Reference	Kalaria 2020 ³
Study type	Cross sectional diagnostic study
Study	Data source: Outpatient assessments
methodology	Recruitment: Between March 2016 and January 2019, consecutive patients attending the endocrine dynamic function clinic for a SST to evaluate HPA recovery were invited to participate
Number of patients	n = 47 (56 SSTs - seven patients underwent two SSTs each and one patient three SSTs)
Patient characteristics	Age, median (IQR): 60 (48.3–69.5) years
01141141011104100	Gender (male to female ratio): 16:31
	Ethnicity: Not reported
	Setting: Single centre, department of endocrinology
	Country: UK
	Inclusion criteria: consecutive patients attending the endocrine dynamic function clinic for a SST to evaluate HPA recovery were invited to participate. All patients had previously been on prolonged supraphysiological therapeutic doses of oral glucocorticoids.
	Exclusion criteria: Those with periodontal disease were excluded.
	Reason for referral: To assess hypothalamic–pituitary–adrenal axis recovery in patients previously treated with prolonged supraphysiological therapeutic doses of oral glucocorticoids.
Target condition(s)	Adrenal insufficiency
Index test(s)	Index tests: Basal serum cortisol, basal salivary cortisol and basal salivary cortisone
and reference standard	SSTs were performed between 09:00 and 10:30 after oral and inhaled glucocorticoid withdrawal for at least 24 h. Baseline salivary sample was collected immediately before the baseline serum sample and then 0.25 mg of tetracosactide acetate
Stallualu	(Synacthen) was injected either intravenously or intramuscularly and a further blood sample collected 30 min later. Saliva was collected in

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	SalivetteVR tubes (plain cotton swab; Sarstedt, Germany) according to manufacturer's instructions. Blood was collected in S-MonovetteVR 4.7 mL Z-gel tubes (Sarstedt, Aktiengesellschaft & Co, Germany).				
	Threshold cut-off value: HPA suppression: Baseline serum cortisol: 170(nmol/L), baseline salivary cortisol: 1.92(nmol/L), baseline salivary cortisone: 9.42 (nmol/L). HPA recovery: Baseline serum cortisol: 365(nmol/L), baseline salivary cortisol: 25.4(nmol/L), baseline salivary cortisone: 37.2 (nmol/L)				
	Assay: Salivary samples were centrifuged at 1500 g for 5 min, the cotton was discarded, and the extracted saliva frozen at 80C until analysed for cortisol and cortisone using liquid chromatography with tandem massspectrometry (LC-MS/MS) on a Shimadzu Prominence HPLC system coupled to an AB Sciex 3200 mass spectrometer based on previously described method. The LC-MS/ MS salivary cortisol and salivary cortisone assays both had a quantitation limit of 0.83 nmol/L. The intra-assay CVs were 4.9% at 9.5 nmol/L for both salivary cortisol and salivary cortisone and the inter-assay CVs were 10.8% at 9.7 nmol/L and 6.0% at 10 nmol/L, respectively.				
	Reference standard Short Synacthen test SSTs were performed between 09:00 and 10:30 after oral and inhaled glucocorticoid withdrawal for at least 24 h. Baseline salivary sample was collected immediately before the baseline serum sample and then 0.25 mg of tetracosactide acetate (Synacthen) was injected either intravenously or intramuscularly and a further blood sample collected 30 min later. Blood was collected in S-MonovetteVR 4.7 mL Z-gel tubes (Sarstedt, Aktiengesellschaft & Co, Germany).				
	was collected in intravenously or	nmediately before the bas intramuscularly and a fu	seline serum sample and rther blood sample colled	I then 0.25 mg of tetracted 30 min later.	acosactide acetate (Synacthen) was injected either
	was collected in intravenously or Blood was collec	nmediately before the base intramuscularly and a fu cted in S-MonovetteVR 4	seline serum sample and rther blood sample collec .7 mL Z-gel tubes (Sarst	I then 0.25 mg of tetra cted 30 min later. cedt, Aktiengesellscha	acosactide acetate (Synacthen) was injected either
	was collected in intravenously or Blood was collected. Threshold: A S Assay: Blood w i2000 (Abbott La	nmediately before the base intramuscularly and a functed in S-MonovetteVR 4 ST was labelled as 'pass' as separated, and serum	seline serum sample and rther blood sample collect. 7 mL Z-gel tubes (Sarst s' (adequate response) if a cortisol measured by a bbott Architect cortisol as	I then 0.25 mg of tetra cted 30 min later. cedt, Aktiengesellscha the 30-min serum cor chemiluminescence r ssay has a quantitatio	acosactide acetate (Synacthen) was injected either aft & Co, Germany). It was >/=450 nmol/L. In the control of the control o
	was collected in intravenously or Blood was collected. Threshold: A S Assay: Blood w i2000 (Abbott La assay coefficien Time between in	nmediately before the base intramuscularly and a further intramuscularly and a further interest in S-MonovetteVR 4 and serum aboratories, USA). The Auts of variation (CV) of 2.5 measurement of index to interest interest interest.	seline serum sample and rther blood sample collect. The Dood sample contisol measured by a bbott Architect cortisol as 5% at 118 nmol/L and 3.5 test and reference stand	I then 0.25 mg of tetracted 30 min later. Leedt, Aktiengesellscharthe 30-min serum conchemiluminescence ressay has a quantitation of the service of the serv	acosactide acetate (Synacthen) was injected either aft & Co, Germany). It was >/=450 nmol/L. In the control of the control o
	was collected in intravenously or Blood was collected. Threshold: A S Assay: Blood w i2000 (Abbott La assay coefficien Time between in	nmediately before the base intramuscularly and a further cted in S-MonovetteVR 4. ST was labelled as 'passes as separated, and serum aboratories, USA). The Auts of variation (CV) of 2.5 measurement of index to a baseline serum cortise.	seline serum sample and rther blood sample collect. The Z-gel tubes (Sarst and cortisol measured by a bbott Architect cortisol as 5% at 118 nmol/L and 3.5 test and reference standol: 170(nmol/L)	I then 0.25 mg of tetracted 30 min later. redt, Aktiengesellschathe 30-min serum corchemiluminescence ressay has a quantitation with at 110 nmol/L, restard: reference stand	acosactide acetate (Synacthen) was injected either aft & Co, Germany). Intisol was >/=450 nmol/L. Inticroparticle immunoassay on an Abbott Architect on limit of 22 nmol/L with an intra-assay and interprectively.
calculated	was collected in intravenously or Blood was collected. Threshold: A S Assay: Blood w i2000 (Abbott La assay coefficien Time between in HPA suppression	nmediately before the base intramuscularly and a functed in S-MonovetteVR 4 and ST was labelled as 'passer's separated, and serum aboratories, USA). The Auts of variation (CV) of 2.5 and serum contists about the baseline serum cortists Reference standard +	seline serum sample and rther blood sample collect i.7 mL Z-gel tubes (Sarst is' (adequate response) if in cortisol measured by a bbott Architect cortisol as 5% at 118 nmol/L and 3.5 test and reference stand sol: 170(nmol/L) Reference standard –	I then 0.25 mg of tetracted 30 min later. redt, Aktiengesellschathe 30-min serum conchemiluminescence resay has a quantitation at 110 nmol/L, restard: reference stand	acosactide acetate (Synacthen) was injected either aft & Co, Germany). Intisol was >/=450 nmol/L. Inticroparticle immunoassay on an Abbott Architect on limit of 22 nmol/L with an intra-assay and interprectively.
2×2 table calculated rom reported ensitivity,	was collected in intravenously or Blood was collected. Threshold: A S Assay: Blood w i2000 (Abbott La assay coefficien Time between in	nmediately before the base intramuscularly and a further cted in S-MonovetteVR 4. ST was labelled as 'passes as separated, and serum aboratories, USA). The Auts of variation (CV) of 2.5 measurement of index to a baseline serum cortise.	seline serum sample and rther blood sample collect. The Z-gel tubes (Sarst and cortisol measured by a bbott Architect cortisol as 5% at 118 nmol/L and 3.5 test and reference standol: 170(nmol/L)	I then 0.25 mg of tetracted 30 min later. redt, Aktiengesellschathe 30-min serum corchemiluminescence ressay has a quantitation with at 110 nmol/L, restard: reference stand	acosactide acetate (Synacthen) was injected either aft & Co, Germany). Intisol was >/=450 nmol/L. Inticroparticle immunoassay on an Abbott Architect on limit of 22 nmol/L with an intra-assay and inter-epectively.

Reference	Kalaria 2020 ³			
(calculated	HPA suppression - baseline salivary cortisol: 1.92(nmol/L)			
from reported sensitivity,		Reference standard +	Reference standard -	Total
	Index test +	15	20	35
specificity and true positive and true negative rates)	Index test -	0	21	21
	Total	15	41	56
(calculated	HPA suppression	n - baseline salivary cort	isone: 9.42(nmol/L)	
from reported		Reference standard +	Reference standard -	Total
sensitivity,	Index test +	15	19	34
specificity and	Index test -	0	22	22
true positive and true negative rates)	Total	15	41	56
(calculated				
from reported	HPA recovery -	baseline serum cortisol:		
sensitivity,		Reference standard +	Reference standard -	Total
specificity and	Index test +	4	0	4
true positive	Index test -	11	41	52
and true negative rates)	Total	15	41	56
(calculated	HPA recovery -	baseline salivary cortisol	: 25.4(nmol/L)	
from reported		Reference standard +	Reference standard -	Total
sensitivity,	Index test +	0	0	0
specificity and	Index test -	15	41	56
true positive and true negative rates)	Total	15	41	56
(calculated	HPA recovery -	baseline salivary cortisor	ne: 37.2(nmol/L)	
from reported		Reference standard +	Reference standard -	Total
sensitivity,	Index test +	4	0	4

Reference	Kalaria 2020 ³				
specificity and	Index test -	11	41	52	
true positive and true negative rates)	Total	15	41	56	
Statistical measures	Sensitivity 100 Specificity 58.5 PPV 0.47h NPV 1 PLR 2.41 NLR 0 AUC 0.772 Index text – HPA Sensitivity 100 Specificity 51.2 PPV 0.43 NPV 1 PLR 2.0408 NLR 0 AUC 0.770	A suppression - baseline A suppression - baseline	serum cortisol: 170(nmo	nol/L)	
	HPA recovery				

Reference	Kalaria 2020 ³
	Index text – HPA recovery - baseline serum cortisol: 365(nmol/L) Sensitivity 26.7 Specificity 100 PPV 1 NPV 0.79 PLR 0 NLR 0.73 AUC Index text – HPA recovery - baseline salivary cortisol: 25.4(nmol/L) Sensitivity 0 Specificity 100 PPV 0 NPV 0.73 PLR 0 NLR 1 AU
	Index text – HPA recovery - baseline salivary cortisone: 37.2(nmol/L) Sensitivity 26.7 Specificity 100 PPV 1 NPV 0.789 PLR 0 NLR 0.73 AUC
Source of funding	The author(s) received no financial support for the research, authorship, and/ or publication of this article.
Limitations	Risk of bias: serious (due to patient flow) Indirectness: none
Comments	Unclear if it was 47 patients or 56 SSTs included in analysis. The cut-offs derived in this study may not be applicable to the evaluation of HPA function in cohorts without HPA suppression or in females on oral oestrogen or premenopausal.