Article:

Supplementary Appendix

Contents

List of Investigators

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1. STARD Checklist

Section and Topic	Item No.		Reported on page No.
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	Page 2
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	Page 5
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	Pages 5,6,7
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	Pages 5,6
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	Pages 5,6
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	Page 5
Test methods	7	The reference standard and its rationale.	Page 3,7
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	Pages 3,4,6,7
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	Pages 7,8
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	Page 7
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	Page 8

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Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	Page 8,9
	13	Methods for calculating test reproducibility, if done.	Page 8,9
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	Page 5
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	Table 1
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	Figure 1
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	Table 2
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	Table 1
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	Table 2
	20	Any adverse events from performing the index tests or the reference standard.	N/A
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	Page 11
	22	How indeterminate results, missing data and outliers of the index tests were handled.	Page 9
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	N/A
	24	Estimates of test reproducibility, if done.	Page 11
DISCUSSION	25	Discuss the clinical applicability of the study findings.	Page 14,15

2. Decision Analytic Model

Model structure

A decision analytic model was developed to describe the outcomes associated with two diagnostic strategies. The current diagnostic strategy is for all patients to be referred for ACTH Stimulation Test (using immunoassay, 15.6µg/dL (430nmol) cutoff), which is undertaken in hospital. The alternative is a two-stage diagnostic strategy that requires a patient undertaking a Waking Salivary Cortisone test at home, using cutoffs derived from this study <251ng/dL & ≥612ng/dL (<7nmol/L & ≥17nmol/L) to confirm or exclude adrenal insufficiency respectively, then if the diagnosis is unclear, they are referred for a hospital-based ACTH Stimulation Test.

For both the waking salivary cortisone test and the ACTH Stimulation Test, patients may not participate; for the waking salivary cortisone test, it is assumed that this leads to a referral for ACTH Stimulation Test, whereas if an ACTH Stimulation Test is missed, no diagnosis is given. Additionally, for the waking salivary cortisone test, an inadequate sample may be given, which again, is assumed to lead to a referral for ACTH Stimulation Test. The resultant decision tree for the two diagnostic strategies is shown in Supplementary Appendix, Figures S2 and S3.

Model outcomes and probabilities

After each diagnostic strategy is complete, patients are assigned as true negative, true positive, false positive, false negative or without diagnosis. These designations are based on our diagnostic accuracy study, as summarized in Supplementary Appendix Table S2.

Two further assumptions are adopted; first, ACTH Stimulation Test is 100% correct, and second; failure to return a waking saliva sample, or give an adequate sample, or failure to attend an ACTH Stimulation Test appointment is not related to the final diagnosis; as such, the underlying adrenal status of these patients is the same as that for the overall sample. The probabilities for patients not returning this salivary cortisol test and not attending the ACTH Stimulation Test are 3%, based on expert opinion.

3. Figures

Figure S1: Receiver-Operating-Characteristic Curves for ACTH Stimulation Test 30-minute cortisol cut-offs A) 14.5µg/dL B) 12.7µg/dL
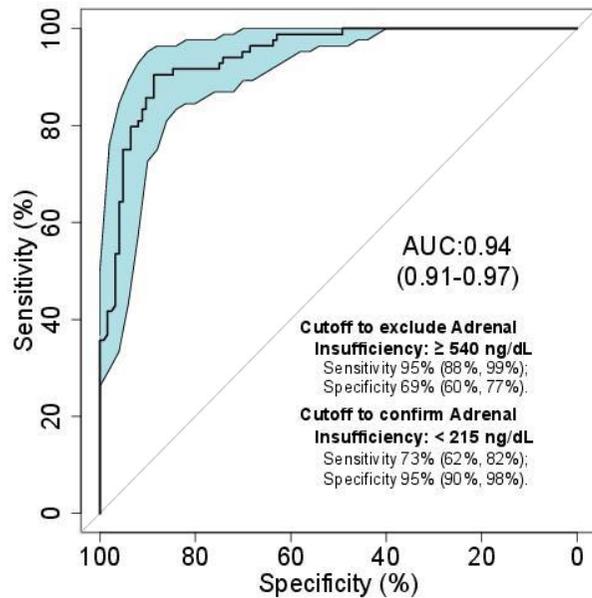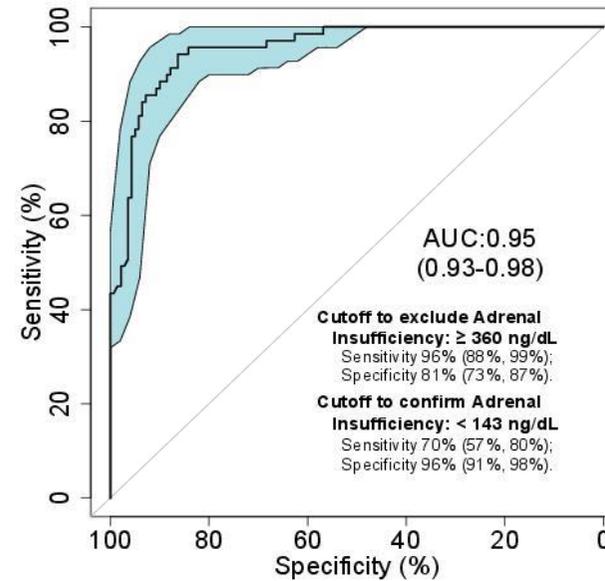
A   **B**

For Waking Salivary Cortisone different cut-offs for the ACTH Stimulation Test 30-minute cortisol are tested including 14.5µg/dL (A) and 12.7µg/dL (B) as part of a sensitivity analysis to confirm Waking Salivary Cortisone predictive power. Conversions: serum cortisol µg/dL=nmol/L/27.6; cortisone ng/dL=(nmol/L/27.8)*1000; salivary cortisol ng/dL=(nmol/L/27.6)*1000; AUC:Area under the Curve

Figure S2: Decision tree for the current diagnostic strategy to assess for adrenal insufficiency

Positive Adrenal Insufficiency

ACTH Stimulation Test

Refer for ACTH Stimulation Test

Negative Adrenal Insufficiency

ACTH Stimulation Test - non-attendance
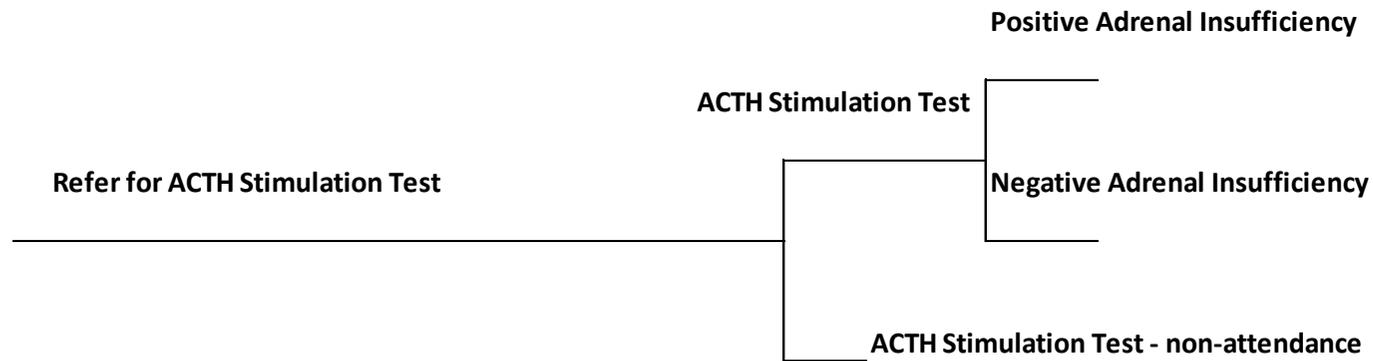
This is the decision tree for current diagnostic strategy where most patients are referred for ACTH Stimulation Test to confirm or exclude adrenal insufficiency. (See 2. Decision Analytic Model for Information)

Figure S3: Decision tree for the alternative diagnostic strategy to assess for adrenal insufficiency

Positive

Negative

Positive Adrenal Insufficiency

ACTH Stimulation Test

Negative Adrenal Insufficiency

Unclear

Test returned

ACTH Stimulation Test - non-atendance

Positive Adrenal Insufficiency

ACTH Stimulation Test

Send Waking Salivary Cortisone

No Waking Salivary Cortisone result

Negative Adrenal Insufficiency

ACTH Stimulation Test - non-atendance

Positive Adrenal Insufficiency

ACTH Stimulation Test

Test not returned

Negative Adrenal Insufficiency

ACTH Stimulation Test - non-atendance

This is the decision tree showing the two-stage diagnostic pathway where all patients are asked to do a waking salivary cortisone and if result does not exclude or confirm adrenal insufficiency that is unclear then an ACTH Stimulation Test is carried out (See 2. Decision Analytic Model for Information)

Figure S4: Questionnaire results to assess patient views on ACTH Stimulation Test and Waking Salivary Cortisone (Percentages (95% CI))

A

B
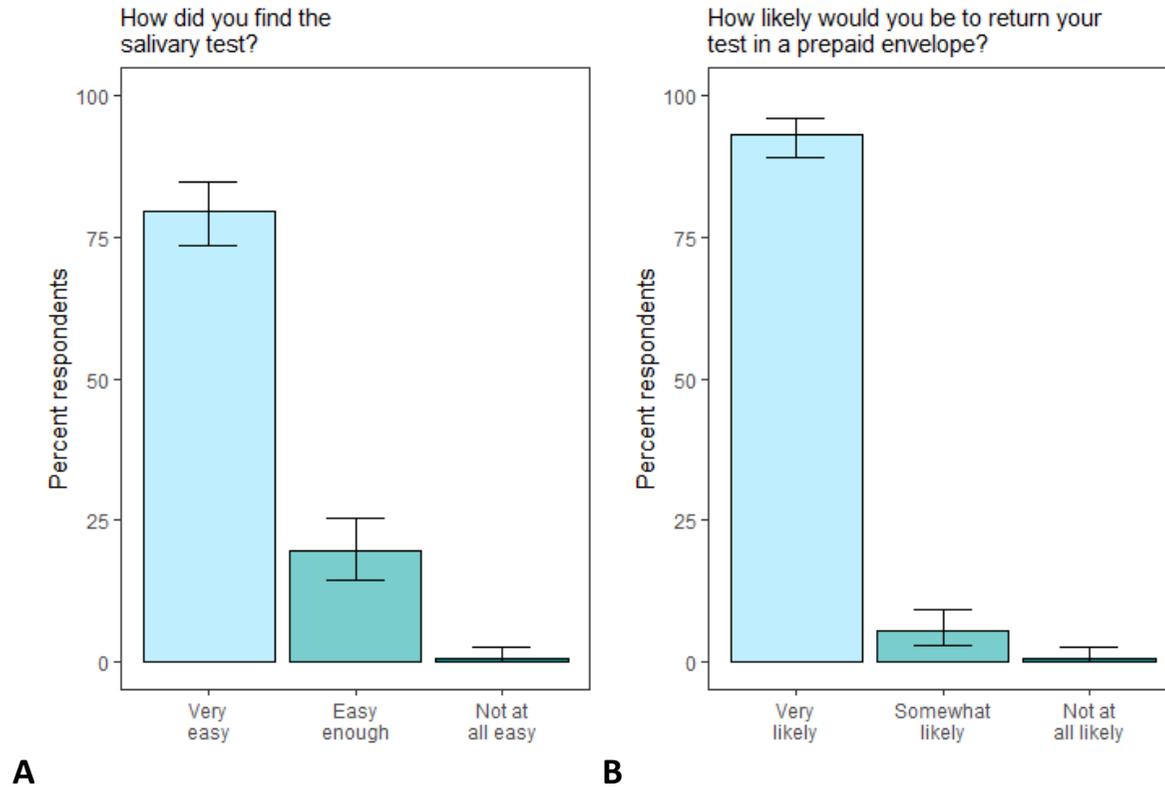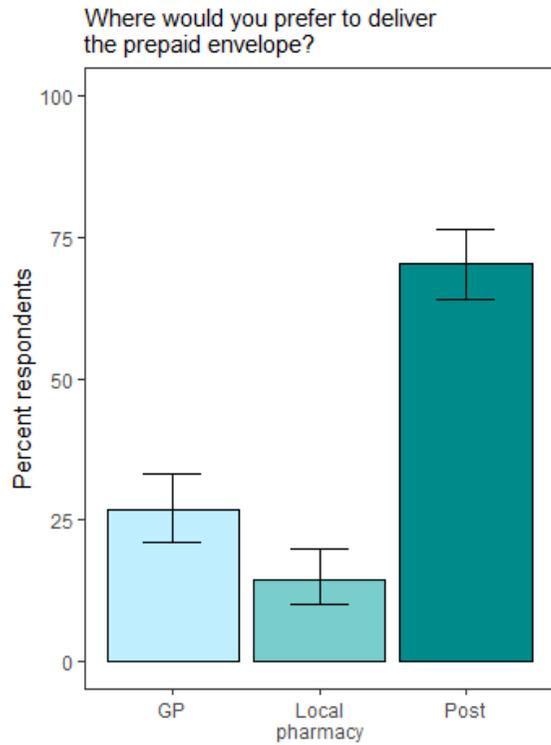
C
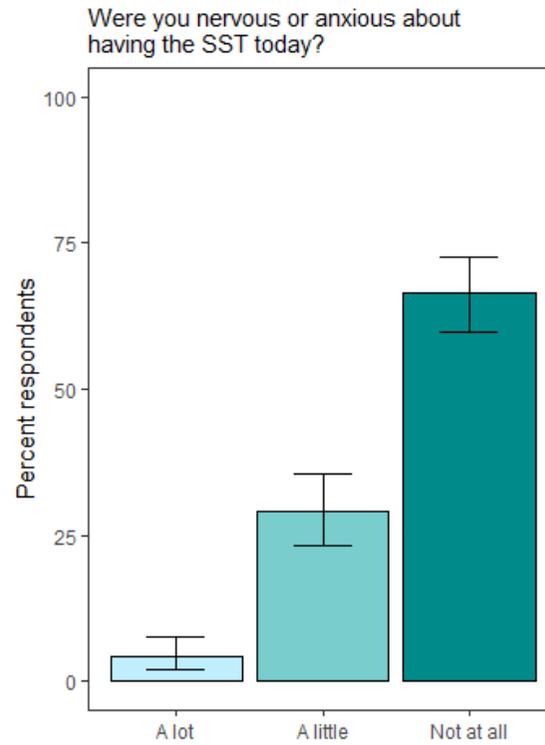
D

ACTH: adrenocorticotropin hormone. SST: short synacthen test (ACTH Stimulation Test)

4. Tables

Table S1: Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value for Index Tests

A. Index test: Waking Salivary Cortisone

a. Diagnostic cutoff for 'gold standard' of 15.6µg/dL

Waking Salivary Cortisone Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30 minute cortisol by LC-MS/MS **Prevalence of Adrenal Insufficiency: 94/208, 45.2%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥612ng/dL	81	96.8 (91.0 – 99.3)	68.4 (59.1, 76.8)	71.7 (63.0, 79.3)	96.3 (90.0, 99.2)
95% Specificity	Confirm Adrenal Insufficiency	<288ng/dL	78	78.7 (69.1, 86.5)	96.5 (91.3, 99.0)	94.9 (87.4, 98.6)	84.6 (77.2, 90.3)
99% Sensitivity	Exclude Adrenal Insufficiency	≥899ng/dL	46	100 (96.2, 100)	40.4 (31.3, 50.0)	58.0 (50.0, 65.7)	100 (92.3, 100)
99% Specificity	Confirm Adrenal Insufficiency	<36ng/dL	26	27.7 (18.9, 37.9)	100 (96.8, 100)	100 (86.8, 100)	62.6 (55.2, 69.7)
ACTH Stimulation Test 30 minute cortisol by Immunoassay **Prevalence of Adrenal Insufficiency: 91/208, 43.8%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥612ng/dL	81	96.7 (90.7, 99.3)	66.7 (57.4, 75.1)	69.3 (60.5, 77.2)	96.3 (89.6, 99.2)

			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
95% Specificity	Confirm Adrenal Insufficiency	<251ng/dL	73	75.8 (65.7, 84.2)	96.6 (91.5, 99.1)	94.5 (86.6, 98.5)	83.7 (76.4, 90.0)
99% Sensitivity	Exclude Adrenal Insufficiency	≥899ng/dL	46	100 (96.0, 100)	39.3 (30.4, 48.8)	56.2 (48.2, 64.0)	100 (92.3, 100)
99% Specificity	Confirm Adrenal Insufficiency	<36ng/dL	26	28.6 (19.6, 39.0)	100 (96.9, 100)	100 (86.8, 100)	64.3 (56.9, 71.2)

b. Diagnostic cutoff for 'gold standard' of 14.5μg/dL

Waking Salivary Cortisone Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30 minute cortisol by LC-MS/MS **Prevalence of Adrenal Insufficiency: 85/208, 40.9%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥540ng/dL	89	95.3 (88.4, 98.7)	69.1 (60.1, 77.1)	68.1 (58.9, 76.3)	95.5 (88.9, 98.8)
95% Specificity	Confirm Adrenal Insufficiency	<216ng/dL	67	71.8 (61.0, 81.0)	95.1 (89.7, 98.2)	91.0 (81.5, 96.6)	83.0 (75.7, 88.8)
99% Sensitivity	Exclude Adrenal Insufficiency	≥647ng/dL	79	100 (95.8, 100)	64.2 (55.1, 72.7)	65.9 (57.0, 74.0)	100 (95.4, 100)

				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
99% Specificity	Confirm Adrenal Insufficiency	<36ng/dL	26	30.6 (21.1, 41.5)	100 (97.1, 100)	100 (86.8, 100)	67.6 (60.3, 74.3)

ACTH Stimulation Test 30 minute cortisol by Immunoassay
Prevalence of Adrenal Insufficiency: 84/208, 40.4%

95% Sensitivity	Exclude Adrenal Insufficiency	≥540ng/dL	89	95.2 (88.3, 98.7)	68.6 (59.6, 76.6)	67.2 (58.0, 75.6)	95.5 (88.9, 98.8)
95% Specificity	Confirm Adrenal Insufficiency	<215ng/dL	67	72.6 (61.8, 81.8)	95.2 (89.8, 98.2)	91.0 (81.5, 96.6)	83.7 (76.5, 89.4)
99% Sensitivity	Exclude Adrenal Insufficiency	≥791ng/dL	61	100 (95.7, 100)	49.2 (40.1, 58.3)	57.1 (48.7, 65.3)	100 (94.1, 100)
99% Specificity	Confirm Adrenal Insufficiency	<36ng/dL	26	31.0 (21.3, 42.0)	100 (97.1, 100)	100 (86.8, 100)	68.1 (60.8, 74.8)

c. Diagnostic cutoff for 'gold standard' of 12.7µg/dL

Waking Salivary Cortisone Threshold		N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30 minute cortisol by LC-MS/MS						
Prevalence of Adrenal Insufficiency: 76/208, 36.5%						

95% Sensitivity	Exclude Adrenal Insufficiency	≥540ng/dL	89	96.1 (88.9, 99.2)	65.2 (56.4, 73.2)	61.3 (52.0, 70.1)	96.6 (90.5, 99.3)
95% Specificity	Confirm Adrenal Insufficiency	<144ng/dL	54	63.2 (51.3, 73.9)	95.5 (90.1, 98.3)	88.9 (77.4, 95.8)	81.8 (74.8, 87.6)
99% Sensitivity	Exclude Adrenal Insufficiency	≥647ng/dL	79	100 (95.3, 100)	59.9 (51.0, 68.3)	58.9 (49.9, 67.5)	100 (95.4, 100)
99% Specificity	Confirm Adrenal Insufficiency	<36ng/dL	26	34.2 (23.7, 46.0)	100 (97.2, 100)	100 (86.8, 100)	72.5 (65.4, 78.9)

ACTH Stimulation Test 30 minute cortisol by Immunoassay
Prevalence of Adrenal Insufficiency: 69/208, 33.2%

95% Sensitivity	Exclude Adrenal Insufficiency	≥360ng/dL	115	95.7 (87.8, 99.1)	80.6 (73.0, 86.8)	71.0 (60.6, 79.9)	97.4 (92.6, 99.5)
95% Specificity	Confirm Adrenal Insufficiency	<143ng/dL	54	69.6 (57.3, 80.1)	95.7 (90.8, 98.4)	88.9 (77.4, 95.8)	86.4 (79.9, 91.4)
99% Sensitivity	Exclude Adrenal Insufficiency	≥647ng/dL	79	100 (94.8, 100)	56.8 (48.2, 65.2)	53.5 (44.5, 62.3)	100 (95.4, 100)
99% Specificity	Confirm Adrenal Insufficiency	<36ng/dL	26	37.7 (26.3, 50.2)	100 (97.4, 100)	100 (86.8, 100)	76.4 (69.5, 82.3)

B. Index test: Waking Salivary Cortisol

a. Diagnostic cutoff for 'gold standard' of 15.6µg/dL

Waking Salivary Cortisol Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30 minute cortisol by LC-MS/MS **Prevalence of Adrenal Insufficiency: 87/197, 44.2%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥181ng/dL	64	95.4 (88.6, 98.7)	54.6 (44.8, 64.1)	62.4 (53.6, 70.7)	93.8 (84.8, 98.3)
95% Specificity	Confirm Adrenal Insufficiency	<36ng/dL	41	43.7 (33.1, 54.7)	97.3 (92.2, 99.4)	92.7 (80.1, 98.5)	68.6 (60.7, 75.8)
99% Sensitivity	Exclude Adrenal Insufficiency	≥399ng/dL	14	100 (95.9, 100)	12.7 (7.1, 20.4)	47.5 (40.1, 55.0)	100 (76.8, 100)
99% Specificity	Confirm Adrenal Insufficiency	<11ng/dL	1	1.2 (0.0, 6.2)	100 (96.7, 100)	100 (2.5, 100)	56.1 (48.9, 63.2)
ACTH Stimulation Test 30 minute cortisol by Immunoassay **Prevalence of Adrenal Insufficiency: 84/197, 42.6%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥180ng/dL	64	95.2 (88.3, 98.7)	53.1 (43.5, 62.6)	60.2 (51.3, 68.5)	93.8 (84.8, 98.3)

95% Specificity	Confirm Adrenal Insufficiency	<35ng/dL	41	45.2 (34.3, 56.5)	97.4 (92.4, 99.5)	92.7 (80.1, 98.5)	70.5 (62.7, 77.5)
99% Sensitivity	Exclude Adrenal Insufficiency	≥399ng/dL	14	100 (95.7, 100)	12.4 (6.9, 19.9)	45.9 (38.5, 53.4)	100 (76.8, 100)
99% Specificity	Confirm Adrenal Insufficiency	<11ng/dL	1	1.2 (0.0, 6.5)	100 (96.8, 100)	100 (2.5, 100)	57.7 (50.4, 64.7)

b. Diagnostic cutoff for 'gold standard' of 14.5µg/dL

Waking Salivary Cortisol Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30 minute cortisol by LC-MS/MS **Prevalence of Adrenal Insufficiency : 78/197, 39.6%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥181ng/dL	64	96.2 (89.2, 99.2)	51.3 (41.9, 60.5)	56.4 (47.5, 65.0)	95.3 (86.9, 99.0)
95% Specificity	Confirm Adrenal Insufficiency	<29ng/dL	38	42.3 (31.2, 54.0)	95.8 (90.5, 98.6)	86.8 (71.9, 95.6)	71.7 (64.0, 78.6)

				Sensitivity	Specificity	PPV	NPV
99% Sensitivity	Exclude Adrenal Insufficiency	≥399ng/dL	14	100 (95.4, 100)	11.8 (6.6, 19.0)	42.6 (35.4, 50.1)	100 (76.8100)
99% Specificity	Confirm Adrenal Insufficiency	<7ng/dL	1	1.3 (0.0, 6.9)	100 (97.0, 100)	100 (2.5, 100)	60.7 (53.5, 67.6)

ACTH Stimulation Test 30 minute cortisol by Immunoassay
Prevalence of Adrenal Insufficiency: 77/197, 39.1%

				Sensitivity	Specificity	PPV	NPV
95% Sensitivity	Exclude Adrenal Insufficiency	≥181ng/dL	64	96.1 (89.0, 99.2)	50.8 (41.6, 60.1)	55.6 (46.8, 64.3)	95.3 (86.9, 99.0)
95% Specificity	Confirm Adrenal Insufficiency	<36ng/dL	41	45.5 (34.1, 57.2)	95.0 (89.4, 98.1)	85.4 (70.8, 94.4)	73.1 (65.4, 79.9)
99% Sensitivity	Exclude Adrenal Insufficiency	≥399ng/dL	14	100 (95.3, 100)	11.7 (6.5, 18.8)	42.1 (34.8, 49.6)	100 (76.8, 100)
99% Specificity	Confirm Adrenal Insufficiency	<7ng/dL	1	1.3 (0.0, 7.0)	100 (97.0, 100)	100 (2.5, 100)	61.2 (54.02, 68.1)

c. Diagnostic cutoff for 'gold standard' of 12.7μg/dL

Waking Salivary Cortisol Threshold	N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)

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ACTH Stimulation Test 30 minute cortisol by LC-MS/MS
Prevalence of Adrenal Insufficiency : 70/197, 35.5%

95% Sensitivity	Exclude Adrenal Insufficiency	≥181ng/dL	64	95.7 (88.0, 99.1)	48.0 (39.1, 57.1)	50.4 (41.6, 59.2)	95.3 (86.9, 99.0)
95% Specificity	Confirm Adrenal Insufficiency	<29ng/dL	38	45.7 (33.7, 58.1)	95.3 (90.0, 98.3)	84.2 (68.8, 94.0)	76.1 (68.7, 82.5)
99% Sensitivity	Exclude Adrenal Insufficiency	≥399ng/dL	14	100 (94.9, 100)	11.0 (6.2, 17.8)	38.3 (31.2, 45.7)	100 (76.8, 100)
99% Specificity	Confirm Adrenal Insufficiency	<7ng/dL	1	1.4 (0.0, 7.7)	100 (97.1, 100)	100 (2.5, 100)	64.8 (57.7, 71.5)

ACTH Stimulation Test 30 minute cortisol by Immunoassay
Prevalence of Adrenal Insufficiency: 73/197, 37.1%

95% Sensitivity	Exclude Adrenal Insufficiency	≥145ng/dL	82	95.4 (86.7, 99.1)	59.0 (50.1, 67.4)	52.2 (42.7, 61.6)	96.3 (89.7, 99.2)
95% Specificity	Confirm Adrenal Insufficiency	<29ng/dL	38	50.8 (37.9, 63.6)	95.5 (90.5, 98.3)	84.2 (68.8, 94.0)	80.5 (73.5, 86.4)
99% Sensitivity	Exclude Adrenal Insufficiency	≥290ng/dL	29	100 (94.3, 100)	21.6 (15.0, 29.6)	37.5 (30.2, 45.3)	100 (88.1, 100)

Baseline Serum Cortisol Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
99% Specificity	Confirm Adrenal Insufficiency	<7ng/dL	1	1.6 (0.0, 8.5)	100 (97.28, 100)	100 (2.5, 100)	68.4 (61.4, 74.8)

C. Index test: Baseline Serum Cortisol by LC-MS/MS

a. Diagnostic cutoff for 'gold standard' of 15.6µg/dL

Baseline Serum Cortisol Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30 minute cortisol by LC-MS/MS **Prevalence of Adrenal Insufficiency: 100/219, 45.7%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥13µg/dL	33	95.0 (88.7, 98.4)	23.5 (16.2, 32.2)	51.1 (43.7, 58.5)	84.9 (68.1, 94.9)
95% Specificity	Confirm Adrenal Insufficiency	<5.3µg/dL	62	58.0 (47.7, 67.8)	96.6 (91.6, 99.1)	93.6 (84.3, 98.2)	73.3 (65.6, 80.0)
99% Sensitivity	Exclude Adrenal Insufficiency	≥16.6µg/dL	7	99.0 (94.6, 100)	5.0 (1.9, 10.7)	46.7 (39.8, 53.7)	85.7 (42.1, 99.6)
99% Specificity	Confirm Adrenal Insufficiency	<4.0µg/dL	49	48.0 (37.9, 58.2)	99.2 (95.4, 100)	98.0 (89.2, 100)	69.4 (61.9, 76.2)
ACTH Stimulation Test 30 minute cortisol by Immunoassay **Prevalence of Adrenal Insufficiency: 97/219, 44.3%**							

95% Sensitivity	Exclude Adrenal Insufficiency	≥13.1µg/dL	34	95.9 (89.8, 98.9)	24.6 (17.3, 33.2)	50.3 (42.8, 57.7)	88.2 (72.6, 96.7)
95% Specificity	Confirm Adrenal Insufficiency	<5.3µg/dL	65	60.8 (50.4, 70.6)	95.1 (89.6, 98.2)	90.8 (81.0, 96.5)	75.3 (67.7, 81.9)
99% Sensitivity	Exclude Adrenal Insufficiency	≥16.6µg/dL	7	100 (96.3, 100)	5.7 (2.3, 11.5)	45.8 (38.9, 52.7)	100 (59.0, 100)
99% Specificity	Confirm Adrenal Insufficiency	<4.0µg/dL	49	49.5 (39.2, 59.8)	99.2 (95.5, 100)	98.0 (89.2, 100)	71.2 (63.7, 77.9)

b. Diagnostic cutoff for 'gold standard' of 14.5µg/dL

Baseline Serum Cortisol Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30 minute cortisol by LC-MS/MS **Prevalence of Adrenal Insufficiency: 91/219, 41.6%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥13.1µg/dL	34	95.6 (89.1, 98.8)	23.4 (16.4, 31.7)	47.0 (39.7, 54.5)	88.2 (72.6, 96.7)

95% Specificity	Confirm Adrenal Insufficiency	<5.3µg/dL	62	61.5 (50.8, 71.6)	95.3 (90.1, 98.3)	90.3 (80.2, 96.4)	77.7 (70.4, 84.0)
99% Sensitivity	Exclude Adrenal Insufficiency	≥20.1µg/dL	2	100 (96.0, 100)	1.6 (0.2, 5.5)	41.9 (35.3, 48.8)	100 (15.8, 100)
99% Specificity	Confirm Adrenal Insufficiency	<2.5µg/dL	40	42.9 (32.5, 53.7)	99.2 (95.7, 100)	97.5 (86.8, 99.9)	71.0 (63.7, 77.5)

ACTH Stimulation Test 30 minute cortisol by Immunoassay
Prevalence of Adrenal Insufficiency: 89/219, 40.6%

95% Sensitivity	Exclude Adrenal Insufficiency	≥11.5µg/dL	54	95.5 (88.9, 98.8)	38.5 (30.1, 47.4)	51.5 (43.6, 59.4)	92.6 (82.1, 97.9)
95% Specificity	Confirm Adrenal Insufficiency	<4.9µg/dL	59	59.60 (48.6, 69.8)	95.4 (90.2, 98.3)	89.8 (79.2, 96.2)	77.5 (70.2, 83.7)
99% Sensitivity	Exclude Adrenal Insufficiency	≥13.8µg/dL	27	100 (95.9, 100)	20.8 (14.2, 28.8)	46.4 (39.2, 53.7)	100 (87.2, 100)
99% Specificity	Confirm Adrenal Insufficiency	<2.5µg/dL	40	43.8 (33.3, 54.8)	99.2 (95.8, 100)	97.5 (86.8, 99.9)	72.1 (64.0, 78.5)

c. Diagnostic cutoff for 'gold standard' of 12.7µg/dL

Baseline Serum Cortisol Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30 minute cortisol by LC-MS/MS **Prevalence of Adrenal Insufficiency: 81/219, 37.0%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥10.9µg/dL	61	95.1 (87.8, 98.6)	41.3 (33.0, 50.0)	48.7 (40.7, 56.8)	93.4 (84.1, 98.2)
95% Specificity	Confirm Adrenal Insufficiency	<4.9µg/dL	59	65.4 (54.0, 75.7)	95.7 (90.8, 98.4)	89.8 (79.2, 96.2)	82.5 (75.7, 88.1)
99% Sensitivity	Exclude Adrenal Insufficiency	≥20.1µg/dL	2	100 (95.6, 100)	1.5 (0.2, 5.1)	37.3 (30.9, 44.1)	100 (15.8, 100)
99% Specificity	Confirm Adrenal Insufficiency	<2.5µg/dL	40	48.2 (36.9, 60.0)	99.3 (96.0, 100)	97.5 (86.8, 99.9)	76.5 (69.6, 82.5)
ACTH Stimulation Test 30 minute cortisol by Immunoassay **Prevalence of Adrenal Insufficiency: 73/219, 33.3%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥8.2µg/dL	112	95.9 (88.5, 99.1)	74.7 (66.8, 81.5)	65.4 (55.6, 74.4)	97.3 (92.4, 99.4)
95% Specificity	Confirm Adrenal Insufficiency	<5.3µg/dL	62	75.3 (63.9, 84.7)	95.2 (90.4, 98.1)	88.7 (78.1, 95.3)	88.5 (82.5, 93.1)

				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
99% Sensitivity	Exclude Adrenal Insufficiency	≥10.9µg/dL	61	100 (95.1, 100)	41.8 (33.7, 50.2)	46.2 (38.3, 54.3)	100 (94.1, 100)
99% Specificity	Confirm Adrenal Insufficiency	<2.5µg/dL	40	53.4 (41.4, 65.2)	99.3 (96.2, 100)	97.5 (86.8, 99.9)	81.0 (74.5, 86.5)

D. Index test: Baseline Serum Cortisol by immunoassay

a. Diagnostic cutoff for 'gold standard' of 15.6µg/dL

Baseline Serum Cortisol Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30 minute cortisol by LC-MS/MS **Prevalence of Adrenal Insufficiency: 100/220, 45.5%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥11.8µg/dL	49	95.0 (88.7, 98.4)	36.7 (28.1, 46.0)	55.6 (47.8, 63.1)	90. 13.4(77.8, 96.6)
95% Specificity	Confirm Adrenal Insufficiency	<5.5µg/dL	65	59.0 (48.7, 68.7)	95.0 (89.4, 98.1)	90.8 (81.0, 96.5)	73.6 (65.9, 80.3)
99% Sensitivity	Exclude Adrenal Insufficiency	≥13.4µg/dL	26	99.0 (94.6, 100)	20.8 (14.0, 29.2)	51.0 (43.8, 58.3)	96.2 (80.4, 99.9)

99% Specificity	Confirm Adrenal Insufficiency	<4.8µg/dL	53	52.0 (41.8, 62.1)	99.2 (95.4, 100)	98.1 (89.9, 100)	71.3 (63.8, 78.0)

ACTH Stimulation Test 30 minute cortisol by Immunoassay
Prevalence of Adrenal Insufficiency: 97/220 , 44.1%

95% Sensitivity	Exclude Adrenal Insufficiency	≥11.2µg/dL	59	95.9 (89.8, 98.9)	44.7 (35.8, 53.9)	57.8 (49.7, 65.5)	93.2 (83.5, 98.1)
95% Specificity	Confirm Adrenal Insufficiency	<5.5µg/dL	65	60.82 (50.39, 70.58)	95.12 (89.68, 98.19)	90.77 (80.98, 96.54)	75.48 (67.94, 82.03)
99% Sensitivity	Exclude Adrenal Insufficiency	≥13.7µg/dL	25	100 (96.3, 100)	20.3 (13.6, 28.5)	49.7 (42.5, 57.0)	100 (86.3, 100)
99% Specificity	Confirm Adrenal Insufficiency	<4.7µg/dL	53	53.6 (43.2, 63.8)	99.2 (95.6, 100)	98.1 (89.9, 100)	73.1 (65.7, 79.6)

b. Diagnostic cutoff for 'gold standard' of 14.5µg/dL

Baseline Serum Cortisol Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30 minute cortisol by LC-MS/MS **Prevalence of Adrenal Insufficiency: 91/220, 41.4%**							

95% Sensitivity	Exclude Adrenal Insufficiency	≥11.1µg/dL	62	95.6 (89.1, 98.8)	45.0 (36.2, 54.0)	55.1 (47.0, 63.0)	93.6 (84.3, 98.2)
95% Specificity	Confirm Adrenal Insufficiency	<5.3µg/dL	62	61.5 (50.8, 71.6)	95.4 (90.2, 98.3)	90.3 (80.1, 96.4)	77.9 (70.6, 84.1)
99% Sensitivity	Exclude Adrenal Insufficiency	≥13.7µg/dL	25	100 (96.0, 100)	19.4 (13.0, 27.3)	46.7 (39.5, 53.9)	100 (86.3, 100)
99% Specificity	Confirm Adrenal Insufficiency	<4.5µg/dL	50	53.9 (43.1, 64.4)	99.2 (95.8, 100)	98.0 (89.4, 100)	75.3 (68.1, 81.6)

ACTH Stimulation Test 30 minute cortisol by Immunoassay
Prevalence of Adrenal Insufficiency: 89/220, 40.5%

95% Sensitivity	Exclude Adrenal Insufficiency	≥10.5µg/dL	73	95.5 (88.9, 98.8)	52.7 (43.8, 61.5)	57.8 (49.4, 65.9)	94.5 (86.6, 98.5)
95% Specificity	Confirm Adrenal Insufficiency	<5µg/dL	59	59.6 (48.6, 69.8)	95.4 (90.3, 98.3)	89.8 (79.2, 96.2)	77.6 (70.4, 83.8)
99% Sensitivity	Exclude Adrenal Insufficiency	≥13.7µg/dL	25	100 (96.0, 100)	19.1 (12.8, 26.9)	45.6 (38.5, 52.9)	100 (86.3, 100)
99% Specificity	Confirm Adrenal Insufficiency	<4.5µg/dL	50	55.1 (44.1, 65.6)	99.2 (95.8, 100)	98.0 (89.4, 100)	76.5 (69.4, 82.6)

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c. Diagnostic cutoff for 'gold standard' of 12.7µg/dL

Baseline Serum Cortisol Threshold			N diagnosed	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ACTH Stimulation Test 30minute cortisol by LC-MS/MS **Prevalence of Adrenal Insufficiency: 81/220, 36.8%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥9.7µg/dL	85	95.1 (87.8, 98.6)	58.3 (49.6, 66.6)	57.0 (48.2, 65.5)	95.3 (88.4, 98.7)
95% Specificity	Confirm Adrenal Insufficiency	<5µg/dL	59	65.4 (54.0, 75.7)	95.7 (90.8, 98.4)	89.8 (79.2, 96.2)	82.6 (75.9, 88.1)
99% Sensitivity	Exclude Adrenal Insufficiency	≥13.7µg/dL	25	100 (95.6, 100)	18.0 (12.0, 25.4)	41.5 (34.5, 48.8)	100 (86.3, 100)
99% Specificity	Confirm Adrenal Insufficiency	<4.5µg/dL	50	60.5 (49.0, 71.2)	99.3 (96.1, 100)	98.0 (89.4, 100)	81.2 (74.5, 86.8)
ACTH Stimulation Test 30 minute cortisol by Immunoassay **Prevalence of Adrenal Insufficiency: 73/220, 33.2%**							
95% Sensitivity	Exclude Adrenal Insufficiency	≥8.1µg/dL	113	95.9 (88.4, 99.1)	74.8 (67.0, 81.6)	65.4 (55.6, 74.4)	97.4 (92.4, 99.5)

95% Specificity	Confirm Adrenal Insufficiency	<5.3µg/dL	62	75.3 (63.9, 84.7)	95.2 (90.4, 98.1)	88.7 (78.1, 95.3)	88.6 (82.6, 93.1)
99% Sensitivity	Exclude Adrenal Insufficiency	≥9.1µg/dL	95	100 (95.1, 100)	64.6 (56.3, 72.3)	58.4 (49.3, 67.2)	100 (96.2, 100)
99% Specificity	Confirm Adrenal Insufficiency	<4.5µg/dL	50	67.1 (55.1, 77.7)	99.3 (96.3, 100)	98.0 (89.4, 100)	85.9 (79.7, 90.7)

Conversion serum cortisol µg/dL=nmol/L/27.6; cortisone ng/dL=(nmol/L/27.8)*1000; salivary cortisol ng/dL=(nmol/L/27.6)*1000. PPV: positive predictive value. NPV: negative predictive value.

Table S2: Clinical results associated with the primary analysis

	30 minute serum cortisol by immunoassay	
	Adrenal insufficiency	No Adrenal insufficiency
Positive Test (Waking Salivary Cortisone <252ng/dL (7nmol/L))	69	4
Negative Test (Waking Salivary Cortisone ≥612ng/dL (17nmol/L))	3	78
Unclear (≥252ng/dL (7nmol/L) Waking Salivary Cortisone <612ng/dL (17nmol/L))	19	35
No Waking Salivary Cortisone	6	6

Table showing the number of patients with adrenal Insufficiency or no adrenal Insufficiency for each waking salivary cortisone outcome. There were 4 patients who were diagnosed with adrenal insufficiency but were falsely positive and there were 3 patients who had adrenal insufficiency excluded but were falsely negative (See 2. Decision Analytic Model for Information**)**

Table S3: Differences in outcomes relative to the current strategy for alternative ACTH Stimulation Test cut-offs

Analysis	Differences in outcomes				
	True positive	True negative	False positive	False negative	No diagnosis
Current (ACTH Stimulation Test only)	94	119	0	0	7
Two stage (Waking Salivary Cortisone ± ACTH Stimulation Test)	93	118	4	3	2
Primary analysis	-1	-1	4	3	-4
SA1	-2	-3	6	4	-5
SA2	-1	-2	6	3	-5

1. Primary Analysis: ACTH Stimulation Test using Immunoassay, 15.6µg/dL cutoff, <251ng/dL & ≥612ng/dL thresholds (95% specificity and sensitivity)
2. SA1: Secondary Analysis 1: ACTH Stimulation Test using Immunoassay, 14.5µg/dL cutoff, <215ng/dL & ≥540ng/dL thresholds (95% specificity and sensitivity)
3. SA2: Secondary Analysis 2: ACTH Stimulation Test using Immunoassay, 12.7µg/dL cutoff, <143ng/dL & ≥360ng/dL thresholds (95% specificity and sensitivity)

Table showing the difference in outcomes between patients having the ACTH Stimulation Test only as per current testing as opposed to the two-stage process, with the waking salivary cortisone followed if necessary by the ACTH Stimulation Test if not diagnostic. This assumes with current testing no false positive or false negative. The "no diagnosis" column represents participants who did not receive a waking salivary cortisone diagnosis or ACTH Stimulation Test diagnosis, which are principally those who don't attend for the test (plus some who's waking salivary cortisone sample is inadequate then don't turn up for the ACTH Stimulation Test). With the two-stage strategy, fewer people are left without a diagnosis because if they don't return their waking salivary cortisone, they then get a second chance for a diagnosis via the ACTH Stimulation Test. Conversions: serum cortisol µg/dL=nmol/L/27.6; cortisone ng/dL=(nmol/L/27.8)*1000; salivary cortisol ng/dL=(nmol/L/27.6)*1000; ACTH: adrenocorticotropin hormone.

Table S4: Supplementary Table on the Representativeness of Study Participants

Category	
Disease, problem, or condition under investigation	Adrenal Insufficiency: Primary, Secondary and Tertiary
Special considerations related to	
Sex and gender	Primary adrenal Insufficiency is more common in women, whereas secondary and tertiary adrenal Insufficiency have equal representation.
Age	Primary adrenal Insufficiency presents usually between 20-50 years of age, secondary adrenal Insufficiency the incidence increases with age and tertiary can present at any age and depends on glucocorticoid exposure.
Race or ethnic group	Primary adrenal Insufficiency is more common in people of European descent than African descent.
Geography	In industrialized countries autoimmune Addison's Disease and congenital adrenal hyperplasia account for the majority of patients with primary adrenal Insufficiency, whereas in regions with high incidence of tuberculosis then infections are the commonest cause. Tertiary adrenal insufficiency is the commonest type of adrenal Insufficiency as up to 3% of population are on systemic glucocorticoids [1,2] and a significant number of patients in the population are on opiates [3]. Geographical distribution depends on glucocorticoid and opiate use in a country.
Overall representativeness of this study	Most patients in our study were assessed for tertiary adrenal Insufficiency, that is glucocorticoid induced adrenal Insufficiency (around 70%). The percentage of adrenal Insufficiency in this group is similar to that of a high-risk population that is around 50% (metaanalysis for glucocorticoid induced adrenal Insufficiency – 1419 patients on oral glucocorticoids [4]). With regards to age and gender, our population is similar to a large epidemiological study of oral glucocorticoids investigating clinical indicators of adrenal Insufficiency when weaning (n=286, 680), with nearly equal representation [5]. 90% of our study group were white, 5% were Asian, and the rest black/Caribbean/African and Mixed. The proportions were equally representative of the prevalence of

Category	
	different races (89.8% white, 2.3% Asian, 0.9% black) in a retrospective record-linkage open-cohort study, investigating the prevalence of glucocorticoid induced adrenal Insufficiency, spanning primary and hospital care in England, including 70, 000 patients on oral glucocorticoids[6].

d. References

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