CUSHING'S SYNDROME: GUIDELINES FOR INVESTIGATION

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Ratified by:	Clinical Biochemistry Senior Staff meeting
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Name of originator/author:	Dr Danni Fan/Dr Sally Stock
Director responsible for implementation:	Dr Edmund Lamb
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Target audience:	Clinical staff (medical, nursing and scientific), Trust wide and primary care

Version Control Schedule

Version	Date	Author	Status	Comment
1.0	28-01-2014	Mrs Ruth Lapworth Dr Susan Vickery	Obsolete	Archived following review, authors no longer work within the trust
2.0	May 2017	Dr Danni Fan/Dr Sally Stock	Obsolete	Archived following review
3.0	July 2019	Dr Danni Fan/Dr Sally Stock	Obsolete	Archived following review
4.0	July 2020	Dr Danni Fan/Dr Sally Stock		

Contents

1 Policy Summary

This policy gives guidance on the investigation of suspected Cushing's syndrome

2 Introduction

The diagnosis of Cushing's syndrome can be difficult and requires close collaboration with the laboratory. Patients with Cushing's syndrome, particularly those with malignant tumours secreting adrenocorticotrophic hormone (ACTH), often present with a hypokalaemic alkalosis.

There are two phases in the investigation; firstly establishing the presence of hypercortisolism in patients with suspected Cushing's syndrome and secondly, identification of the source of the hypercortisoloism. Cushing's syndrome may be ACTH-dependent due to a pituitary tumour (Cushing's disease) or an ectopic source or may be ACTH independent due to an adrenal tumour or in rare cases macronodular adrenocortical hyperplasia.

3 Purpose and Scope

This policy outlines the biochemical investigation required to confirm or exclude a diagnosis of Cushing's syndrome and further investigation of its origin. It may be used for patients both within the EKHUFT and in primary care and the community.

4 Definitions

Cortisol: A steroid hormone produced by the adrenal glands
ACTH: adrenocorticotrophic hormone
UFC: urinary free cortisol
Cushing's syndrome: A disease characterised by excessive and inappropriate endogenous cortisol secretion caused either by excess ACTH secretion (from a pituitary or other ectopic tumour) or independent adrenal overproduction of cortisol.
Dexamethasone: a synthetic steroid with 25 times the glucocorticoid activity of cortisol. It does not interfere with cortisol measurement. Dexamethasone leads to suppression of adrenal cortisol secretion in normal individuals.

5 Duties

All staff involved in the investigation of Cushing's syndrome, whether clinical or laboratory must adhere to this policy.

6 Cushing's syndrome: guidelines for investigation

A. INITIAL TESTING

Please state on the request form all relevant clinical details (including symptoms and signs and drug history) and that hypercortisolism is suspected.

For the initial testing for Cushing's syndrome, we recommend one of the following tests based on its suitability for a given patient:

(i) Measurement of urinary free cortisol (UFC) in a 24 hour urine sample

Collect urine for 24 hours in a plain collection bottle and send to the laboratory for analysis. Ensure the patient is not receiving steroids.

(ii) An overnight dexamethasone suppression test (1 mg)

This test is carried out as described in section 7.

Interpretation

If the above test results are normal, then Cushing's syndrome can be excluded. However, if the diagnosis is strongly suspected, a diagnosis of cyclical Cushing's syndrome should be considered.

An elevated UFC excretion and/or failure to suppress in response to dexamethasone (1 mg) is consistent with Cushing's syndrome.

There are several situations in which false positive or false negative test results may occur. Table 1 details the drugs that may interfere with evaluation of tests for the diagnosis of Cushing's syndrome (6). Table 2 details non drug related causes of false positive and false negative test results. It important to consider these limitations when interpreting UFC and dexamethasone suppression test results.

If the results from the above tests are abnormal, it is important to exclude alternative causes of hypercortisolaemia such as steroid administration, stress, alcoholism, obesity and endogenous depression.

Table 1 Selected drugs that interfere with the evaluation of tests for the diagnosis of Cushing's syndrome

Drugs that accelerate dexamethasone metabolism by induction of CYP 3A4	*Drugs that impair dexamethasone metabolism by inhibition of CYP 3A4*
• Phenytoin • Cabamazepine • Rifampin • Phenobarbital • Pioglitazone • Primidone • Ethosuximide	• Intraconazole • Ritonavir • Fluoxetine • Diltiazem • Cimetidine
Drugs that increase CBG and may falsely elevate cortisol results	*Drugs that increase UFC results*
• Estrogens • Mitotane	• Carbamazepine (increase) • Some synthetic glucocorticoids • Drugs that inhibit 11b-HSD2 (liquorice, carbenoxolone)

Table 2. Cushings Syndrome diagnostic tests - uncertainty and limitations (modified from references 5, 6, 8, 11)

	False positives	**False negatives**
UFC sensitivity 76 – 100% specificity 98%	• Physical stress e.g. trauma, exercise, malnutrition, hospitalization, surgery, pain • Mental stress e.g. depression, alcohol or drug abuse/withdrawal • Metabolic e.g. raised cortisol binding globulin (CBG), glucocorticoid resistance, complicated diabetes, pregnancy	• Renal failure • Incomplete urine collection • Cyclical Cushing's
Dexamethasone suppression test (1mg) sensitivity 95 – 100% specificity 80%	• Depression • Severe systemic illness • Renal failure on dialysis • Chronic alcohol abuse • Old age • Anorexia nervosa	(Very rare in patients with Cushing's syndrome – if clinical suspicion is high continue investigating)

B. CONFIRMATION OF DIAGNOSIS

Further investigations to confirm or exclude Cushing's syndrome following an elevated UFC excretion and/or lack of suppression of the serum control concentration following 1 mg dexamethasone include:

(i) Repeat the urinary free cortisol (UFC) measurement in a 24 hour urine sample

(ii) Assess circadian rhythm

Take samples for measurement of serum cortisol at 09.00 and 24:00 (midnight). It may be appropriate at this stage to take samples for ACTH in addition to those for cortisol, but ACTH will only be measured once the diagnosis of Cushing's syndrome has been made.

In patients with Cushing's syndrome there is a loss of the circadian rhythm of cortisol secretion: the 09:00 sample however may be within the reference range, but midnight cortisol sampling had high sensitivity for Cushing's syndrome.

(iii) Measure midnight salivary cortisol

In patients whom the initial test results do not support a diagnosis of Cushing's syndrome, but there is a strong clinical suspicion, it is possible to measure midnight salivary cortisol. This should be requested by a Consultant Endocrinologist. Please contact the duty biochemist (01233 616287 or exn 723 6287) to discuss testing protocol and obtain salivettes.

C. DIFFERENTIAL DIAGNOSIS OF CUSHING'S SYNDROME

The biochemical tests which provide useful information in determining the aetiology of Cushing's syndrome include the measurement of plasma ACTH and assessment of the response of plasma cortisol concentration to dexamethasone administration. However, the clinical and biochemical differentiation of pituitary-dependent Cushing's disease from ectopic ACTH secretion is often extremely difficult, and repeated investigations are often required to make this differential diagnosis, which is essential for appropriate therapy.

i) ACTH

Sample requirements for ACTH measurement and interpretation of results is described in section 8.

ii) High-dose dexamethasone suppression test

This test is carried out as described in section 9.

iii) Other Investigations

A variety of alternative laboratory and imaging procedures can be utilised in the differentiation of pituitary from ectopic ACTH secretion. Biochemical indices include the plasma potassium concentration, glucose intolerance, ACTH response to CRH, venous sampling catheterisation and inferior petrosal sinus catheterisation. It is essential that these specialised procedures are carried out in a recognised centre after full consultation with the appropriate staff on the patient's history and relevant results.

7 Dexamethasone suppression test (1 mg)

Indications for test:
An overnight dexamethasone suppression test (ONDST) is performed to confirm the diagnosis of hypercortisolism. Indications include a raised urinary free cortisol (UFC) cortisol concentration, and/or clinical signs of Cushing's Syndrome (e.g. weight gain, menstrual irregularity, hirsutism, depression, impotence, muscle wasting, red striae, easy bruising, and thin skin in the young).

Contraindications:
- Patients on enzyme inducing drugs (e.g. anti-convulsants) that may rapidly metabolise dexamethasone (see table 1) or malabsorption syndromes that may lead to insufficient dexamethasone absorption.
- Oestrogens (e.g. pregnancy, HRT or COC) may induce cortisol binding globulin (CBG) and artefactually increase total cortisol levels.
- Care should be taken in patients with severe depression or hypomania.

Examples for false positive or false negative results are listed in Table 2.

Patient preparation:
Stop all oral oestrogen therapy 6 weeks prior to test. Hormonal implants can also interfere with test results (9).

Side effects:
None.

Procedure:
- Instruct patient to take 1 mg dexamethasone orally (20 µg/kg in children) between 23:00 and midnight.
- Take blood sample (gold tube) at 09:00 the following day for serum cortisol measurement. Please ensure that the request form/clinical information states that the blood has been taken post dexamethasone.

Interpretation:
A serum cortisol concentration <40 nmol/L at 09:00 post dexamethasone administration is an adequate response.

A cortisol concentration > 40 nmol/L suggests an inadequate response. Please check that dexamethasone was taken and rule out drug effects. The Duty Biochemist is available on 01233 616287/x723 6287 to discuss further investigations.

8 Adrenocorticotrophic hormone (ACTH): guidelines for requesting in suspected Cushing's syndrome

Indications for test:
ACTH measurement is required in patients with confirmed Cushing's syndrome of unknown aetiology.

Patient preparation:
Avoid stress.

Procedure:

Please note, samples for ACTH cannot be collected in primary care, as samples must reach the laboratory within 15 minutes of collection.

- Collect the blood samples between 08:00am and 10:00am.
- Take a 4 mL blood sample into an EDTA tube (purple tube) for plasma ACTH and a 4 mL blood sample (gold tube) for serum cortisol.
- Clearly state on both the request form and sample tubes the actual time the blood was collected.
- Send both blood samples with a request form for ACTH and cortisol to the laboratory immediately. Plasma for ACTH must be separated from the red blood cells and frozen within 15 minutes of venepuncture.
- Samples that are haemolysed or not separated within 15 minutes are unsuitable for analysis.
- ACTH measurement is performed at a referral laboratory.
- Requests will be vetted by the Duty Biochemist before being sent away for analysis, to ensure the request is clinically indicated.
- The turnaround time for the result is 2-3 weeks.

Interpretation:

ACTH concentration will be inappropriately elevated relative to the serum cortisol concentration in patients with Cushing's syndrome due to secondary causes:

- hypothalamic dysfunction
- pituitary tumour
- ectopic ACTH secretion. The higher the ACTH concentration the more likely there is an ectopic source.

ACTH concentration will be low or undetected in primary hypercortisolism whether due to an adrenal adenoma, carcinoma or macronodular adrenocortical hyperplasia.

9 Low dose dexamethasone suppression test, 48-Hour

Indications for test:
A low dose dexamethasone suppression test (LDDST) is performed in patients with suspected Cushing's syndrome. However 1mg overnight suppression test (described in section 7) may occasionally be sufficient in low probability patients. The LDDST is also performed to diagnose glucocorticoid-remediable aldosteronism (GRA) by measuring plasma aldosterone levels 6 hours after the last dexamethasone dose.

Contraindications:
(Similar to the contraindications described in section 7)

Patient preparation:
Stop all oral oestrogen therapy 6 weeks prior to test. Hormonal implants can also interfere with test results (9).

Side effects:
None.

Procedure (12):

- On day 1 take blood sample (gold tube) at 09:00 for measurement of serum cortisol.
- Administer 0.5 mg dexamethasone orally (10 µg/kg in children) every 6 h for 48 hours (e.g. 09:00, 15:00, 21:00, 03:00).
- A second plasma cortisol is drawn (gold tube) at 9 AM, 6 hours after the last dexamethasone dose.
- On day 3 take blood sample (gold tube) at 09:00 for measurement of serum cortisol measurement, 6 hours after the last dexamethasone dose.

Interpretation:
A serum cortisol concentration <40 nmol/L on day 3 is an adequate response to the low dose of dexamethasone, which essentially exclude Cushing syndrome.

Please contact the Duty Biochemist (01233 616287/x723 6287) to discuss further investigation if necessary.

10 High dose dexamethasone suppression test

Indications for test:
A high dose dexamethasone suppression test may be used in patients with confirmed Cushing's syndrome of unknown aetiology. It is useful in differentiation of pituitary ACTH-dependent Cushing's disease from ectopic ACTH secretion. However, the clinical and biochemical differentiation of Cushing's disease can be extremely difficult and repeated investigations may be required.

Contraindications:
(Similar to the contraindications described in section 7)

Patient preparation:
Stop all oral oestrogen therapy 6 weeks prior to test. Hormonal implants can also interfere with test results (9).

Side effects:
None.

Procedure (12):

- On day 1 take blood sample (gold tube) at 09:00 for measurement of serum cortisol.
- Administer 2 mg dexamethasone (50 µg/kg in children) orally every 6 h for 48 hours (e.g. 09:00, 15:00, 21:00, 03:00).
- A second plasma cortisol is drawn (gold tube) at 9 AM, 6 hours after the last dexamethasone dose.
- On day 3 take blood sample (gold tube) at 09:00 for measurement of serum cortisol measurement.

Interpretation:
In the majority of cases of pituitary ACTH-dependent Cushing's disease, the serum cortisol on day 3 will fall to <50 % of its basal value.

In patients with ectopic ACTH secretion, adrenal adenomas and carcinomas, inadequate suppression of serum cortisol by the high dose dexamethasone is most likely to be observed.

Please note this test should not be used in isolation for differential diagnosis. Suppression occurs in 75% of patients with Cushing's disease, 10-25% of patients with ectopic ACTH and 0-6% of patients with adrenal tumours. Unexpected responses can be seen in both pituitary ACTH-dependent and ectopic ACTH secretion patient groups. Patients with ectopic ACTH who show suppression tend to have occult and relatively benign tumours with lower levels of ACTH and cortisol. These patients are very hard to differentiate from Cushing's disease (10). Therefore all biochemical and radiological investigations must be interpreted together with the clinical findings before the differential diagnosis can be made.

Please contact the Duty Biochemist (01233 616287/x723 6287) to discuss further investigation if necessary.

11 Dexamethasone suppression test (2 mg)

Indications for test:
2 mg overnight dexamethasone suppression test (ONDST) protocol is commonly used by the Kings College Hospital Adrenal MDT as an alternative to the low dose dexamethasone suppression test, especially in patients who cannot adhere to its strict 0.5mg 6 hourly dosing regimens. 2mg ONDST has better sensitivity and specificity especially in obese patients and hence preventing further extensive testing because of false positives.

Contraindications:
[same as Dexamethasone suppression test (1 mg)]

Patient preparation:
[same as Dexamethasone suppression test (1 mg)]

Side effects:
None.

Procedure:
(same as Dexamethasone suppression test (1 mg), apart from 2 mg dexamethasone taken orally)

Interpretation:
[same as Dexamethasone suppression test (1 mg)]

Inadequate suppression will indicate the possibility of a cortisol producing low grade adrenocortical carcinoma and the need for tertiary referral

12 Key Stakeholders, Consultation, Approval and Ratification Process

East Kent Hospitals University NHS Foundation Trust is the key stakeholder for this policy.

Consultation has been through e-mail and face-to-face communication between clinical biochemistry staff and Trust consultant endocrinologists (Dr S Joseph, Dr C McGettigan). Copies of correspondence are held on the shared drive.

13 Review and Revision Arrangements

Three years from implementation date.

14 Dissemination and Implementation

Dissemination to relevant staff within Pathology via Q Pulse. Dissemination to users of the service via documentation hosted in the healthcare professionals zone of Trustnet.

15 Document Control including Archiving Arrangements

Archive of this document will be through QPulse.

16 Monitoring Compliance

Within the Trust, compliance with this policy must rest with the requesting Divisions with vetting of requests in Clinical Biochemistry. Compliance will also be subject to occasional audit within Clinical Biochemistry.

17 References

1. Tsigos C & Chrousos GP. Clinical presentation, diagnosis and treatment of Cushing's syndrome. Current Opinion in Endocrimology & Diabetes 1995; **2**:203-213.
2. Wood P, Barth JH, Freedman DB, Perry L & Sheridan B. Evidence for the low dose dexamethasone suppression test to screen for Cushing's syndrome – recommendations for a protocol for UK biochemistry. Ann Clin Biochem; 1997: **34**: 222-9.
3. Perry LA & Grossman AB. The role of the laboratory in the diagnosis of Cushing's syndrome. Ann Clin Biochem; 1997; **34**: 345-359.
4. Ross RJM & Trainer. PJ Endocrine investigation: Cushing's syndrome. Clin Endo 1998; **49**: 153-55.

5. Barth J, Butler GE & Hammond P. Biochemical investigations in Laboratory Medicine. ACB Venture Publications, 2001.
6. Nieman LK et al. The diagnosis of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. J Clin Endo Met 2008, **93**:1526-1540.
7. Arnaldi G et al. Diagnosis and complications of Cushing's syndrome: A consensus statement. J Clin Endo Met 2003, **88**:5593-5601.
8. Ma RCW, Chan WB, So WY, Tong PCY, Chan JCN, Chow CC. Carbamazepine and false positive dexamethasone suppression tests for Cushing's syndrome. BMJ : British Medical Journal. 2005;330(7486):299-300.
9. Endocrinology Handbook Imperial Centre for Endocrinology, Imperial College Healthcare NHS Trust Charing Cross, Hammersmith and St. Mary's Hospitals, Updated: April 2016
10. Aron, Raff and Findling, Effectiveness Versus Efficacy: The Limited Value in Clinical Practice of High Dose Dexamethasone Suppression Testing in the Differential Diagnosis of Adrenocorticotropin-Dependent Cushing's Syndrome (1997) (JCEM 82: 1780-1785).
11. Ma RCW, Chan WB, So WY, Tong PCY, Chan JCN, Chow CC. Carbamazepine and false positive dexamethasone suppression tests for Cushing's syndrome. BMJ : British Medical Journal. 2005;330(7486):299-300.


Dexamethasone Suppression Tests - fi

12. Communication with local endocrine team -
13. Sahin M1 et al. Comparison of 1 mg and 2 mg overnight dexamethasone suppression tests for the screening of Cushing's syndrome in obese patients. Intern Med. 2009;48(1):33-9. Epub 2009 Jan 1.

18 Associated Documentation

Not applicable.

Appendix A - Equality Impact Assessment

Equality and Human Rights Impact Analysis (EHRIA)

Part One – Screening Tool

Name of the policy, strategy, function or methodology:	Cushing's syndrome: Guidelines for investigation

Details of person completing the EHRIA	
Name	Dr Danni Fan
Job Title	Senior Clinical Scientist
Department/Specialty	Pathology/Clinical Biochemistry
Telephone Number	ext 723 6287

1. Identify the policy, strategy, function or methodology aims

What are the main aims, purpose and outcomes of the policy, strategy, function or methodology?
To ensure appropriate investigation of suspected Cushing's syndrome across the health service in East Kent.
Does it relate to our role as a service provider and/or an employer?
Service provider.

2. Assess the likely impact on human rights and equality

Use this table to check if the policy, strategy, function or methodology:

- could have a negative impact on human rights or on any of the equality groups, or
- could have a positive impact on human rights, contribute to promoting equality, equal opportunities or improve relations.

It is not necessary to complete each box, nor to mark whether it is positive or negative, although you can do this if you find it helpful.

	Protected Characteristic								
	Race	**Sex**	**Disability**	**Sexual Orientation**	**Religion or belief**	**Age**	**Gender reassignment**	**Marriage & Civil Partnership**	**Pregnancy & Maternity**
Could this policy, procedure, project or service affect this group differently from others? **YES/NO**									
Could this policy, procedure, project or service promote equal opportunities for this group? **YES/NO**									
Right to life *e.g. decisions about life-saving treatment, deaths through negligence in hospital*									
Right not to be tortured or treated in an inhuman or degrading way *e.g. dignity in care, abuse or neglect of older people or people with learning disabilities.*									
Right to respect for private and family life e.g. *respecting lgb relationships, confidentiality*									
Right to freedom of thought, conscience and religion *e.g. respect for cultural and religious requirements*									
Right to freedom of expression *e.g. access to appropriate communication aids*									
Right to freedom of assembly and association *e.g., right to representation, to socialise in care settings*									
Right to education *e.g. access to basic knowledge of hygiene and sanitation*									
Right to liberty e.g. informal detention of patients who do not have capacity									

3. How does it impact on people's human rights and equality?

Using the table above, explain anticipated impacts. If a full EHRIA is recommended, you can summarise the impacts - it is not necessary to set these out in detail,

Could people's human rights be impacted negatively? Could the policy, strategy, function or methodology result in inequality or discrimination?
No
Could this policy, strategy, function or methodology result in positive impacts on people's human rights or equality? Could it present opportunities to promote equality?
No

4. Recommendations

Is a full EHRIA recommended? If not, give reasons
No. The policy has equal impact.

5. Publication of EHRIA

Give details of where Screening Tool or the full EHRIA will be published and when this will take place
With document.

Details of person completing the EHRIA	
Name	Dr Danni Fan, Senior Clinical Scientist

Signed …… Date: ……

Approval and sign-off	Name
Head of Department/Director	Dr Sally Stock, Head of Service Clinical Biochemistry

Signed …… Date: ……

	Name
Trust Board approval and sign-off	not applicable

Appendix B – Author's Checklist of compliance with the Policy for the Development and Management of Organisation Wide Policies and Other Procedural Documents

POLICY:

To be completed and attached to any policy when submitted to the appropriate committee for consideration and approval.

	Requirement:	Compliant Yes/No/ Unsure	Comments
1.	**Style and format**	Yes	
2.	**An explanation of any terms used in documents developed**	Yes	
3.	**Consultation process**	Yes	
4.	**Ratification process**	Yes	
5.	**Review arrangements**	Yes	
6.	**Control of documents, including archiving arrangements**	Yes	
7.	**Associated documents**	n/a	
8.	**Supporting references**	Yes	
9.	**Relevant NHSLA criterion specific requirements**	n/a	
10.	**Any other requirements of external bodies**	n/a	
11.	**The process for monitoring compliance with NHSLA and any other external and/or internal requirements**	n/a	

Appendix C – Plan for Dissemination of Policies

To be completed and attached to any policy when submitted to the appropriate committee for consideration and approval.

Acknowledgement: University Hospitals of Leicester NHS Trust (Amended)

Title of document:	Cushing's syndrome: Guidelines for investigation		
Version Number:	4.0		
Approval Date:	July 2020	**Dissemination lead:**	Dr Danni Fan
Previous document already being used?	Yes		
If yes, in what format (paper / electronic) and where (e.g. Directorate / Trust wide)?	Electronic version hosted on Q pulse (document management system within pathology) and within the healthcare professionals zone of Trustnet		
Proposed instructions regarding previous document:	This document will replace version 2.0		

To be disseminated to:	**How will it be disseminated, who will do it and when?**	**Format (i.e. paper or electronic)**	**Comments:**
Trust clinical staff	Trustnet	electronic	
Primary care	Trustnet	electronic	
Clinical Biochemistry staff	Q Pulse	electronic	

Author's Dissemination Record - to be used once document is approved – to be kept with the master document

Date document forwarded to be put on the Trust's central register / in SharePoint:		**Date document put on Directorate register (if appropriate) / on Directorate webpage (if applicable)**	

Disseminated to: (either directly or via meetings, etc.)	**By Whom?**	**Format (i.e. paper or electronic)**	**Date Disseminated:**