INVESTIGATION OF CUSHING'S SYNDROME

Making the Diagnosis of Cushing's Syndrome

Recently reviewed in

Arnaldi et al. 'Diagnosis and complications of Cushing's Syndrome: a consensus statement.' JCEM 2003; 88: 5593-5602

Niemann et al ' The diagnosis of Cushing's syndrome; an Endocrine Society Clinical Practice Guideline' JCEM 2008; 93: 1526-1540

Selection of patients for investigation

Follow *screening protocol* when:

- clinical suspicion of Cushing's syndrome
- adrenal incidentaloma
- osteoporosis or hypertension out of keeping for patient's age
- (no recent glucocorticoid administration)

Proceed to *formal Cushing's protocol* when:

- out-patient screening tests suggest Cushing's syndrome or are equivocal
- clinical suspicion is high
- in most circumstances when the patient is referred from another Unit

If screening all negative and clinical suspicion still high, consider cyclical Cushing's

- repeat 24 hr urine free cortisol at weekly intervals for 3 months
- (longest recorded cycle was 83 days)
- if no cyclical hypercortisolism detected, re-assess clinically after 6 months and repeat screening tests if clinical features have progressed

Out-patient Screening Tests

Overnight dexamethasone suppression test

- 1 mg dexamethasone taken orally at 23.00
- 0900 plasma cortisol the following am
- *Cushing's excluded by plasma cortisol < 60 nmol/l*
- *do not use dexamethasone in pregnancy, in patients on OCP, or in patients using anti-epileptic or other medications that induce dexamethasone metabolism (see appendix 2)*

Only when clinical suspicion is high, proceed to:

Urinary free cortisol X 2

- 24 h collection in plain container
- *Cushing's excluded by cortisol within reference range*
- *do not rely on urinary free cortisol in renal failure*

Formal Cushing's Protocol

The flow diagram in the Appendix shows the logical sequence of investigation for Cushing's syndrome. Additions to this protocol which are not normally necessary, but which may need to be considered, include Insulin Tolerance Test and period of abstinence from alcohol.

IN CLINIC

Measure ACTH and cortisol in plasma. Two detectable ACTHs are required to confirm ACTH-dependent Cushing's and this is the first test.

Arrange:

DEXA scan

Clinical Photos

CXR

Nursing and pharmacy for protocols below

DAY 1 (Sunday)

<u>24 h urine free cortisol (baseline for the high dose dex test)</u>

DAY 2 (Monday)

<u>Low-dose dexamethasone suppression test – day 1</u>

- 0900 cortisol and ACTH (the second sample to confirm ACTH-dependent Cushing's) with Glucose, U/E, DHEAS, Androstenedione
- 0.5 mg dexamethasone at 0900, 1500, 2100, and 0300

DAY 3 (Tuesday)

<u>Low-dose dexamethasone suppression test – day 2</u>

- 0.5 mg dexamethasone at 0900, 1500, 2100, and 0300
- 24h urine free cortisol

DAY 4 (Wednesday)

- 0900 h plasma cortisol
- *Cushing's suggested if plasma cortisol on day 4 is >50 nmol/L. Supportive evidence is provided if urine cortisol on day 4 > 100 nmol/day*

<u>High-dose dexamethasone suppression test –day 1</u>

- 2 mg dexamethasone at 0900, 1500, 2100, and 0300
- 24 h urine free cortisol

DAY 5 (Thursday)

<u>High-dose dexamethasone suppression test –day 2</u>

- 2 mg dexamethasone at 0900, 1500, 2100, and 0300
- 24 h urine free cortisol

DAY 6 (Friday)

0900 plasma cortisol

- *Pituitary disease suggested with following confidence, comparing urine cortisol on days 1 and 6:*

50% suppression	*4.2:1*
80% suppression	*10.1:1*
90% suppression	*infinite*

*LATER DAY **if ACTH detectable twice***

CRH test

- Usually performed at 09.00
- 100 µg human CRH iv at time 0
- Sample for cortisol and ACTH at times -15, 0, 15, 30, 45, 60, 90, 120 min

- *Basal is mean of time -15 and 0*
- *Pituitary Cushing's suggested by peak cortisol > 120% baseline or by peak ACTH > 150% baseline*
- *NOT useful in establishing presence of Cushing's syndrome, except to exclude depression*

Further Investigation When Cushing's Syndrome is Confirmed

Probable adrenal disease

- If results suggest adrenal disease (2 undetectable ACTHs; no suppression with high-dose dexamethasone; flat CRH test) proceed to CT scan of adrenals.
- If equivocal or bilateral abnormalities on CT, consider adrenal vein sampling or fluorodeoxyglucose PET scan.

Probable Pituitary-dependent Cushing's disease

- If above results all suggest an ACTH-secreting pituitary adenoma (at least one detectable ACTH; clearcut suppression with high-dose dexamethasone; brisk cortisol response to CRH) then proceed to MRI pituitary with Gadolinium enhancement.
- If ACTH is detectable, but above results are equivocal or inconsistent in distinguishing pituitary from ectopic ACTH secretion, proceed to MRI pituitary with Gadolinium enhancement and consider inferior petrosal sinus sampling (refer to neuroradiologists in DCN, WGH).

Probable Ectopic ACTH Syndrome

- If above results suggest ectopic ACTH secretion (normal or high ACTH, no suppression with high-dose dexamethasone and flat CRH test) then further

investigation depends on the clinical presentation. Relevant tests include CXR, CT chest, USS liver, other tumour markers etc.

- Other markers may be useful in follow-up, so screening the following is justified (70% of ectopic tumours secrete more than one peptide): Chromogranin A and B; CEA; somatostatin; gastrin; calcitonin; pancreatic polypeptide; VIP; glucagon; hCG-ß; AFP; alpha-subunit; synaptophysin, AVP. Some markers are reported in the literature but more difficult to source assays and will only be assayed in exceptional cases: bombesin; GHRH; CRH. Also consider measuring ACTH pre-cursors by Anne White in Manchester.

Medical Therapy For Cushing's Syndrome

In preparation for surgery, medical therapy is usually indicated and is especially important when the patient is ill, when complications of Cushing's are difficult to control (eg diabetes, hypertension), or when there is to be a long delay till surgery.

Metyrapone 250 mg bd, increasing by 250 mg/day to maximum 6 g/day, is used as first-line. Monitor weekly 24 h urine cortisol (aim within reference range); U/E; and s/e BP. Also monitor blood glucose as required. Metyrapone therapy alone may cause hypokalaemia and paradoxical worsening of hypertension. Ketoconazole (200 - 400 mg 3 times daily, with monitoring of liver function) can be added if Metyrapone alone is insufficient or causes complications. Supplementary hydrocortisone therapy may be required if medical therapy causes too much adrenal suppression (analogous to a block and replace regimen).

Peri-Operative Management of Cushing's Disease

1. Discontinue metyrapone and ketoconazole the day before surgery

2. Prescribe peri-operative thromboprophylaxis unless specifically contra-indicated

3. Hydrocortisone replacement should be instituted on the day of surgery:
    - l00mg hydrocortisone to be given intramuscularly immediately pre-operatively then 8 hourly for the first 24 hours.

- On the second post-operative day 50mg intramuscularly 8 hourly should be given.

4. Measure plasma cortisol at 0900 (before hydrocortisone dose) on the third post-operative day and, if the patient is well, conventional replacement dosage of oral hydrocortisone, 20mg in the morning and 10mg in the evening should be started.

5. Discharge all patients on replacement oral hydrocortisone doses and arrange follow up after 4 weeks.

6. Following bilateral adrenalectomy, observe the above protocol, except once undetectable cortisol is confirmed, convert to physiological hydrocortisone replacement and monitor ACTH.

Post-discharge Management (after Transphenoidal Surgery)

After discharge, if post-operative cortisol was undetectable:

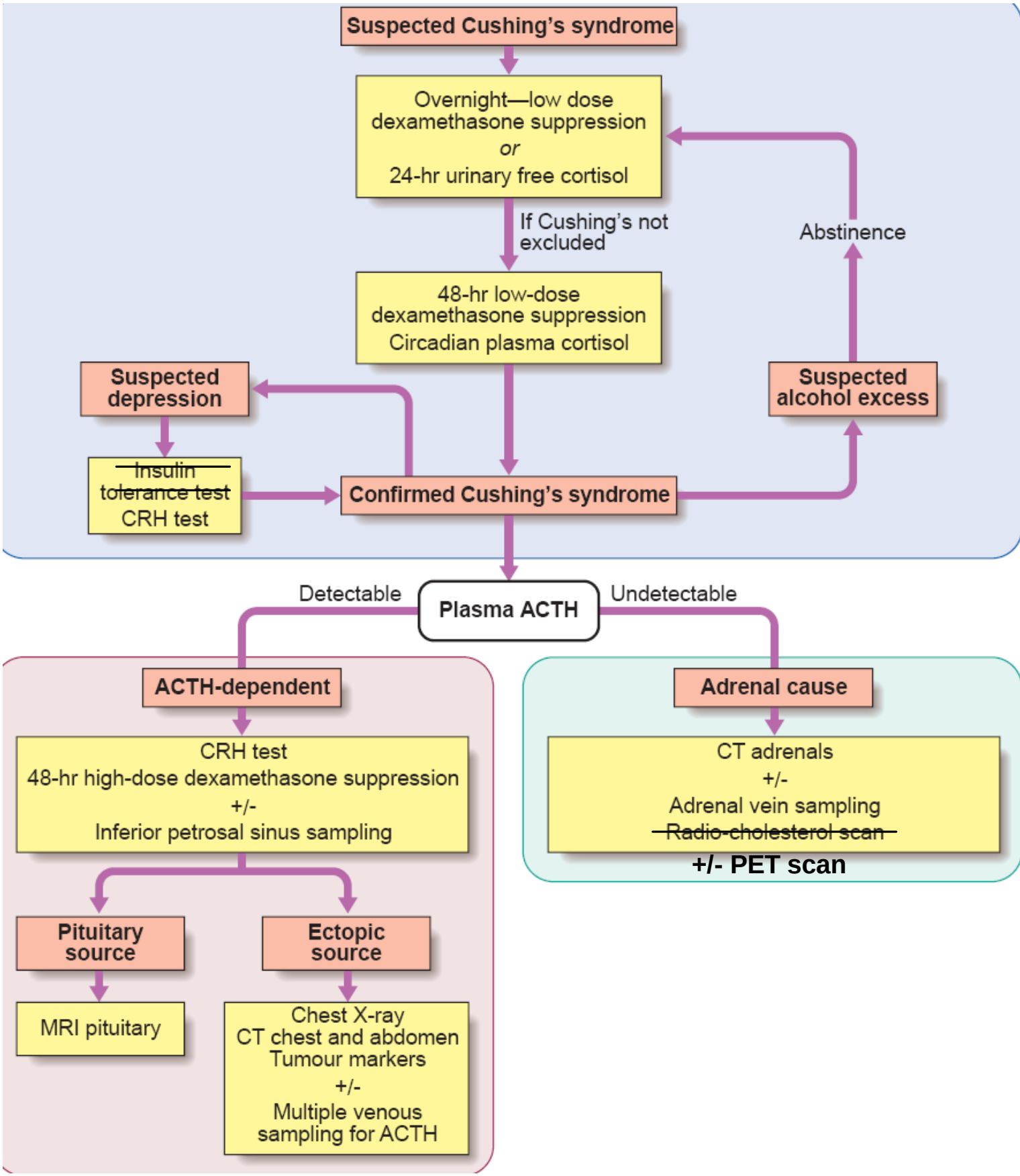
- Arrange 'routine' pituitary testing for 6 weeks post surgery.
- 9am cortisol levels and U&E's (pre-hydrocortisone) should be checked monthly until cortisol levels become detectable.
- When cortisol is detectable a short synacthen test should be performed (in the morning before hydrocortisone) prior to withdrawing glucocorticoid, to ensure adequate adrenocortical reserve.

After discharge, if post-operative cortisol was detectable:

- Arrange 'routine' pituitary testing for 6 weeks post surgery, including a short synacthen test as above.
- If plasma cortisol >430 nmol/l, discontinue hydrocortisone therapy and re-assess Cushing's status with out-patient screening tests.

All patients:

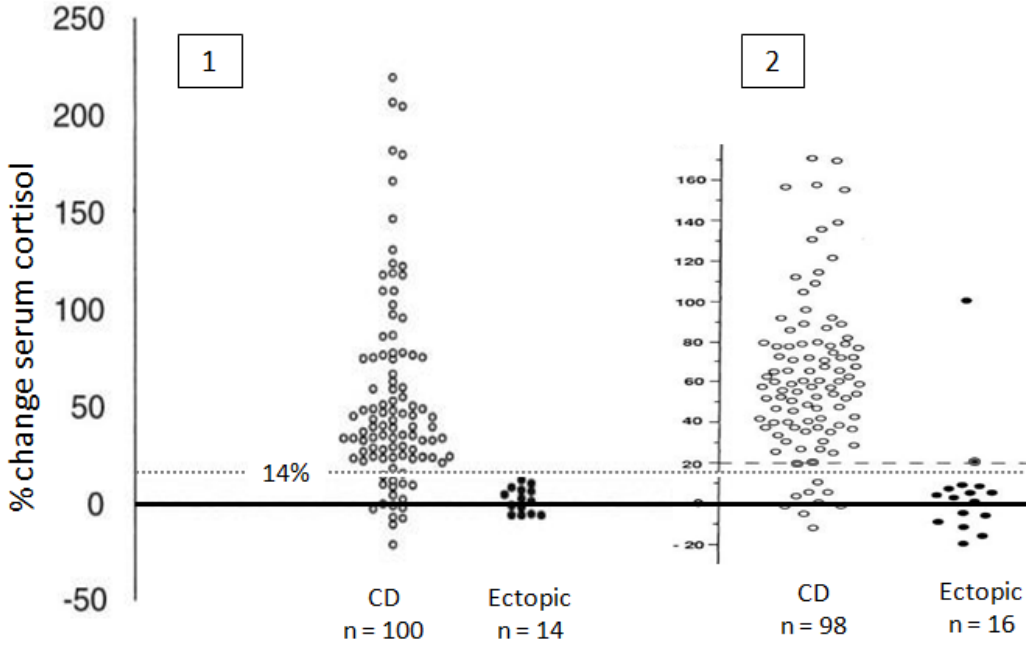
- *Consider* calcium supplementation, and bisphosphonates, for osteoporosis.
- Reassess diabetes, amenorrhoea, hypertension etc prn
- Repeat clinical photos at 6 months
- Repeat DEXA scanning at 12 months

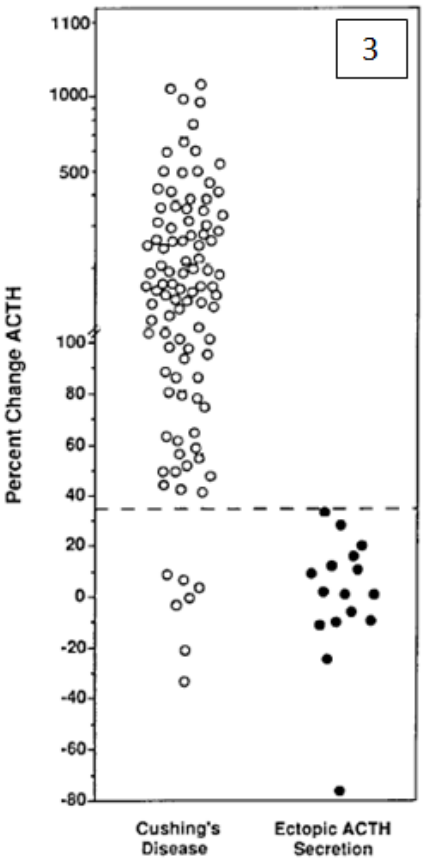

Suspected Cushing's syndrome
Overnight—low dose dexamethasone suppression
or
24-hr urinary free cortisol
If Cushing's not excluded
Abstinence
48-hr low-dose dexamethasone suppression
Circadian plasma cortisol
Suspected depression
Suspected alcohol excess
~~Insulin tolerance test~~
CRH test
Confirmed Cushing's syndrome
Detectable
Plasma ACTH
Undetectable
ACTH-dependent
Adrenal cause
CRH test
48-hr high-dose dexamethasone suppression
+/-
Inferior petrosal sinus sampling
CT adrenals
+/-
Adrenal vein sampling
~~Radio-cholesterol scan~~
+/- PET scan
Pituitary source
Ectopic source
MRI pituitary
Chest X-ray
CT chest and abdomen
Tumour markers
+/-
Multiple venous sampling for ACTH

Appendix: Criteria for interpretation of tests and their resultant specificity and sensitivity

	Cut-off	*Sensitivity (%)*	*Specificity (%)*
o/n Dexamethasone suppression	>60 nM	98	99
Urinary free cortisol	>450 nmol/d	94	97
Diurnal cortisol	2300 >75% of 0900	77	94
Low-dose Dex suppression	plasma cortisol >60 nM	96	70-87
	urine cortisol >100 nmol/d	94	100
Insulin Tolerance Test	peak cortisol > 120% baseline	82	?
CRH test	peak cortisol >120% baseline	91	95
	peak ACTH >150% baseline	86	95
High-dose Dex suppression	urine cortisol <50% of basal	92	94
	plasma cortisol ?		
Inf Pet Sinus Sampling	Central:peripheral ACTH > 2:1		?
	no CRH	46	
	with CRH	85	
MRI with Gadolinium		74	80

Appendix: CRH test





1) Percentage change in serum cortisol from a mean basal at -15 and 0 mins to a mean value calculated from the levels at 15 and 30 mins after the administration of hCRH (100µg IV) in 100 patients with Cushing's disease and 14 patients with ectopic ACTH secretion.

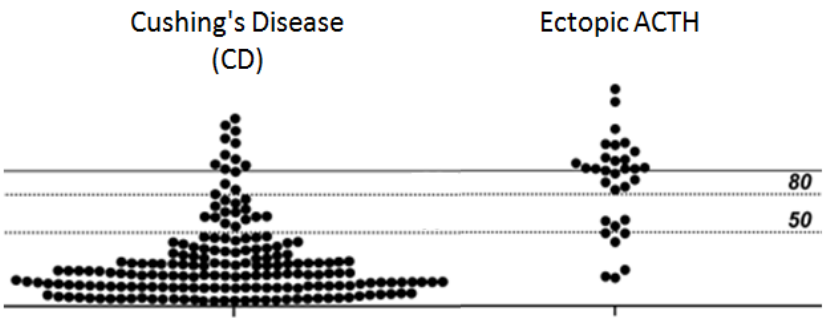
From Newell-Price J, JCEM 87:1640–1645, 2002

2&3) Responses of plasma cortisol (2) and CRH (3) in 100 patients with Cushing's disease and 16 patients with ectopic ACTH secretion. Responses are expressed as % change in mean value 15 and 30 mins after ovine CRH administration (for ACTH) and 30 and 45 mins (for cortisol) from the mean basal value 1 and 5 min before the injection.

From Nieman LK , JCEM 77:1308–1312, 1993

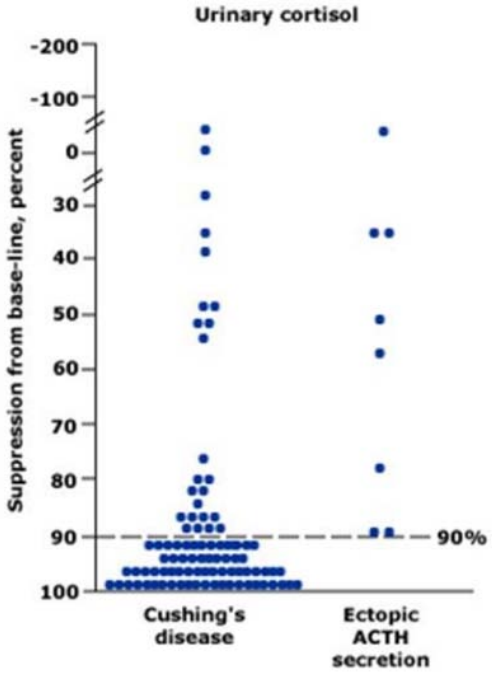
Appendix: High dose dexamethasone suppression test

a) Serum



Isidori, Grossman JCEM 2003 88:5299-5306

b) Urine



Flack, Oldfield. Ann Intern Med 1992 116:211

TABLE 3. Selected drugs that may interfere with the evaluation of tests for the diagnosis of Cushing's syndrome[a]

Drugs that accelerate dexamethasone metabolism by induction of CYP 3A4

- Phenobarbital
- Phenytoin
- Carbamazepine
- Primidone
- Rifampin
- Rifapentine
- Ethosuximide
- Pioglitazone

Drugs that impair dexamethasone metabolism by inhibition of CYP 3A4

- Aprepitant/fosaprepitant
- Itraconazole
- Ritonavir
- Fluoxetine
- Diltiazem
- Cimetidine

Drugs that increase CBG and may falsely elevate cortisol results

- Estrogens
- Mitotane

Drugs that increase UFC results

- Carbamazepine (increase)
- Fenofibrate (increase if measured by HPLC)
- Some synthetic glucocorticoids (immunoassays)
- Drugs that inhibit 11β-HSD2 (licorice, carbenoxolone)

[a]*This should not be considered a complete list of potential drug interactions. Data regarding CYP3A4 obtained from http://medicine.iupui.edu/flockhart/table.htm.*

Original protocol prepared by Brian Walker, February 1994; Revised July, 2007; Revised December 2009