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Home Waking Salivary Cortisone to Screen for Adrenal Insufficiency

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Abstract

Background: Worldwide, adults and children are at risk of adrenal insufficiency due to infectious diseases and adrenal suppression from use of anti-inflammatory glucocorticoids and opiates. The Adrenocorticotropin (ACTH) Stimulation Test is the reference standard for diagnosis but requires clinic attendance and venesection. Salivary cortisone reflects free serum cortisol and can be collected at home and posted to a laboratory. We tested whether home waking salivary cortisone could be used to screen for adrenal insufficiency.

Methods: A prospective, diagnostic accuracy study was performed in patients at high risk of adrenal insufficiency. Patients collected a salivary sample on waking and then attended the clinical facility for an ACTH Stimulation Test. Salivary cortisone was measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). Receiver Operating Characteristics (ROC) curves were computed and positive (PPV) and negative (NPV) predictive values calculated.

Results: As measured by an ACTH Stimulation Test, the prevalence of adrenal insufficiency was 44%. The area under ROC curve (AuROC) for waking salivary cortisone as a predictor of adrenal insufficiency was 0.95 (95%CI 0.92 to 0.97). Cut-offs to ensure a minimum of 95% sensitivity and specificity gave a NPV of 96% (95%CI 90 to 99%) and a PPV of 95% (95%CI 87 to 99%) to exclude and confirm adrenal insufficiency, respectively. Waking salivary cortisone data provided similar information as an ACTH Stimulation Test in 70% of participants. Eighty-two percent of patients preferred home salivary collection to clinic attendance.

Conclusion: Home waking salivary cortisone has accuracy for the diagnosis of adrenal insufficiency similar to that of a standard ACTH Stimulation Test. Patients found the “at home” test more convenient than the hospital based test.

Introduction

Adrenal insufficiency, or cortisol deficiency, is a life-threatening condition which can be primary (adrenal), secondary (pituitary) and tertiary (adrenal suppression)[1]. Prevalence is rising due to the increased prescription of glucocorticoid and opioid therapies that suppress adrenal function[2,3 4-6]. Fifty percent of patients on oral glucocorticoids have adrenal suppression[4 7,8], and approximately 10% of patients on a morphine equivalent >20mg/day are at risk of adrenal insufficiency[5]. In low- and middle-income countries (LMIC), tuberculosis remains a common cause of adrenal insufficiency[9]. If left untreated, adrenal insufficiency can result in an adrenal crisis, which carries a 6% mortality rate[1], and 6-8% of individuals with adrenal insufficiency have an adrenal crisis each year[1]. Recognition of adrenal insufficiency is essential but diagnosis is often delayed[10-12 13]; the majority of patients are only diagnosed during an acute hospital admission[14], which implies that patients are at risk of dying from an adrenal crisis before diagnosis. Therefore, a simple, cost-effective screening test to diagnose adrenal insufficiency is needed.

The standard test for adrenal insufficiency is the Adrenocorticotropin (ACTH) Stimulation Test, also called the Short Synacthen Test[13 15 16]. While this test is considered accurate, it requires a clinic visit and venesection. Cortisol has a circadian rhythm that peaks shortly after waking and declines over the day to low levels in the evening[17], and a morning serum cortisol level can also identify adrenal insufficiency[18], followed by an ACTH Stimulation Test if results are indeterminate[19 20]. However, this screening approach still requires a clinic visit and venesection.

Salivary glucocorticoid sampling has a number of advantages over serum sampling. Since salivary glucocorticoids are stable at room temperature, patients can collect their own

sample at home and mail it to the laboratory[21]. Salivary cortisol is derived from serum free cortisol, and late-night salivary cortisol is used in the diagnosis of Cushing's syndrome[22,23]. The salivary gland has high levels of 11β-hydroxysteroid dehydrogenase 2, which converts free cortisol to cortisone. Thus, salivary cortisone correlates better with serum cortisol than salivary cortisol, as salivary cortisone levels are higher than salivary cortisol and are detectable at low serum cortisol levels[24].

The current diagnostic strategy is for patients to be referred for an ACTH stimulation test, using a cut-off to confirm (<15.6μg/dL (430nmol/L)) or exclude (≥15.6μg/dL (430nmol/L)) adrenal insufficiency. We hypothesised that a home waking salivary cortisone could predict a normal or abnormal ACTH Stimulation Test when screening for adrenal insufficiency.

Methods

Study Design and Oversight

We performed a prospective, diagnostic accuracy study to assess the relationship between waking salivary cortisone and the 30-minute cortisol on an ACTH Stimulation Test in patients with adrenal insufficiency. The study protocol was approved by the South Yorkshire and the Humber Research Ethics Committee, Reference 19/YH/0333. Written informed consent was obtained from all participants. The study is reported as per Standards for Reporting of Diagnostic Accuracy (STARD) guidelines (1.STARD, Supplementary Appendix, page 3) [25].

The first and last authors wrote the first draft and together with statisticians vouch for the accuracy, completeness of the data and analyses. All authors critically reviewed the manuscript and participated in the design of the trial. There was no commercial support for this study.

Participants

Patients were recruited by consecutive sampling at Sheffield Teaching Hospitals NHS Foundation Trust, UK (a University Hospital), between November 2019 and December 2021. All patients referred for an ACTH Stimulation Test to assess for a new diagnosis of adrenal insufficiency or for recovery from a previous diagnosis of adrenal insufficiency were considered for the study. Patients greater than 18 years of age with a high probability of either primary, secondary or tertiary adrenal insufficiency as determined by the investigators were eligible for enrolment. This included patients dependent on any type of glucocorticoid, who had been on an oral glucocorticoid prednisolone equivalent dose of ≥5mg/day for 4 weeks or more and were referred for adrenal testing only after they had

been weaned down to prednisolone ≤5mg/day or equivalent or converted to physiological doses of hydrocortisone ≤25mg/day. Patients receiving any intermediate or long-acting intramuscular or intra-articular glucocorticoid injections were recruited at least 3 months after their last injection. Other patients included those with pituitary disease, such as tumors, inflammatory disease, or those with a history of cranial radiotherapy (Table1).

Exclusion criteria included patients unable to produce a suitable saliva sample, night shift workers, patients with known protein losing disorders, known or suspected alcohol dependence, known severe liver disease, patients with uncontrolled active infection and patients on estrogens or those who were pregnant. Patients on drugs that influence the hypothalamo-pituitary-adrenal axis (e.g opioids) had the medications omitted on the day of testing as per routine clinical practice. In view of COVID measures resulting in limited staff and fewer appointment slots to enable study tasks, some patients were excluded from study participation so as not to hinder their clinical care (Figure 1). All patients enrolled received an information pack, which included an invitation letter, participant information sheet, and a Salivette.

Procedures

On the day of their scheduled ACTH Stimulation Test, all individuals provided a salivary sample on waking using Salivette tubes containing synthetic swabs (Salivette® Cortisol, Sarstedt, Numbrecht, Germany). A total of 500µL of saliva were necessary to ensure a good representative sample, and 50µL were used for the assay. All patients were given written instructions with images and a video (https://www.youtube.com/watch?v=OIylGyEmP-c&t=36s) on how to collect their salivary sample. Patients were advised to refrain from smoking/vaping on the day of the test. Patients on glucocorticoids were asked to omit these

medicines the evening before and the day of the test until all samples were collected. Patients were allowed to follow their usual waking routines but were asked to collect their waking salivary sample at the moment they got out of bed to commence the day and before cleaning teeth, eating, or drinking.

At the endocrine clinic, participants completed the first part of a patient questionnaire to assess patient views on salivary testing at home. They also completed a Case Report Form to collect demographic data (Table 1 and Table S4, Supplementary Appendix, page 30). An intravenous cannula was inserted, and a baseline serum cortisol was measured. An ACTH Stimulation Test was performed with intravenous injection of 250µg of Synacthen (Atnahs Pharma UK Limited, Essex, UK) followed by a 30-minute serum cortisol level blood draw. Tests were carried out by specialized, experienced endocrine nurses at the clinic. On completing the ACTH Stimulation Test, patients completed the final part of the questionnaire assessing their views on the ACTH Stimulation Test and salivary sample collection. Results of the ACTH Stimulation Test were interpreted by a consulting endocrinologist or a specialized endocrine nurse who were unaware of the waking cortisol data. An a priori criterion of a peak cortisol of ≥15.6µg/dL (430nmol/l) measured by immunoassay indicated adequate adrenal reserve, whereas those below this value were considered as having adrenal insufficiency, according to current clinical practice in our center[13,26].

Outcome Measures

The primary outcome measure was waking salivary cortisone to exclude adrenal insufficiency identified by Receiver Operating Characteristic (ROC) analysis. As secondary outcome measures, we also assessed waking salivary cortisol and baseline serum cortisol

levels at ACTH Stimulation Test to exclude adrenal insufficiency. The aim was to assess whether we can use waking salivary cortisone in a two-stage diagnostic process to exclude and diagnose adrenal insufficiency with the patient undertaking a waking salivary cortisone test at home, assigning a diagnosis based on waking salivary cortisone level cut-offs derived from the study to confirm or exclude adrenal insufficiency with intermediate values triggering a referral for an ACTH Stimulation Test (additional details are given in the Decision Analytic Model, Supplementary Appendix, page 5, with the associated decision trees for the two diagnostic strategies being shown in Figures S2 and S3, Supplementary Appendix, pages 7, 8).

Assays

Serum cortisol was analysed by Immunoassay (Elecsys Cortisol II assay from Roche) and interpreted immediately at Sheffield Teaching Hospitals NHS Foundation Trust. An extra serum cortisol was stored at -80^{o}C and together with the salivary sample was then analysed and interpreted by liquid chromatography and tandem mass spectrometry (LC-MS/MS) as a batch at the end of the study in a different laboratory in Manchester University NHS Foundation Trust [24].

Statistical Analysis

An ROC curve was computed to assess the diagnostic accuracy of waking salivary cortisone to detect an abnormal ACTH stimulation test. The area under the receiver operating characteristics curve (AuROC) was reported with associated 95% confidence intervals (CIs). Summaries of diagnostic accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were reported with 95% Cis calculated using the Clopper-

Pearson method. Linear regression models were used to investigate variables' effects on waking salivary cortisone. The study sample size for estimating the AuROC, sensitivity and specificity of the test with a given degree of precision, assuming a prevalence of adrenal insufficiency (ACTH Stimulation Test 30-minute cortisol <15.6µg/dL(<430nmol/L)) around 50% in the proposed target population [4], and a sensitivity of 80% and specificity of 95%, was around 200 participants (100 with adrenal insufficiency; 100 without). In this way, the AuROC curve can be estimated within ±0.06 with 95% CIs (i.e. 0.74 to 0.86) assuming the new test has an AuROC of 0.80. We therefore aimed to recruit consecutive patients until there were 100 patients with adrenal insufficiency or until a total maximum of 300 patients were recruited. As part of a sensitivity analysis to test whether waking salivary cortisone levels had similar predictive power to the 30-minute ACTH Stimulation Test cortisol cut-off of 15.6µg/dL (430nmol/L), we explored other cut-off values, including 14.5µg/dL (400nmol/L) and 12.7µg/dL (350nmol/L) derived from the literature[26 27]. Analyses were performed on a complete case basis.

Results

Patients

Two hundred and eighty-one patients were screened, and 220 patients were recruited. By ACTH Stimulation Test result measured by immunoassay, 96 patients were classified as having adrenal insufficiency and 124 patients had no adrenal insufficiency. After recruitment, 12/220 (5.5%) samples were excluded due to inadequate salivary sample collection (Figure 1). A total of 208 individuals, 91 patients (44%) with adrenal insufficiency and 117 patients (56%) with no adrenal insufficiency, were analysed for the primary outcome measure. Eleven of the 220 (5%) saliva samples were contaminated with hydrocortisone from residual oral hydrocortisone last administered the day before, and salivary cortisol measurements for these individuals were therefore excluded from analysis (their salivary cortisone was still utilized in analysis). Baseline demographics are represented in Table 1. Participants had a mean age (standard deviation [SD]) of 55.1 (15.8) years and were 49% female. Overall, 67% of patients were being screened for glucocorticoid-induced adrenal insufficiency, with 81% on a glucocorticoid formulation, including glucocorticoid replacement for adrenal disease or pituitary disease post-surgery (Table 1). One hundred percent of patients diagnosed with adrenal insufficiency were on glucocorticoids. Eleven patients were on opioids; of these, three patients had adrenal insufficiency and were also on glucocorticoids. The study population, when considering age, sex, and race, was broadly representative of the population with adrenal insufficiency, particularly tertiary adrenal insufficiency, as reported in large epidemiological studies. Ninety percent of our study group were white, 5% were Asian, and the remaining were Black/Caribbean/African and Mixed (Table S4, Supplementary Appendix, page 30).

Waking saliva cortisone as a predictor of adrenal insufficiency

The median waking salivary cortisone was 828.9ng/dL (intraquartile range, 509.6 to 1046.4ng/dL) for participants with no adrenal insufficiency and 92.4ng/dL (31.7 to 245.2ng/dL) for patients with adrenal insufficiency (Table 2). Waking salivary cortisone predicted the ACTH Stimulation Test 30-minute serum cortisol >15.6µg/dL (>430nmol/L) as measured by immunoassay with an AuROC of 0.95 (95%CI, 0.92 to 0.97) (Figure 2A). Using a waking salivary cortisone cut-off value of ≥612ng/dL (17nmol/L) excluded adrenal insufficiency with a sensitivity of 97% (95%CI, 91 to 99%) and a NPV 96% (95%CI, 90 to 99%), while using a cut-off of <251ng/dL (7nmol/L) confirmed adrenal insufficiency with a specificity of 97% (95%CI, 92 to 99%) and PPV 95% (95%CI, 87 to 99%). The results were reproduced and similar when using the LC-MS/MS for ACTH Stimulation Test (Table S1, Supplementary Appendix, page 11) and when using different cut-offs for the 30-minute serum cortisol including 14.5µg/dL (400nmol/L) and 12.7µg/dL (350nmol/L) (Table S1, Figure S1, Supplementary Appendix, page 11, 6) as part of a sensitivity analysis. To achieve at least 99% sensitivity to exclude adrenal insufficiency and 99% specificity to confirm adrenal insufficiency one would need to use the cut-offs ≥899ng/dL (25nmol/l) and <36ng/dL (1nmol/L), respectively.

Secondary measures

Waking salivary cortisol was a slightly weaker predictor of the ACTH Stimulation Test 30-minute serum cortisol >15.6µg/dL (>430nmol/L), with an AuROC of 0.89 (95%CI, 0.85 to 0.94) (Figure 2B). A cut-off value of ≥180ng/dL (5nmol/L) excluded adrenal insufficiency with a sensitivity of 95% (95%CI, 88 to 99%) and NPV of 94% (95%CI, 85 to 98%), while a cut-off value of <35ng/dL (1nmol/L) confirmed adrenal insufficiency with a specificity of 97%

(95%CI, 92 to 100%) and a PPV of 93% (95%CI, 80 to 99%). Similarly, baseline serum cortisol measured by immunoassay was a slightly weaker predictor than waking salivary cortisone, with an AuROC of 0.90 (95%CI, 0.86 to 0.94) (Figure 2C). A cut-off of ≥11.2µg/dL (310nmol/L) excluded adrenal insufficiency with a sensitivity of 96% (95%CI, 90 to 99%) and a NPV of 93% (95%CI, 84% to 98%) whereas a cut-off <5.5ug/dL (152nmol/L) confirmed adrenal insufficiency with a specificity of 95% (95%CI, 90 to 98%) and a PPV 91% (95%CI, 81 to 97%). The results were reproduced and similar when using LC-MS/MS for ACTH Stimulation Test cortisol tests (Figure 2D), including using different cut-offs for the 30-minute serum cortisol including 14.5µg/dL (400nmol/L) and 12.7µg/dL (350nmol/L) as part of a sensitivity analysis (Table S1, Supplementary Appendix, page 11).

Further analysis examined the percentage of ACTH Stimulation Tests that could have been avoided using waking salivary cortisone cut offs as a screening test in a two-stage process. The ACTH Stimulation Test would have been avoided in 70% (154/220) of participants, 73 patients with waking salivary cortisone <251ng/dL (7nmol/L) and 81 patients with waking salivary cortisone ≥612ng/dL (17nmol/L). This two-stage process resulted in 4 patients who were falsely positive and 3 patients who were falsely negative (Table S2, S3, Supplementary Appendix, pages 28, 29).

Factors influencing waking salivary cortisone

We carried out univariable regression analysis and found no significant influence on waking salivary cortisone for a one year increase in age (β-0.09; 95%CI -0.2 to 0.01), sex (females) (β, 0.65; 95%CI -2.61 to 3.91), for a one unit increase in BMI (β, 0.06; 95%CI -0.18 to 0.3), smoking status (Previous smoking history β, 3.02; 95%CI -0.48 to 6.51; Current smoker β, 1.72; 95% CI -4.38 to 7.81) and alcohol intake (Y/N β, 1.28; 95%CI -2.01 to 4.56; Average

units β, -0.11; 95%CI -0.53 to 0.31). In a multivariable regression analysis examining whether different glucocorticoid formulations influence waking salivary cortisone, use of oral glucocorticoids (β, -12.66; 95%CI -15.65 to -9.67) was significant, whereas inhaled (β, -0.73; 95%CI -3.87 to 2.41), topical (β, -3.99; 95%CI -9.25 to 1.27) and nasal (β, -5.62; 95%CI -12.67 to 1.44) glucocorticoids had no impact.

Patient preferences

Two hundred eighteen out of two hundred twenty (99%, 95%CI 97 to 100%) patients stated that it was acceptable to do a salivary test at home and 175/219 (80%, 95%CI 74 to 85%) responded that performing the test was very easy. One hundred eighty out of two hundred sixteen (83%, 95%CI 78 to 88%) patients preferred the salivary test at home to the ACTH Stimulation Test in the clinical facility (Figure 3).

Discussion

We have shown that waking salivary cortisone was a strong predictor of the 30-minute ACTH stimulation test serum cortisol level; data from this test would have been sufficient to allow the provider to make an accurate diagnosis in 70% of individuals at risk of adrenal insufficiency. The home waking salivary cortisone was preferred by patients to an ACTH Stimulation Test.

Defining cut-offs for the waking salivary cortisone as an adrenal insufficiency screening test is likely to vary among centers as the criteria for diagnosis of adrenal insufficiency varies among doctors according to etiology, cortisol assay and reference ranges applied. Commonly used cortisol immunoassays give variable results and require individual reference ranges; therefore, LC-MS/MS assays are now considered the reference standard for measuring levels of glucocorticoids in biological fluids [28]. Our results were validated by retesting using an LC-MS/MS assay. The major challenge in the diagnosis and treatment of adrenal insufficiency is that there are no established biomarkers of normal cortisol levels used clinically, and clinicians usually decide when to replace cortisol based on the cortisol level in response to an ACTH Stimulation Test and symptoms such as fatigue. The Endocrine Society guidelines suggest a peak cortisol level <18.0 ug/dL (500nmol/L) (which is assay dependent) at 30-minutes of an ACTH Stimulation Test indicates adrenal insufficiency[29]. In our study, we defined adrenal insufficiency as a peak cortisol of <15.6µg/dL (<430nmol/L) measured by immunoassay. This definition of adrenal insufficiency was based on our routine laboratory immunoassay reflective of the widespread use of immunoassays globally in clinical care [26]. The prevalence of adrenal insufficiency in our study was 44%. Cut-offs to ensure a minimum of 95% sensitivity and specificity gave a NPV of 96% (95%CI 90 to 99%)

and a PPV of 95% (95%CI 87 to 99%) to exclude and confirm adrenal insufficiency, respectively. Using these values, we were able to establish cut-offs for waking salivary cortisone that defined normal cortisol secretion and diagnosed adrenal insufficiency. In a sensitivity analysis, we have shown waking salivary cortisone works equally well at different cut-offs of ACTH Stimulation Test including 14.5µg/dL (400nmol/L) and 12.7µg/dL (350nmol/L) and gave similar results when we analysed the serum cortisol samples by LC-MS/MS.

Using these identified cut-offs, ≥612ng/dL (17nmol/L) and <251ng/dL (7nmol/L) there was a very small number of patients who had false negative results (n=3), that is, had a falsely high waking salivary cortisone, or false positive (n=4), that is, had a falsely low waking salivary cortisone. We believe the false positive and false negative rate will have minimal overall clinical impact on the individual patient. Our false negative waking salivary cortisone values varied between 647 - 863ng/dL (18 to 24nmol/L), which are borderline levels considering the waking salivary cortisone threshold of ≥612ng/dL (17nmol/l). In most settings, patients with borderline results will be given advice about steroid sick day rules and retested.

The ACTH Stimulation Test is an indirect test of adrenal reserve and function and has a specificity of 95% and sensitivity of 97% in primary adrenal insufficiency, but for secondary adrenal insufficiency the sensitivity to diagnose adrenal insufficiency is lower compared to the insulin tolerance test [30,31]. The waking salivary cortisone directly reflects physiologic serum cortisol levels, and with increasing adoption of salivary glucocorticoid assay platforms, the ability to test will become more mainstream. If this is the case, then the waking salivary cortisone has the potential to supersede the ACTH Stimulation Test as the diagnostic test for adrenal insufficiency. Major reference laboratories across the world

report salivary cortisol measured by LC-MS/MS, reference standard for measuring glucocorticoids, but do not report salivary cortisone. Analysis by LC-MS/MS can separately measure salivary cortisol and cortisone in the same sample, at the same time, and at no incremental cost[24]. The measurement of salivary cortisol in many laboratories is by immunoassay and in laboratories that cannot measure salivary cortisone by LC-MS/MS the use of waking salivary cortisol could be an acceptable alternative screening test. In our study, waking salivary cortisol was only a slightly weaker predictor of the ACTH Stimulation Test than waking salivary cortisone: AuROC 0.89 vs 0.95. However, clinicians should be aware that taking oral hydrocortisone in the 24 hours prior to testing can give a falsely elevated salivary cortisol due to oral contamination.

Five percent of patients returned an inadequate salivary sample and further work is required to identify the cause of this occurrence and whether better instruction could reduce this figure. The strength of our study is that we have tested the use of waking salivary cortisone measured by LC-MS/MS in a high-risk group of patients for adrenal insufficiency recruited prospectively. The limitations of this study include the fact we only studied a high-risk population; however, this was important to have sufficient patients with adrenal insufficiency to determine the PPV and NPV. It is likely that the incidence of adrenal insufficiency will vary in different patient populations, but the reference ranges used clinically for cortisol are similar across ages, sexes and most populations. We have not separately analysed patients with potentially reversible adrenal suppression from those with likely permanent adrenal suppression. This would be unlikely to alter the results since we are measuring adrenal status at one point in time, and the clinical decision regarding management depends on whether the clinician believes the adrenal insufficiency is

reversible, in which case the test will be repeated. One needs to take into consideration the ACTH Stimulation Test may give false negative results in secondary adrenal insufficiency such as immediately after pituitary surgery, where the insulin tolerance test may be more accurate; however, all our patients had an ACTH Stimulation Test at least 6 weeks from a pituitary event and therefore the risk of a false negative result is likely small. Some studies show the 60-minute cortisol value in the ACTH Stimulation Test improves diagnostic performance albeit overall in a small number of individuals (5%)[32]. Future studies can explore this aspect to further validate our data.

In this study, we have shown that waking salivary cortisone provides data that allows a reasonably accurate prediction of whether a given patient has adrenal sufficiency or insufficiency.

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Figure Legends

1. **Recruitment Flowchart for Primary Analysis**: Figure indicates total number of patients who were at high risk for adrenal insufficiency recruited by consecutive sampling between November 2019 and December 2021. Two hundred and eight were analyzed for the primary endpoint.
2. **Receiver Operator Curves (ROCs) to assess Predictive Value for the 30-minute ACTH Stimulation Test Cortisol reference test by the Index Tests:** Waking Salivary Cortisone (A), Waking Salivary Cortisol (B), Baseline Serum Cortisol by Immunoassay (C) and Baseline Serum Cortisol by LC-MS/MS (D). The 30-minute ACTH Stimulation Test cortisol cut-off is 15.6µg/dL. Area under the receiver operator curves indicate the strongest predictive value for waking salivary cortisone with lower predictive power for waking salivary cortisol and baseline serum cortisol. Conversions: serum cortisol µg/dL=nmol/L/27.6; salivary cortisone ng/dL=(nmol/L/27.8)*1000; salivary cortisol ng/dL=(nmol/L/27.6)*1000; AI: Adrenal Insufficiency
3. **Questionnaire results to assess patient views on ACTH Stimulation Test and waking saliva cortisone:** Bar charts indicate percentage respondents (95% CI) for survey results. Questions included patient views on salivary test acceptability (A), test preference (B, C) and response likelihood and ACTH Stimulation Test anxiety (Figure S4, Supplementary Appendix, page 9); SST: Short Synacthen Test or ACTH Stimulation Test. [1] For panel C, the specific question asked was "Do you prefer the SST in hospital or the Salivary test at home?". We have reported this as clinical facility since an ACTH Stimulation Test can take place in a number of different clinical venues.

Tables

Table 1 Baseline demographics and clinical characteristics for patients providing a valid waking salivary cortisone

	Overall (N=208)	adrenal insufficiency negative (N=117)	adrenal insufficiency positive (N=91)	p-value
Age (years)†	55.1 (15.8)	52.4 (16.7)	58.6 (13.9)	0.004
Sex*				
Male	106 (51.0%)	59 (50.4%)	47 (51.6%)	0.86
Female	102 (49.0%)	58 (49.6%)	44 (48.4%)	
Weight (kg)†	86.2 (20.3)	88.4 (21.4)	83.3 (18.6)	0.86
BMI†	29.9 (6.92)	30.3 (7.42)	29.3 (6.23)	0.30
On glucocorticoids*				
No	40 (19.2%)	40 (34.2%)	0 (0%)	<0.001
Yes	168 (80.8%)	77 (65.8%)	91 (100%)	
Primary glucocorticoid delivery route				
Oral	140 (67.3%)	52 (44.4%)	88 (96.7%)	
Other§	28 (13.5%)	25 (21.4%)	3 (3.3%)	
Reason for testing				
Glucocorticoid induced adrenal insufficiency	139 (66.8%)	73 (62.4%)	66 (72.5%)	
Pituitary Disease	42 (20.2%)	25 (21.4%)	17 (18.7%)	
Unilateral Adrenalectomy	13 (6.3%)	5 (4.3%)	8 (8.8%)	
Symptomatic/low cortisol×	14 (6.7%)	14 (12.0%)	0 (0.0%)	

Results are shown as Mean (SD) †or N (%)* .

§ 'Other' includes patients not on oral glucocorticoids but on inhalers, nasal, subcutaneous, intravenous, eye drops or topical glucocorticoids

Statistical tests used: †t-test, *Chi-squared test

×Low cortisol defined as <11µg/dL; <300nmol/L; symptomatic: typical symptoms of adrenal insufficiency

Table 2: Outcome measures for index test (waking salivary cortisone/cortisol) and reference standard (ACTH Stimulation Test) for patients providing a valid waking salivary cortisone

	Overall (N=208)	adrenal insufficiency negative (N=117)	adrenal insufficiency positive (N=91)	p-value
Waking salivary cortisone¥				
ng/dL	441.3 [123.1, 870.9]	828.9 [509.6, 1046.4]	92.4 [31.7, 245.2]	<0.001
Waking salivary cortisol¥				
ng/dL	112.4 [42.0, 227.3]	187.7 [112.4, 283.8]	37.5 [13.9, 75.3]	<0.001
Baseline ACTH stimulation test cortisol¥				
µg/dL	8.4 [4.8, 11.5]	10.9 [8.8, 12.9]	4.3 [0.6, 6.9]	<0.001
30 minute ACTH stimulation test cortisol¥				
µg/dL	16.9 [8.8, 21.3]	21.1 [18.6, 23.3]	7.9 [3.3, 11.8]	<0.001
ACTH (pg/ml)¥	24.0 [13.3, 35.8]	25.0 [19.0, 35.0]	20.0 [6.0, 42.0]	0.07
Time waking salivary test¥ was undertaken as reported by participants	06:51 [06:19, 07:30]	06:47 [06:15, 07:30]	06:54 [06:30, 07:30]	
Time ACTH Stimulation Test was undertaken¥ (baseline cortisol)	09:50 [09:15, 10:20]	09:50 [09:20, 10:20]	09:45 [09:10, 10:18]	

Results are shown as Median [IQR] ¥
Statistical test used: ¥Mann-Whitney test
ACTH: Adrenocorticotropin Hormone
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