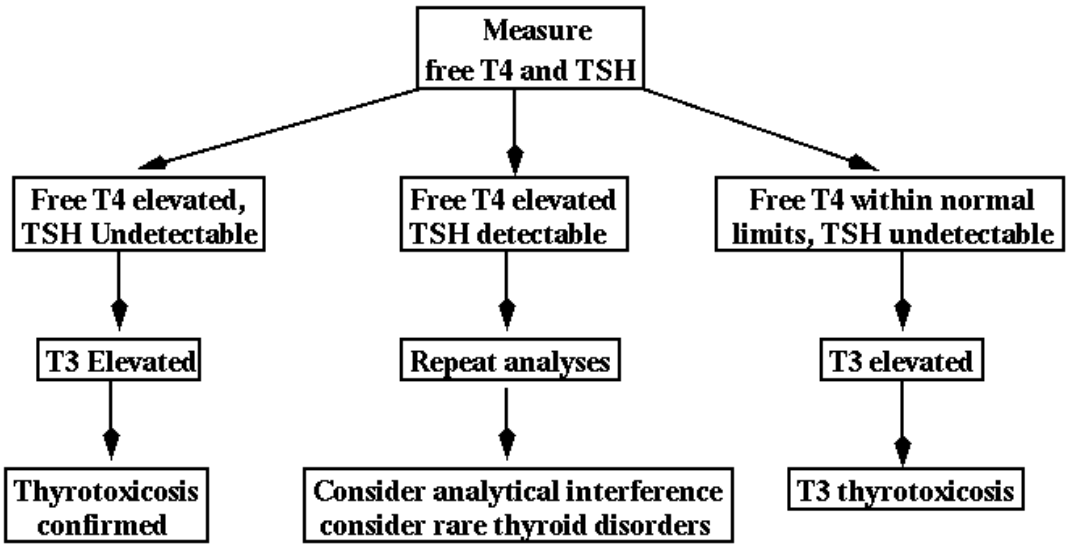

Note: non-thyroidal illness can cause a raise fT4 and detectable TSH, so repeat when well. Heparin can also increase fT4 levels.

TESTS FOR HYPOTHYROIDISM

1. Basal blood tests

In routine clinical practice most centres use an elevated TSH concentration as the single biochemical test in the diagnosis of hypothyroidism. This may be supported by a low T4 with a low or normal T3 concentration.

Subclinical hypothyroidism is defined as an elevated TSH, with a normal T3 level in the absence of clinical features of hypothyroidism. The conversion to frank hypothyroidism is 3% pa in thyroid autoantibody negative patients and 6% in those with positive antibodies.

Amiodarone induced hypothyroidism is noted by a persistently elevated TSH and a low fT4 levels. FT3 levels will be reduced in all patients on Amiodarone due to the reduced conversion of T4 to T3 and in itself does not suggest hypothyroidism.

2. Associated biochemical features.

Other biochemical features may include hypercholesterolaemia, raised serum CK, hypergammaglobulinaemia, anaemia and raised ESR.

3. Screen for associated conditions.

Primary autoimmune hypothyroidism may be associated with Addison's disease, Type 1 DM, hypoparathyroidism, pernicious anaemia, vitiligo, alopecia areata, primary Gonadal failure, Sjogrens syndrome, SLE, coeliac disease, chronic active hepatitis, and rheumatoid arthritis.

TESTS FOR GOITRE.

Thyroid dysfunction is excluded by checking TFTs. In those with a multinodular goitre the TSH may be low with a normal ft3 and fT4 concentration. Autoantibodies may be elevated in those with autoimmune disease but are of little value in the euthyroid goitre. Serum thyroglobulin is a marker for well-differentiated thyroid tumours but is only of use in the monitoring of disease in the presence of a suppressed TSH and not in the diagnosis. Serum calcitonin is a marker for medullary cell carcinoma of the thyroid but is of no proven benefit in screening of goitres unless there is a specific clinical indication, such a familial disease.

TESTS FOR THYROIDITIS.

As thyroiditis may go through phases of both hyperthyroidism and hypothyroidism, TFTs need to be repeated at intervals. A raised ESR suggests subacute thyroiditis and microbiological screens for enterococcus, coxackie, mumps, measles and EBV may confirm the cause, although this is of little clinical use.

SECTION 5
ADRENAL DISEASE

TESTS FOR CUSHINGS SYNDROME

As for hypercortisolaemia

TESTS FOR ADDISONS DISEASE

As for hypocortisolaemia

TESTS FOR CONNS SYNDROME

Indicated in those with:

Resistant hypertension
Blood pressure not controlled on 3 or more drugs
Those with persistent hypokalaemia
Patients below 40 years plus any of the above

1. Measurement of lying and standing renin and aldosterone

Purpose of test: investigation of suspected primary hyperaldosteronism; to distinguish patients with aldosterone secreting adenomas (Conn's Syndrome) from those with bilateral adrenal hyperplasia, low renin hypertension or essential hypertension

Rationale: primary hyperaldosteronism is suggested by elevated levels of aldosterone and suppressed renin, together with loss of the normal response to standing. Some patients with an adenoma show a fall in plasma aldosterone after 4h ambulation or a normal/marginally high aldosterone level, which is inappropriate for the accompanying renin level, may be considered for the 3day aldosterone suppression test (see below)

Patient preparation: **Stop the following drugs prior to test:**
Spironolactone and Oestrogen 6weeks, diuretics 4wks, ACEI 2 wks, NSAIDs 2wks, calcium channel blockers, betablockers and sympathomimetics 1wk.Can use alpha-blockers. Restore plasma K^+ to normal and stop these 24h prior to the test. Eat 50 - 100 mmol K^+ /d and 100 mmol Na^+ /d prior to study.

Protocol:
0800 fasting renin and aldosterone taken whilst lying down
0800 - 0830 patient to walk around the ward
0830 take blood for ambulant plasma renin, give breakfast
1200 take blood for ambulant plasma aldosterone, give lunch

Interpretation: Aldosterone/renin ratio: >2000 almost certainly Conns, >1000 possibly Conns, <800 excludes Conns. Plasma renin activity of <0.5pmol/ml/h, aldosterone>250pmol/l (normal or high) is suggestive of Conns.

2. Aldosterone suppression test

Purpose of study: to distinguish patients with primary hyperaldosteronism from those with essential hypertension when the first test gives equivocal results

Rationale: in patients with essential hypertension maximal extracellular fluid expansion suppresses aldosterone concentration

Patient preparation: as for measurement of renin and aldosterone

Protocol: Admit to PIU, follow a normal diet. Give 17 slow sodium tablets (175 mmol) and 0.5mg fludrocortisone orally once a day for 2 days. On day 3, commence 24h urine collection at 0800h give diet, sodium and fludrocortisone as Days 1 and 2

Day 4 measure plasma renin , aldosterone & U & E's, urine electrolytes, volume & aldosterone as described earlier

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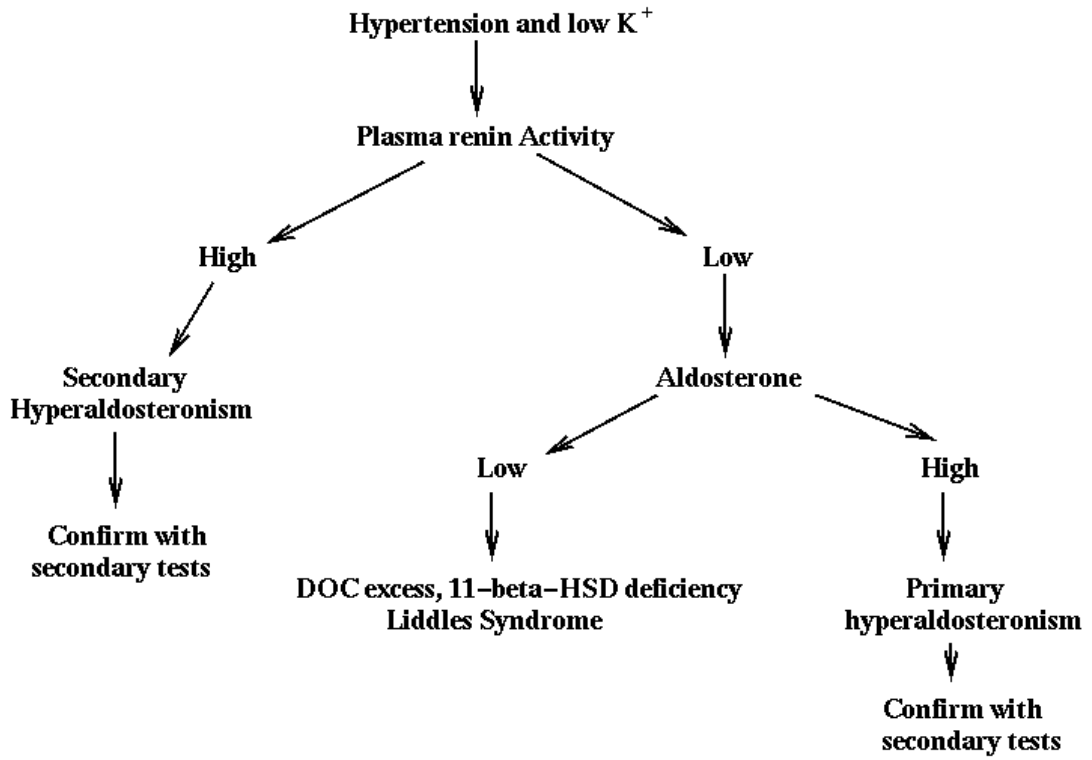


TESTS FOR PHAEOCHROMOCYTOMA

1. 24hour urinary catecholamines and plasma catecholamines

A 24hour collection of acidified urine (special bottle required from laboratory) for the measurement of catecholamines is a 95% sensitive test, but may need to be repeated, as secretion can be variable.

Plasma catecholamines concentrations are rarely used, as measurements are fraught with difficulties. Discussion with the laboratory prior to sampling is necessary. For the same reasons, interpretation of the following two test may be difficult. Where necessary, plasma catecholamines may be assessed by taking a cannulated heparinised blood sample in the supine position after 30mins rest. Plasma noradrenaline levels >10nmol/l (NR=1.0-3.07nmol/l), and adrenaline levels >1.5nmol/l (NR0.05-1.07nmol/l) give a specificity of 95% and a sensitivity of 85%.

2.Clonidine suppression test.

Indication	to exclude phaeo in patients with hypertension and borderline catecholamines levels
Rationale	Clonidine acts via the alpha pre-ganglionic receptors to reduce catecholamines secretion
Preparation	stop hypotensive agents for a minimum of 48hrs before test Fast overnight, ensure well hydrated as hypotension may occur. For this reason the pentolinium suppression test is preferred in patients with renal failure.
Protocol	insert cannula Rest for 30 mins Monitor BP and pulse every time blood is taken Take 2 baseline plasma catecholamines samples at 5min intervals Give 0.3mg of clonidine po Take blood for catecholamines hourly for 3 hours
Interpretation	Normally catecholamines will suppress into the normal range 3 hours after clonidine. A Phaeochromocytoma is suggested by lack of suppression.

3. Pentolinium suppression test.

Indication	to exclude phaeo in patients with hypertension and borderline catecholamines levels.
Rationale	Pentolinium reduces catecholamines secretion
Preparation	stop hypotensive agents for a minimum of 48hrs before test Fast overnight, ensure well hydrated as hypotension may occur
Protocol	insert cannula, Rest for 30 mins Monitor BP and pulse every time blood is taken Take 2 baseline plasma catecholamines samples at 5min intervals Give 2.5mg iv of pentolinium Take blood for catecholamines 5 and 10 minutes after injection
Interpretation	Normally catecholamines will suppress into the normal range 3 hours after Pentolinium. A Phaeochromocytoma is suggested by lack of suppression.

4. Imaging.

CT	Resolution of most CTs is ~5mm, so some small tumours may be missed. Avoid intravenous contrast media as they may precipitate a crisis.
MRI	Phaeos are bright on T2 weighted MRI images
MIBG	meta-iodobenzylguanidine is taken up by chromaffin cells and is useful in the localisation of extra-adrenal Phaeos. 10% false negative rate and 1-3% false positive rate. Specificity is 100% in malignant and familial tumours. MIBG given iv and patient is scanned at 24hours (to detect normal physiological uptake) and 72hours (to detect tumour uptake) after injection. Drugs interefering with uptake include tricyclic antidepressants, labetalol, calcium channel blockers, reserpine, cocaine, phenylpropanolamine containing drugs should be stopped several weeks prior to scans
Octreotide	the use has a similar rationale as above, but the spleen may confound good adrenal imaging.

TESTS FOR CONGENITAL ADRENAL HYPERPLASIA

A short synacthen test with 17 OH-progesterone levels should be performed as described earlier and is used as a diagnostic test.

TESTS FOR HYPERANDROGENISM

See section on hirsuitism in reproduction

SECTION 6
Ix FOR NEUROENDOCRINE TUMOURS.

TESTS FOR INSULINOMA

Include: fasting glucose measurements
72 hour fast
C-peptide suppression test

All are described in the section on hypoglycaemia.

TESTS FOR CARCINOID SYNDROME.

1. 24 hour urine collection for 5HIAA

Rationale: Carcinoid syndrome is a clinical syndrome resulting from excessive secretion of serotonin. Those arising from the foregut may also secrete ACTH, gastrin, histamine and calcitonin. Hindgut carcinoids never secrete serotonin, but may produce somatostatin and peptide YY. Serotonin is metabolised to 5-hydroxyindolacetic acid, which is excreted in the urine

Patient preparation: Ensure patient is not taking SSRI antidepressants.

Protocol: A 24-hour collection of urine for 5HIAA measurement must be performed 3 times before Carcinoid syndrome can be excluded, as secretion may be intermittent.

Interpretation: 5-HIAA concentrations will be markedly increased in carcinoid syndrome.

TESTS FOR GASTRINOMAS.

Include: Plasma gastrin level
Basal acid secretion
Secretin test.

1. Plasma Gastrin levels.

Rationale: Gastrinomas are gut tumours secreting excessive amounts of gastrin, resulting in acid production and ulceration. Gastrin levels can be measured.

Patient preparation: PPIs must be stopped for 3 weeks prior to test, and H_2-blockers must be stopped at least 3 days prior to the test.

Protocol: fasting plasma gastrin levels, after liaison with the laboratory (a special tube is required). Exlude hypercalcaemia prior to test as this will stimulate gastrin secretion. A separate blood sample for calcium and urea should be collected at the same time.

Interpretation: A raised gastrin level is suggestive of gastrinoma.

2. Basal acid secretion output.

Rationale: A high basal acid production usually would suppress gastrin levels; this is not the case in gastrinomas where high basal acid secretion is a result of gastrin excess.

Patient preparation: PPIs must be stopped for 3 weeks prior to test, and H_2-blockers must be stopped at least 3 days prior to the test.

Interpretation: Basal acid production is usually in excess of 15 mmol/h in gastinomas and can be greater than 5mmol/h in patients with gastrinomas who have had previous acid reducing surgery.

3. Secretin test.

Rationale: Secretin suppresses gastrin levels in normals. However, a paradoxical rise is seen in those with a gastrinoma. This test is reserved for borderline cases or those in whom it is difficult to wean medication

Patient preparation: PPIs preferably stopped for 3 weeks prior to test, and H_2-blockers preferably stopped at least 3 days prior to the test.

Protocol: Warn lab in advance.
Patient fasted overnight, IV cannula inserted.
Blood (10mls in lithium heparin tubes to which 200ul of trasylol has been added) taken for gastrin level, put on ice and immediately taken to laboratory.
2 U/g Secretin IV given as bolus injection.
Blood taken as above immediately following injection, then 2, 10, 15, 20 and 30 mins after injection.
Blood samples must be spun within 15 minutes of taking from patient thus lab needs to be prepared and a 'runner' available.

Interpretation: a rise of gastrin from baseline by 100 pmol/l gives a sensitivity of 85% when performed in patients with a fasting gastrin level of < 400pmol/l. A rise of 50% greater than baseline gives a sensitivity of 78%.

TESTS FOR VIPomas, Somatostatinomas, GAWK, chromogranin A.

A fasting level of VIP, somatostatin, GAWK and chromogranin A may indicate the diagnosis. As secretion may be intermittent, tests should be performed 3 times at least.

Samples must be as follows:
10mls from overnight fasted patient collected into lithium heparin tubes to which 200ul of Trasylol has been added, taken on ice, sent to lab immediately and spun within 15 minutes.

SECTION 7–
ASSESSMENT OF ENDOCRINE REPLACEMENT

Tests of cortisol replacement.

Hydrocortisone/Cortisol day curves

Purpose of test: To assess plasma cortisol levels in patients on hydrocortisone therapy or to assess cortisol reserve when the IST is contraindicated or to assess adequacy of treatment

Patient preparation Rest for 20mins post-insertion of cannula, prior to commencing test.
Patient advised not to take Hydrocortisone prior to the test, but to take immediately after first blood test and at the patient's normal times thereafter.

Protocol IV cannula inserted and flushed regularly
Cortisol samples in Lithium heparin tubes collected at 9am, 11am, 1pm and 3pm. Put times of doses on request form. All samples should be retained until collections are completed, when they should be sent to the laboratory as one request.

Interpretation Adequate replacement suggested by basal cortisol level of less than 200nmol/l, never exceeding 500nmol/l throughout the test.

TESTS OF TESTOSTERONE REPLACEMENT

Testosterone profile.

Purpose of test: to evaluate plasma testosterone concentrations during im testosterone replacement (not for oral replacement)

Rationale: under-replacement results in inadequate symptomatic improvement and risk of osteoporosis in the long term

Patient preparation: none

Protocol:

For men already having testosterone by injection

Patient first seen on the day before he is due his next testosterone injection. A sample for testosterone is taken, labelled as baseline and sent to the laboratory.

The patient receives his testosterone as usual the following day

Testosterone levels are to be rechecked on days 3, 7, 14 etc after injection until 1 week before the next due injection (label the tubes and forms 'day 3', 'day 7' etc

For men who are about to start treatment

check baseline testosterone

Give 100 mg sustanon IM

check testosterone levels on Days 3, 7, 14 and 21

label forms and bottles as above

Interpretation: dosage and/or interval of injections can be adjusted according to the plasma levels and the test can be repeated on the changed regimen if necessary

NB A prostate specific antigen (PSA) followed by a rectal examination should be performed in all patients starting treatment and repeated approximately annually. Patients complaining of urinary symptoms while on testosterone replacement should be investigated appropriately without delay.

TESTS FOR LH/FSH REPLACEMENT.

In females, adequate therapy can be assessed by the presence of a regular bleed, the occurrence of ovulation (confirmed by an elevated day 21 progesterone), normal FSH and oestradiol levels and the maintainence of normal bone mineral density.

In males, adequacy of replacement may be assessed by normal testosterone and LH concentrations, the production of sperm, maintenance of secondary sex characteristics and of normal bone mineral density.

TESTS FOR GH REPLACEMENT.

Adequacy of replacement is determined by maintainence of the IGF-1 concentration within the upper quartile of the age and sex matched normal range, together with clinical observation and the maintenance of normal bone mineral density.

SECTION 8
ASSESSING CALCIUM & BONE METABOLISM

TESTS FOR HYPERCALCAEMIA.

Tests include: plasma calcium, PTH, Po4, ALP, creatinine, TFTs and 24hr urinary calcium. Plasma ACE may be considered at a later date

The calcium clearance to creatinine clearance should be calculated to exclude familial benign hypercalcaemia (FHH), as this ratio should be >0.01. It can be calculated as:

$$\frac{\text{Urine Ca (mmol/l) x [plasma creatinine (}u\text{mol/l)/1000]}}{\text{Plasma calcium (mmol/l) x urine creatinine (mmol/l)}}$$

Interpretation:

Cause of hypercalcaemia	serum						Urine			Others
	PO4	ALP	Cl	Bic	PTH	Vit D	Ca	PO4	cAMP	
hyperPTH	↓	→	↑	↓	↑→	↓→	↑	↑	↑	
Vit D toxicity					↑					
Sarcoidosis					↓	↑1,25				↑ ACE
FHH					→		↓			
thyrotoxicosis					↓					↓TSH
Milk-alkali					↓					
Malignancy					↓					↑PTHrP

TESTS OF HYPOCALCAEMIA

1. Basal Blood Tests

These include plasma calcium, PTH, Po4, ALP, vit D, creatinine as first line tests. Rarely Mg and cAMP may be helpful. The table below may be useful

Cause of hypocalcaemia	serum				Urine			Other
	PO4	ALP	PTH	Vit D	Ca	PO4	cAMP	
Vit D deficiency	↓	↑	↑	↓	↓	↓		
Renal failure	↑	↑	↑	↓	↓			
Hypoparathyroid	↑	→	↓					
PseudohypoPTH	↑	→	↑				↓	
Hypomagnesaemia	↑	→	↓					↓Mg

2. Parathyroid hormone infusion test

Purpose of test: to distinguish hypoparathyroidism from pseudo-hypoparathyroidism in patients with unexplained hypocalcaemia

Rationale: patients with hypoparathyroidism respond to exogenous PTH by increasing plasma and urinary cAMP levels and increasing urinary phosphate excretion

Patient preparation: fast from midnight
maintain diuresis (give 150 ml water every 30 min from 0700 - 1100h)

Protocol: 0800h patient empties bladder, insert IV cannula
1/2 hourly urine collections for cAMP, creatinine and phosphate 0800-0830h, 0830-0900h etc until 1200h
1/2 hourly bloods at 0815h, 0845h, 0915h etc until 1200h for cAMP, creatinine and phosphate

0900 - 0930h give 200 units of PTH in 50 ml 0.9% saline containing 2.5 ml human serum albumin

TESTS FOR RENAL STONES

Test include:

Stone analysis
Fresh MSU for culture, urinalysis and cystine
Blood (Calcium, phosphorus, Na, K, Cl, HCO3, urate, creatinine)
24 hr urine (volume, pH, Mg, Ca, PO4, creat, oxalate, urate)
confirmation of abnormalities found above
PTH with calcium if calcium elevated
urine for cyclic AMP.

OSTEOPOROSIS

1. Basal blood tests.

To exclude secondary causes of osteoporosis that can be corrected or treated.

Serum bone profile, liver function tests, creatinine and electrolytes.
Serum PTH
Serum TSH
Serum testosterone (in males)
Serum total proteins, electrophoresis and ESR
Full blood count
Myeloma screen Ig electrophoresis

2.Imaging

In patients suspected of vertebral deformities or fractures a lateral thoraco-lumbar spine X-ray is indicated. All patients need a lumbar spine and femoral neck Bone Mineral Density measurement Results are expressed as the numbers of standard deviations from the Young Adult (Z score)

0 to -1	Normal
-1 to -2.5	Low bone mass, osteopenia
-2.5 or below	Osteoporosis

PAGET'S DISEASE OF BONE

1. Blood and urine tests

Serum creatinine and electrolytes
bone profile
liver function tests
prostatic specific antigen

2. Imaging.

X-ray of appropriate part of skeleton
Bone Scintigraphy - using 99m Tc-bisphosphonate

3. Others.

Audiometry, if disease involves the skull

SECTION 9
INVESTIGATION OF HYPERTENSION

INDICATIONS FOR INVESTIGATING HYPERTENSION

Clinical features of an underlying cause
Onset before age 40
Rapid progression
Proteinuria, haematuria, glycosuria
Severe hypertension; difficult to control
Vascular disease - peripheral, carotid, coronary
Heart failure

1. Blood tests

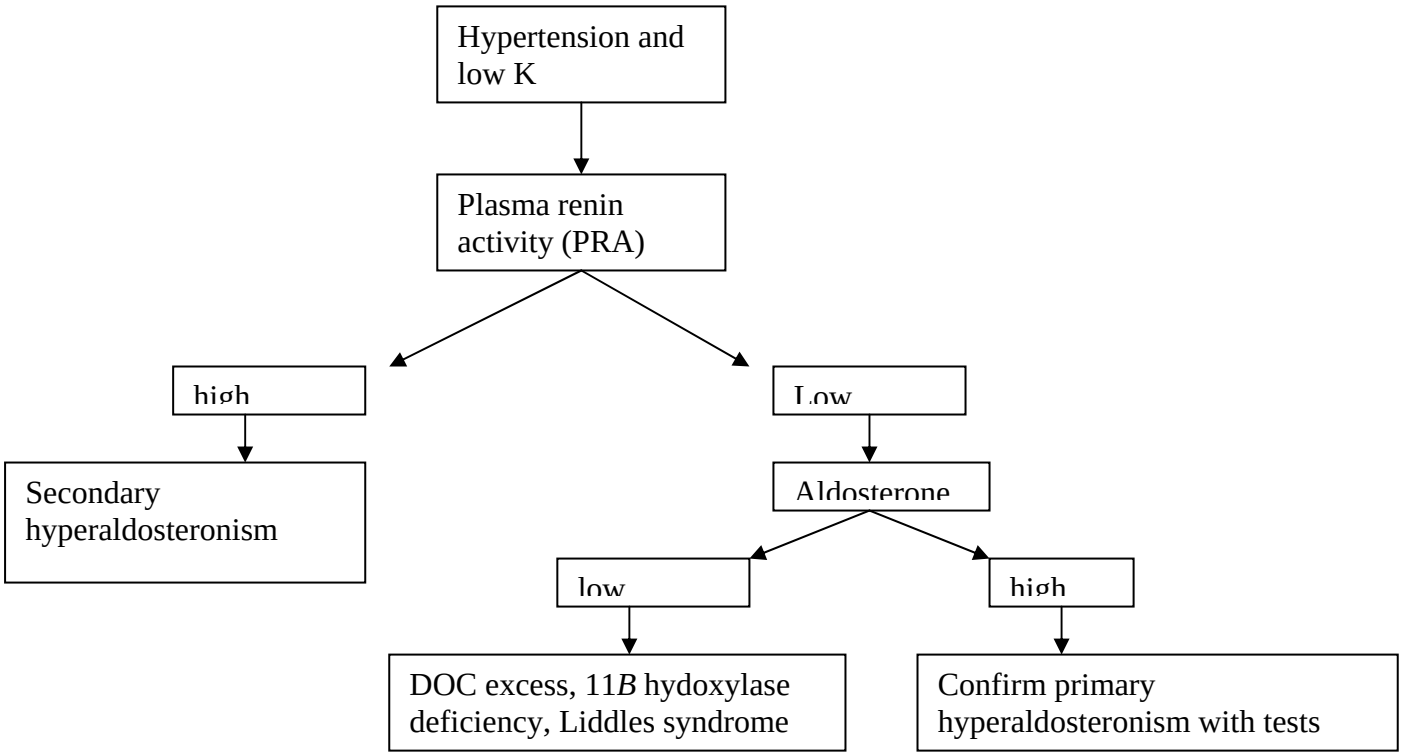
U&Es
FBC
ESR
Glucose
LFTS
Lipids
Renin, Aldosterone if hypokalaemic (see below)

2. Urine tests

Stix' tests: blood, protein, sugar → microscopy

3. Cardiac investigations

ECG
Echo

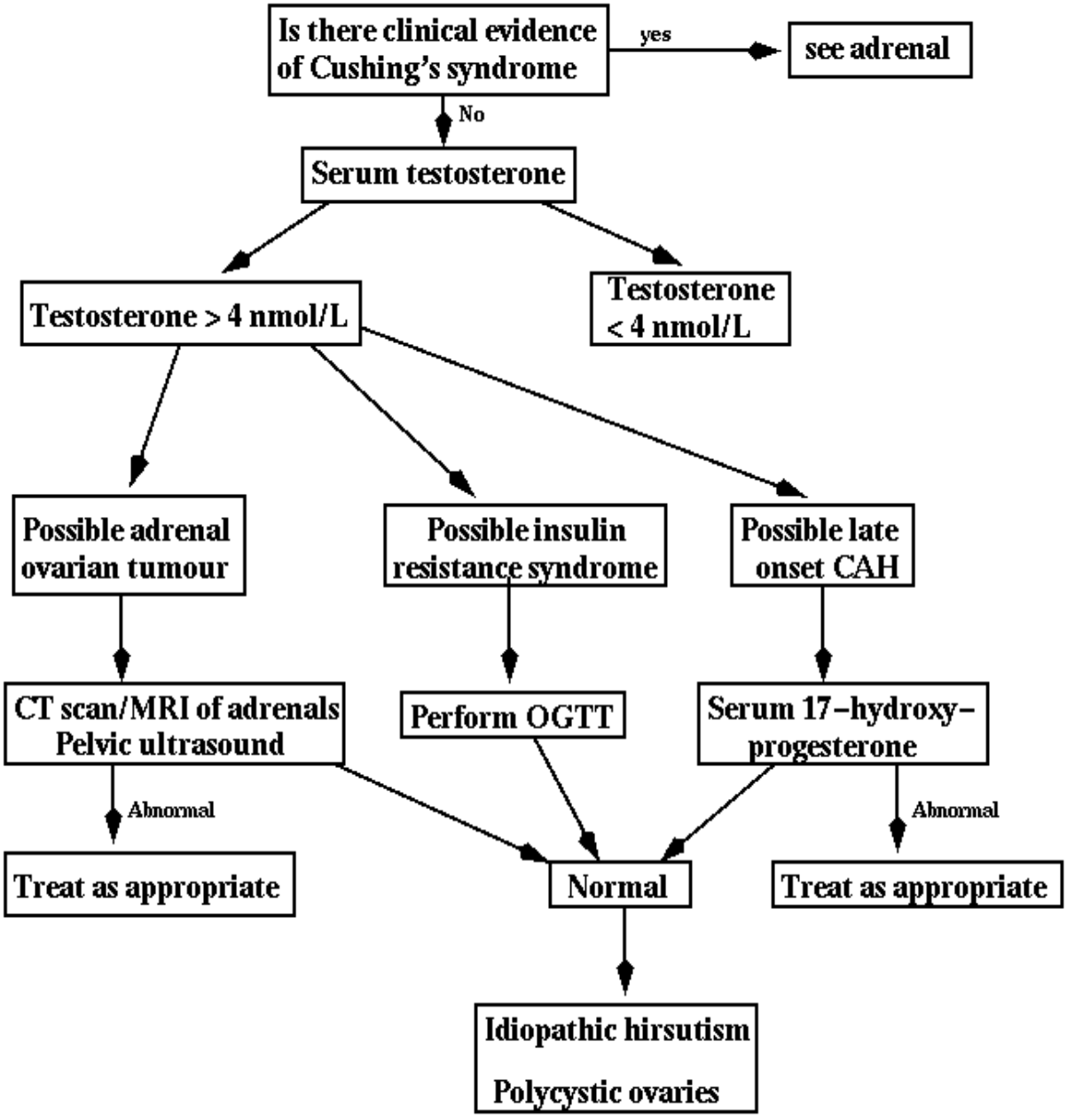


SECTION 10
INVESTIGATION OF REPRODUCTION

HIRSUTISM

Tests

Clinical case	Appropriate test
Longstanding hirsuitism with regular cycles	Testosterone, ovarian U/S
Short history with cycle disturbance	As above, plus LH, FSH, ?UFC, progesterone challenge
Testosterone>4	DHEA-S, test for CAH



TEST FOR INTERSEX

Initial baseline tests
Peripheral karyotype
Plasma 17OH-progesterone
Plasma testosterone

Karyoype 46XX
Consider congenital adrenal hyperplasia and perform the test described earlier for this condition:

Karyotype 46XY
Consider deficient androgen production or abnormal androgen action.
hCG stimulation test. Samples pre and post for plasma 17OH-progesterone, androstenedione and testosterone

Tests for precocious or delayed puberty

Initial baseline tests

1. Basal LH, FSH, testosterone/oestradiol
2. GnRH stimulation test
3. Free T4/TSH
4. Prolactin
5. hCG

Test for Delayed Puberty

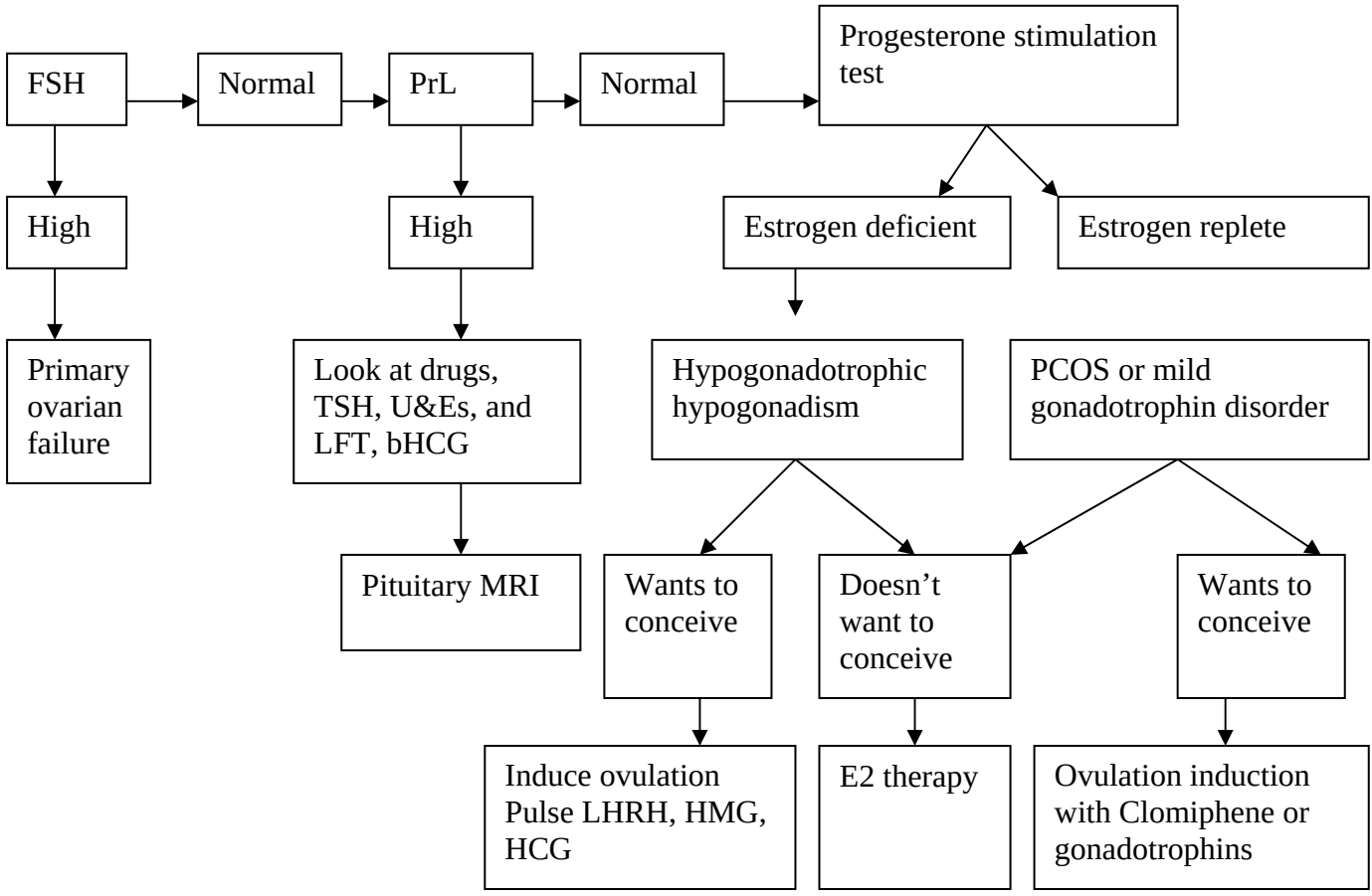
1. Karyotype
2. Free T4/TSH
3. Basal LH, FSH, testosterone/oestradiol
4. GnRH stimulation test
5. Full blood count
6. Urea and electrolytes
7. Investigation for short stature as indicated

Investigation of Amenorrhoea

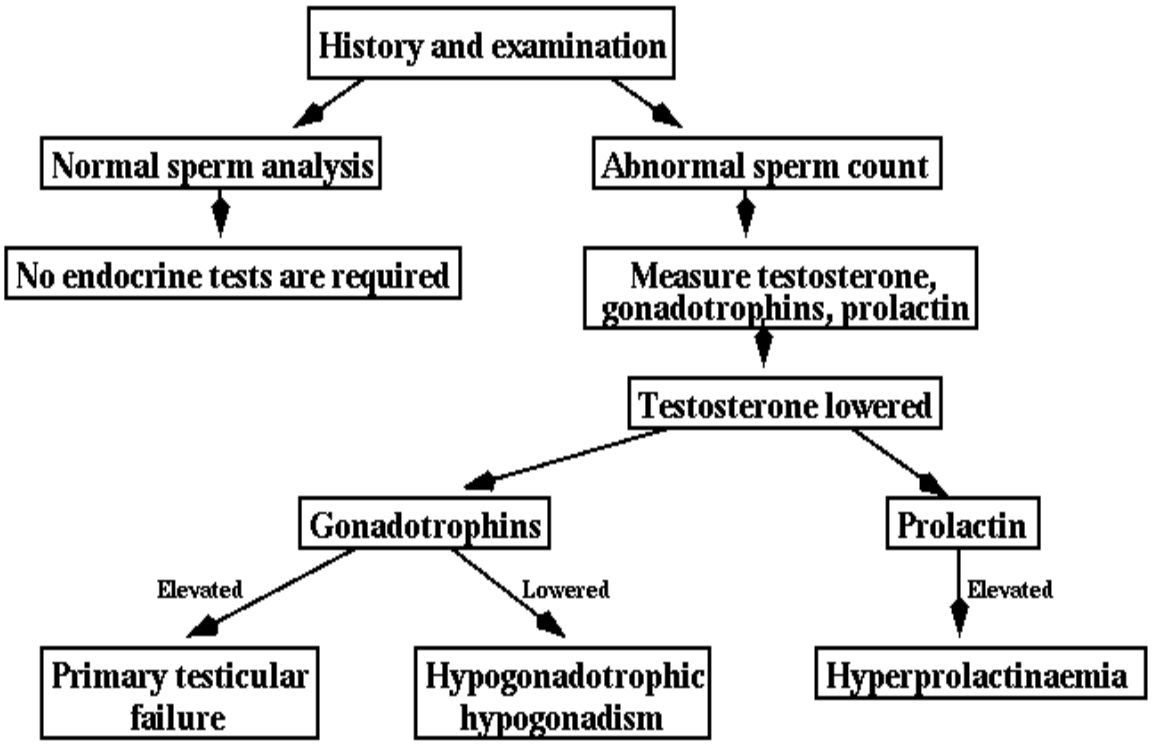
Primary Amenorrhoea
1. Pregnancy test
2. Chromosomes
3. LH/FSH
4. Prolactin
5. Free T4/TSH
6. Androgens
7. Others: see secondary Amenorrhoea

Secondary Amenorrhoea
1. Pregnancy test
2. FSH/LH
3. Prolactin
4. Free T4/TSH
5. Testosterone, other androgens
6. Sex hormone binding globulin
7. 17OMP

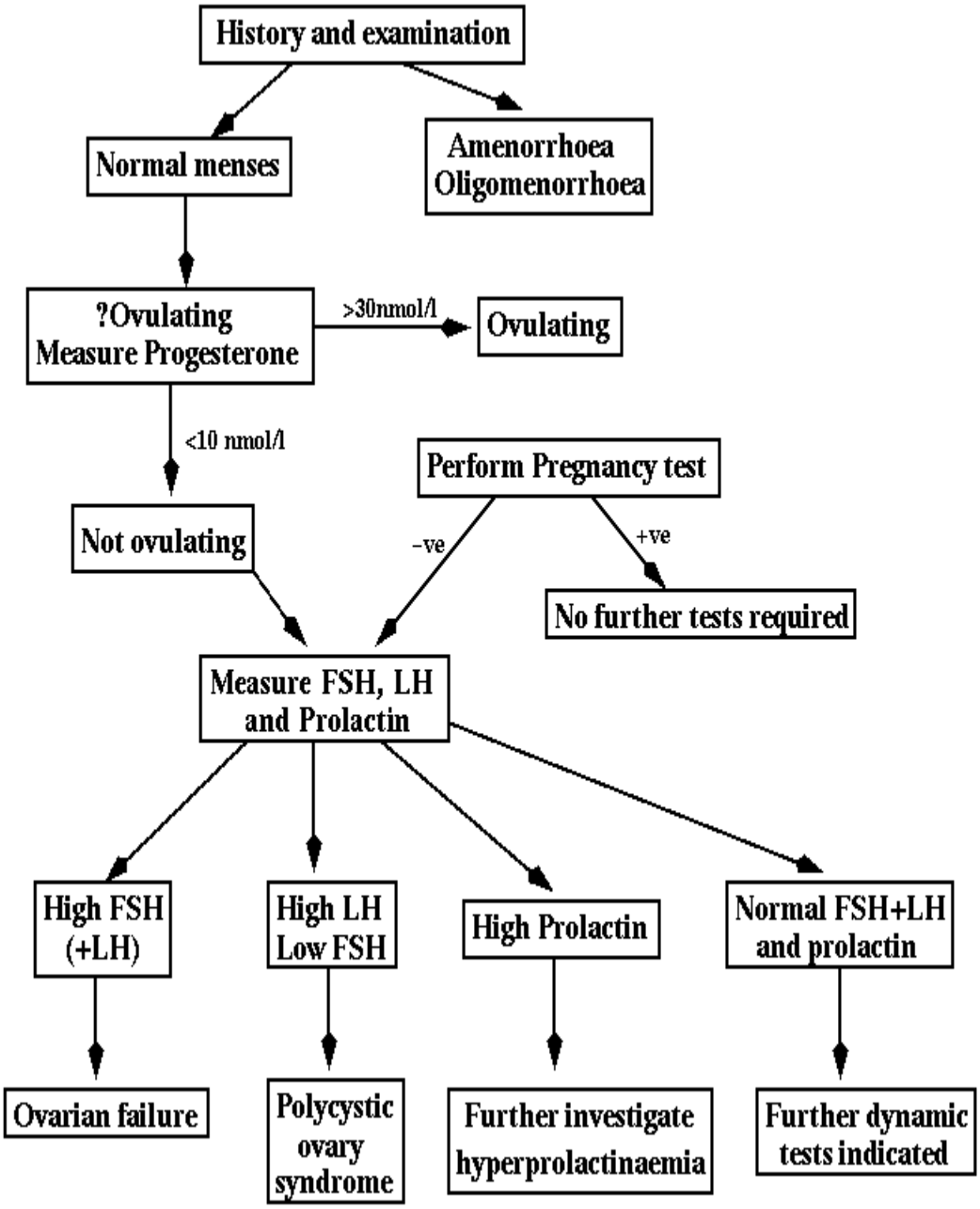
Investigations for secondary amenorrhoea



Tests for sub-fertility in the man

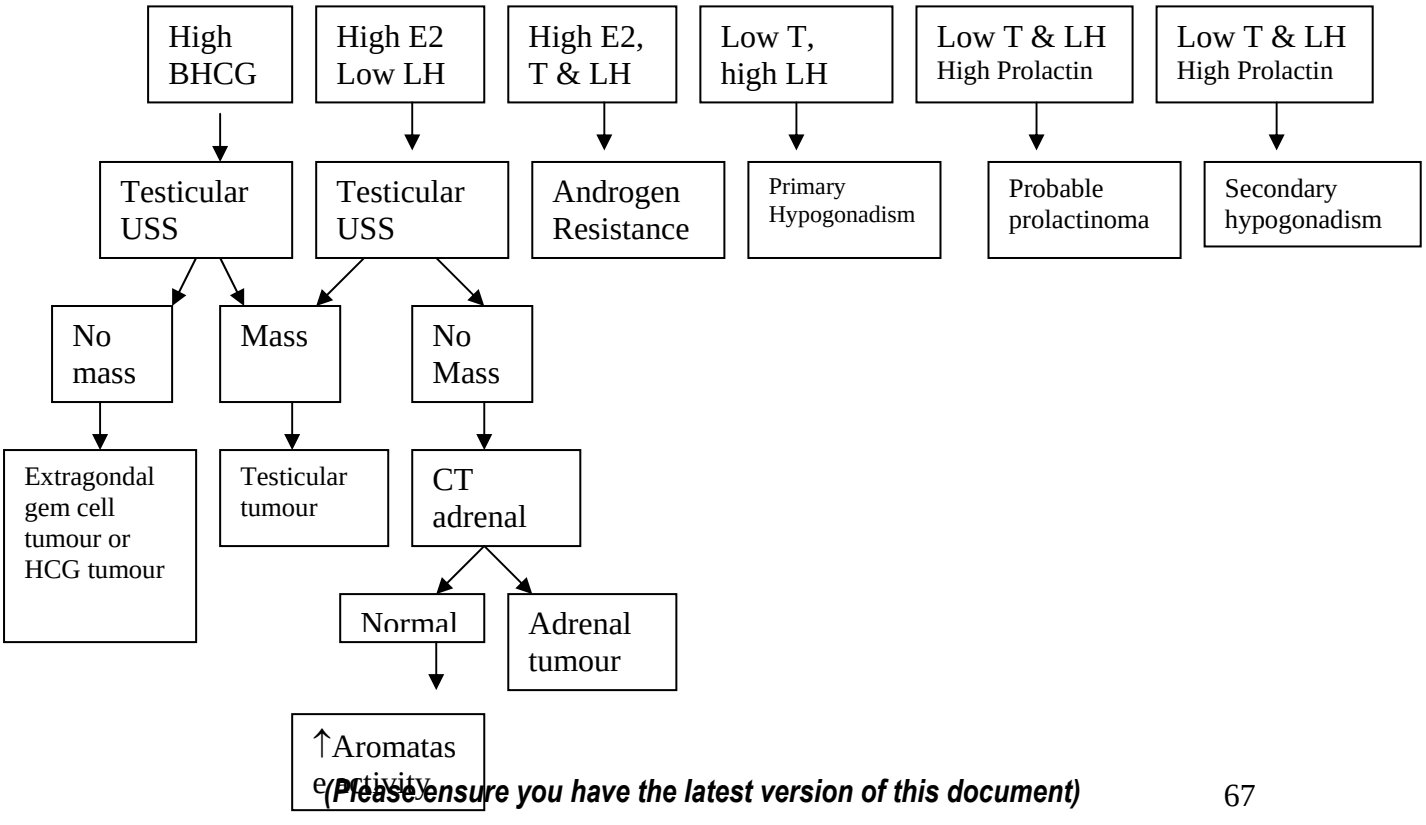


Diagnostic approach to sub-fertility in the woman



Investigation of Gynaecomastia

	Testosterone	LH	FSH	SHBG	Oestradiol (E2)	hCG	PrL
Testicular Failure (Primary)	↓	↑	↑			N	
Testicular Failure (secondary)	↓	↓	↓		↑	N	
Adrenal Tumour		↓			↑		
Inc.Aromatase activity		↓			↑		
Androgen Resistance	↑	↑	↑		↑		
Primary oestrogen-secreting tumour	↓	↓			↑	N	
hCG-secreting tumour		↓			↑	↑	
Chronic liver disease	↓	↓	↓	↑		N	
Thyrotoxicosis	↑	↑	↑	↑		N	
Prolactinoma	↓	↓	↓				↑



SECTION 11
MISCELLANEOUS TESTS

TEST DOSE SHORT-ACTING SOMATOSTATIN ANALOGUE (OCTREOTIDE)

Purpose of test: to assess GH response to a single dose of short-acting somatostatin analogue in patients with acromegaly.

Rationale: Octreotide is a short-acting synthetic analogue of somatostatin, the physiological inhibitor of GH release. 70% of patients with acromegaly will have a useful suppression of GH levels with octreotide and will do so following a single dose of short-acting octreotide.

Patient preparation: A light breakfast can be taken at 6am on morning of test, but patient must then remain fasting until completion of the test.

Protocol:
0800h insert IV cannula, take blood for GH and IGF-1
Give octreotide 50 mcg sub cut
Bloods for GH at 10:00, 12:00, 14:00, 16:00
Feed patient, test completed

Interpretation: GH levels should suppress to less than 5 mU/l to suggest effective treatment.

TEST DOSE LONG-ACTING SOMATOSTATIN ANALOGUE (OCTREOTIDE LAR)

Purpose of test: to assess GH response to a single dose of a long-acting somatostatin analogue in patients with acromegaly.

Rationale: Octreotide LAR is a long-acting synthetic analogue of somatostatin, the physiological inhibitor of GH release. 70% of patients with acromegaly will have a useful suppression of GH levels with octreotide LAR and will do so following a single dose.

Patient preparation: A light breakfast can be taken at 6am on morning of test, but patient must then remain fasting until completion of the test.

Protocol: Perform test 4 weeks administration of Octreotide LAR.
0800h insert IV cannula, blood for GH and IGF-1 then hourly blood test levels for GH only for another 3 hours to determine the mean of 4 GH samples.

Interpretation: Mean GH level less than 5 mU/l suggest effective treatment.

AUTONOMIC FUNCTION TESTS

Purpose of test: assessment of autonomic function

Rationale: cardiovascular responses depend upon normally functioning autonomic nervous system

Patient preparation: none

Protocol:

heart rate response to Valsalva
ECG leads connected and machine running
Patient seated, blows into a mouthpiece connected to a sphygmomanometer at 40 mm Hg for 15 seconds
Repeat 3 times
measure ratio of longest R-R interval within 20 beats of stopping valsalva to shortest R-R interval during manoeuvre

Normal ratio > 1.2 abnormal < 1.1

Heart rate response to standing
ECG leads connected and machine running
Patient lies on couch then stands unaided
Measure ratio of longest R-R interval around 30^{th} beat after standing to shortest R-R interval around 15^{th} beat (30 - 15 ratio)

Normal ratio > 1.04 abnormal < 1.0

BP response to standing
Measure systolic BP lying down and 1 min after standing

Normal difference<10 mm Hg, abnormal > 30 mm Hg

BP response to hand grip
Measure resting BP
Use hand pump from the sphygmomanometer connected directly to the mercury column and close the valve
squeeze handpump to maintain pressure of 300 mm Hg and measure the BP every minute for at least 5 minutes
measure difference between maximum diastolic and resting diastolic BP before hand grip

Normal difference>16 mm Hg, abnormal<10 mm Hg

AUTONOMIC FUNCTION TEST RESULTS

NAME
HOSPITAL NUMBER
DATE

1. **Heart rate response to valsalva**

	Longest RR	Shortest RR	Ratio
i			
ii			
iii			

Mean ratio:
Normal > 1.21
Abnormal < 1.2

2. **Heart rate response to standing**

Longest RR (30th beat) Shortest RR (15th beat)

30:15 ratio:
Normal > 1.04
Abnormal < 1.0

3. **Blood pressure response to standing**

Lying systolic Standing systolic

Difference:
Normal < 10
Abnormal > 30

4. **Blood pressure response to handgrip**

Resting diastolic Maximum diastolic

Difference:
Normal > 16
Abnormal < 10

SECTION 12
Assessment of treatment of excess hormone production

ASSESSMENT OF SUPPRESSION OF GH

Aims: Random GH<5mU/l
Suppression of GH <2mU/l during OGTT
IGF-1 in normal age and sex matched range

1. Random GH, IGF-1 and GH profile

Random GH levels are of limited value unless they are clearly extremely low or extremely high. However, a GH series is of benefit.

Rationale: A mean GH level is determined over the course of the day

Patient Preparation: None required. Eat, drink, have medication as normal

Protocol: Admit to PIU, bed rest. Blood taken for GH and IGF-1 level at 9am and then samples for GH only taken hourly for five hours thereafter. The mean GH is calculated.

Interpretation: Mean GH<5mU/l and an IGF-1 within the age and sex matched normal range suggests cure or effective suppression

2. OGTT with GH level

The protocol described for the diagnosis of Acromegaly can also be used to assess effectiveness of treatment. A suppression of GH to <2mU/l suggests cure

ASSESSMENT OF SUPPRESSION OF PROLACTIN

A cannulated prolactin level within the normal range suggests effective treatment

ASSESSMENT OF SUPPRESSION OF CORTISOL

Suppression of cortisol can be assessed with:

24 hour urinary cortisol
cortisol day curve

1. 24 hour urinary cortisol.

Patient Preparation: None required. Eat, drink, have medication as normal

Protocol: all urine passed within a 24 hour period is collected and the cortisol concentration measured.

Interpretation: a urinary cortisol level of < 400nmol/l is suggestive of good control of cortisol production

2. Cortisol day curve.

Patient Preparation: None required. Eat, drink, have medication as normal

Protocol: Patient is admitted to PIU, a cannula is inserted and samples for the measurement of cortisol are taken at 9am, 11am, 1pm and 3 pm.

Interpretation: the suppression o f cortisol levels to below 400 nmol/l is suggestive of good control