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ORIGINAL ARTICLE

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 as a result of adrenal suppression from use of anti-inflammatory glucocorticoids and opiates, as well as infectious diseases. The adrenocorticotropin (ACTH) stimulation test is the reference standard for diagnosis of adrenal insufficiency but requires clinic attendance and venesection. Salivary cortisone reflects free serum cortisol, and samples can be collected at home and posted to a laboratory. We tested whether home waking salivary cortisone level 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 home 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. Receiver-operating characteristic curves were computed, and positive and negative predictive values were calculated.

RESULTS Two hundred twenty patients were recruited. As measured by an ACTH stimulation test, the prevalence of adrenal insufficiency was 44%. The area under the receiver-operating characteristic curve for waking salivary cortisone as a predictor of adrenal insufficiency was 0.95 (95% confidence interval [CI], 0.92 to 0.97). Cutoffs to ensure a minimum of 95% sensitivity and specificity gave a negative predictive value of 96% (95% CI, 90 to 99) and a positive predictive value of 95% (95% CI, 87 to 99) to exclude and confirm adrenal insufficiency, respectively. Waking salivary cortisone data provided information similar to that of an ACTH stimulation test in 70% of participants. Eighty-three percent of patients preferred home salivary collection to clinic attendance.

CONCLUSIONS Home waking salivary cortisone sampling has accuracy for the diagnosis of adrenal insufficiency similar to that of a standard ACTH stimulation test. Patients found the at-home test to be more convenient than the hospital-based test. (Funded by the National Institute for Health Research.)

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Introduction

Adrenal insufficiency, or cortisol deficiency, is a life-threatening condition that can be primary (adrenal), secondary (pituitary), or tertiary (adrenal suppression).[1] Prevalence is rising as a result of the increased prescription of glucocorticoid and opioid therapies that suppress adrenal function.[2-6] Fifty percent of patients taking oral glucocorticoids have adrenal suppression,[4,7,8] and approximately 10% of patients taking opioids at a morphine-equivalent dose of >20 mg/d are at risk of adrenal insufficiency.[5] In low- and middle-income countries, 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% to 8% of individuals with adrenal insufficiency have an adrenal crisis each year. Recognition of adrenal insufficiency is essential, but diagnosis is often delayed[10-13]; the majority of patients are only diagnosed during an acute hospital admission,[14] which implies that patients are at risk of dying of 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] Although 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 several advantages over serum sampling. Because salivary glucocorticoids are stable at room temperature, patients can collect their own samples at home and mail them 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 for adrenal insufficiency is for patients to be referred for an ACTH stimulation test, using a cutoff to confirm (<15.6 μg/dl [430 nmol/l]) or exclude (≥15.6 μg/dl [430 nmol/l]) adrenal insufficiency. We hypothesized that a home waking salivary cortisone sample could predict a normal or abnormal ACTH stimulation test result when screening for adrenal insufficiency.

Methods

STUDY DESIGN AND OVERSIGHT

This prospective, diagnostic accuracy study was performed to assess the relationship between waking salivary cortisone level and the 30-minute cortisol level on an ACTH stimulation test in patients with adrenal insufficiency. The study protocol was approved by the South Yorkshire and Humber Research Ethics Committee (Reference 19/YH/0333). Written informed consent was obtained from all participants. The study is reported per the Standards for Reporting of Diagnostic Accuracy guidelines (STARD, Supplementary Appendix available with the full text of this article at evidence.nejm.org).[25]

The first and last authors wrote the first draft and together with statisticians vouch for the accuracy and 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 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 older than age 18 years with a high probability of either primary, secondary, or tertiary adrenal insufficiency as determined by the investigators were eligible for enrollment. This included patients who were dependent on any type of glucocorticoid, who had been on an oral glucocorticoid prednisolone-equivalent dose of ≥5 mg/d for ≥4 weeks, and who were referred for adrenal testing only after they had been weaned down to prednisolone ≤5 mg/d or equivalent or converted to physiologic doses of hydrocortisone ≤25 mg/d. Patients receiving any intermediate- or long-acting intramuscular or intra-articular glucocorticoid injections were recruited at least 3 months after their last injection. Patients with pituitary disease, such as tumors,

inflammatory disease, or those with a history of cranial radiotherapy, were considered eligible for inclusion.

Patients were excluded who were unable to produce a suitable saliva sample; night shift workers; patients with known protein-losing disorders, known or suspected alcohol dependence, and known severe liver disease; patients with uncontrolled active infection; and patients taking estrogens or those who were pregnant. Patients taking drugs that influence the hypothalamic-pituitary-adrenal axis (e.g., opioids) had their medications omitted on the day of testing, per routine clinical practice. In view of the coronavirus disease 2019 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 (Fig. 1). All patients enrolled received an information pack, which included an invitation letter, participant information sheet, and a Salivette (Salivette Cortisol; Sarstedt).

PROCEDURES

On the day of their scheduled ACTH stimulation test, all individuals provided a salivary sample upon waking using Salivette tubes containing synthetic swabs (Salivette Cortisol; Sarstedt). A total of 500 μl of saliva was necessary to ensure a good representative sample, and 50 μl was used for the assay. All patients were given written instructions with images and a video on how to collect their salivary sample (Video 1, included with the full text of this article at evidence.nejm.org). Patients were advised to refrain from smoking/vaping on the day of the test. Patients taking 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 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. An intravenous cannula was inserted, and baseline serum cortisol was measured. An ACTH stimulation test was performed with intravenous injection of 250 μg of Synacthen (Atnahs Pharma UK Limited), followed by a serum cortisol level blood draw at 30 minutes. Tests were performed by specialized 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 was unaware of the waking cortisol data. An a priori criterion of a peak cortisol level

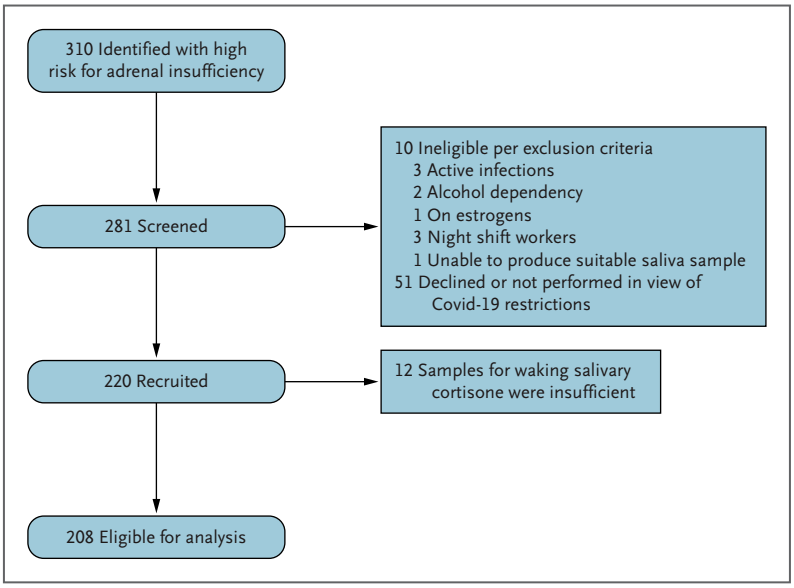

Figure 1. Recruitment Flowchart for Primary Analysis.

Graphic indicates the total number of patients who were at high risk for adrenal insufficiency recruited by consecutive sampling between November 2019 and December 2021. A total of 208 patients were analyzed for the primary end point. Covid-19 denotes coronavirus disease 2019.

of ≥15.6 μg/dl (430 nmol/l) measured by immunoassay indicated adequate adrenal reserve, whereas those patients with levels less than this value were considered to have adrenal insufficiency according to current clinical practice in our center.[13,26]

OUTCOME MEASURES

The primary outcome measure was waking salivary cortisone level 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 testing to exclude adrenal insufficiency. The aim was to assess whether we can use the waking salivary cortisone level 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 on the basis of waking salivary cortisone level cutoffs derived from the study to confirm or exclude adrenal insufficiency; intermediate values triggered a referral for an ACTH stimulation test. (Additional details are given in the Decision Analytic Model, with the associated decision trees for the two diagnostic strategies shown in Figs. S2 and S3.)

ASSAYS

Serum cortisol was analyzed by immunoassay (Elecsys Cortisol II assay; Roche) and interpreted immediately at Sheffield Teaching Hospitals NHS Foundation Trust. An extra serum cortisol sample was stored at −80°C and, together with the salivary sample, was then analyzed and interpreted by liquid chromatography–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 level to detect an abnormal ACTH stimulation test result. The area under the ROC 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 by using the Clopper-Pearson method. Linear regression models were used to investigate the effects of variables on waking salivary cortisone level. 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 <15.6 μg/dl [<430 nmol/l]) of 50% in the proposed target population[4] and a sensitivity of 80% and specificity of 95%, was approximately 200 participants (100 patients with adrenal insufficiency and 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 a predictive power similar to that of the 30-minute ACTH stimulation test cortisol cutoff of 15.6 μg/dl (430 nmol/l), we explored other cutoff values, including 14.5 μg/dl (400 nmol/l) and 12.7 μg/dl (350 nmol/l) derived from the literature.[26,27] Analyses were performed on a complete case basis.

Results

PATIENTS

A total of 281 patients were screened, and 220 patients were recruited. According to ACTH stimulation test results measured by immunoassay, 96 patients were classified as having adrenal insufficiency, and 124 patients had no adrenal insufficiency. After recruitment, 12 (5.5%) of 220 samples were excluded because of inadequate salivary sample collection (Fig. 1). A total of 208 individuals — 91 patients (44%) with adrenal insufficiency and 117 patients (56%) with no adrenal insufficiency — were analyzed for the primary outcome measure. Eleven (5%) of the 220 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 used in analysis). Baseline demographic characteristics are presented in Table 1. Participants had a mean age of 55.1±15.8 years, and 49% were female. Overall, 67% of patients were being screened for glucocorticoid-induced adrenal insufficiency, with 81% taking a glucocorticoid formulation, including glucocorticoid replacement for adrenal disease or pituitary disease postsurgery. One hundred percent of patients diagnosed with adrenal insufficiency were taking glucocorticoids. Eleven patients were taking opioids. Of these, three patients had adrenal insufficiency and were also taking 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 epidemiologic

Table 1. Baseline Demographic and Clinical Characteristics for Patients Providing a Valid Waking Salivary Cortisone Sample.*				
Demographic or Clinical Characteristic	Overall (N=208)	Adrenal Insufficiency — Negative (n=117)	Adrenal Insufficiency — Positive (n=91)	P
Age — yr†	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
Taking glucocorticoids‡				
No	40 (19.2)	40 (34.2)	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	

* Values are presented as the mean (±SD) or no. (%). BMI denotes body-mass index (the body-mass index is the weight in kilograms divided by the square of the height in meters).

† t-test used.

‡ χ^2 test used.

§ Includes patients not taking oral glucocorticoids but on inhalers, nasal, subcutaneous, intravenous, eye drops, or topical glucocorticoids.

¶ Symptomatic indicates typical symptoms of adrenal insufficiency; low cortisol level defined as <11 μg/dl (<300 nmol/l).

studies. Ninety percent of the study group were White, 5% were Asian, and the remaining were Black/Caribbean/African and multiracial (Table S4).

WAKING SALIVA CORTISONE AS A PREDICTOR OF ADRENAL INSUFFICIENCY

Median waking salivary cortisone level was 828.9 ng/dl (interquartile range [IQR], 509.6 to 1046.4 ng/dl) for participants with no adrenal insufficiency and 92.4 ng/dl (IQR, 31.7 to 245.2 ng/dl) for patients with adrenal insufficiency (Table 2). Waking salivary cortisone levels predicted an ACTH stimulation test 30-minute serum cortisol level >15.6 μg/dl (>430 nmol/l) as measured by immunoassay with an AuROC of 0.95 (95% CI, 0.92 to 0.97) (Fig. 2A). Using a waking salivary cortisone cutoff value of ≥612 ng/dl (17 nmol/l) excluded adrenal insufficiency with a sensitivity of 97% (95% CI, 91 to 99) and an NPV of 96% (95% CI, 90 to 99); using a cutoff of <251 ng/dl (7 nmol/l) confirmed adrenal insufficiency with a specificity of 97% (95% CI, 92 to 99) and a PPV of 95% (95% CI, 87 to 99). The results were reproduced and similar when using LC-MS/MS for the ACTH stimulation test (Table S1) and when using different cutoffs for the 30-minute serum cortisol measurement, including 14.5 μg/dl (400 nmol/l) and 12.7 μg/dl (350 nmol/l) (Fig. S1 and Table S1) 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 waking salivary cortisone cutoffs of ≥899 ng/dl (25 nmol/l) and <36 ng/dl (1 nmol/l), respectively.

SECONDARY MEASURES

Waking salivary cortisol level was a slightly weaker predictor of an ACTH stimulation test 30-minute serum cortisol level of >15.6 μg/dl (>430 nmol/l), with an AuROC of 0.89 (95% CI, 0.85 to 0.94) (Fig. 2B). A waking salivary cortisol cutoff value of ≥180 ng/dl (5 nmol/l) excluded adrenal insufficiency with a sensitivity of 95% (95% CI, 88 to 99) and an NPV of 94% (95% CI, 85 to 98); a cutoff value of <35 ng/dl (1 nmol/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 using immunoassay was a slightly weaker predictor than waking salivary cortisone, with an

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 Sample.*

Outcome Measure	Overall (N=208)	Adrenal Insufficiency — Negative (n=117)	Adrenal Insufficiency — Positive (n=91)	P
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 that waking salivary test was undertaken as reported by participants†	6:51 a.m. (6:19 a.m.–7:30 a.m.)	6:47 a.m. (6:15 a.m.–7:30 a.m.)	6:54 a.m. (6:30 a.m.–7:30 a.m.)	
Time that ACTH stimulation test was undertaken (baseline cortisol)†	9:50 a.m. (9:15 a.m.–10:20 a.m.)	9:50 a.m. (9:20 a.m.–10:20 a.m.)	9:45 a.m. (9:10 a.m.–10:18 a.m.)	

* Values are presented as median (interquartile range), unless otherwise noted. 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 denotes adrenocorticotropin.

† Mann–Whitney test used.

AuROC of 0.90 (95% CI, 0.86 to 0.94) (Fig. 2C). A baseline serum cortisol cutoff of ≥11.2 μg/dl (310 nmol/l) excluded adrenal insufficiency with a sensitivity of 96% (95% CI, 90 to 99) and an NPV of 93% (95% CI, 84 to 98); a cutoff <5.5 μg/dl (152 nmol/l) confirmed adrenal insufficiency with a specificity of 95% (95% CI, 90 to 98) and a PPV of 91% (95% CI, 81 to 97). The results were reproduced and similar when using LC-MS/MS for the ACTH stimulation test cortisol tests (Fig. 2D), including using different cutoffs for the 30-minute serum cortisol such as 14.5 μg/dl (400 nmol/l) and 12.7 μg/dl (350 nmol/l) as part of a sensitivity analysis (Table S1).

Additional analysis examined the percentage of ACTH stimulation tests that could have been avoided using waking salivary cortisone cutoffs as a screening test in a two-stage process. The ACTH stimulation test would have been avoided in 70% (154 of 220) of participants — 73 patients with waking salivary cortisone <251 ng/dl (7 nmol/l) and 81 patients with waking salivary cortisone ≥612 ng/dl (17 nmol/l). This two-stage process resulted in four patients who were falsely positive and three patients who were falsely negative (Table S2 and Table S3).

FACTORS INFLUENCING WAKING SALIVARY CORTISONE LEVELS

We conducted univariable regression analysis and found no significant influence on waking salivary cortisone level for a 1-year increase in age (β, −0.09; 95% CI, −0.2 to 0.01), sex (female β, 0.65; 95% CI, −2.61 to 3.91), for a one-unit increase in body-mass index (β, 0.06; 95% CI, −0.18 to 0.3), smoking status (history of smoking 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 (yes/no 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 consistent with an effect, 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

A total of 218 (99%) of 220 patients (95% CI, 97 to 100) stated that it was acceptable to do a salivary test at home, and 175 (80%) of 219 (95% CI, 74 to 85) responded that performing the test was very easy. A total of 180 (83%) of 216 patients (95% CI, 78 to 88) preferred the at-home salivary test versus the ACTH stimulation test in the clinical facility (Fig. 3).

Discussion

We have shown that waking salivary cortisone level 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 test was preferred by patients over a hospital-based ACTH stimulation test.

Definitions of cutoffs for waking salivary cortisone as an adrenal insufficiency screening test are likely to vary

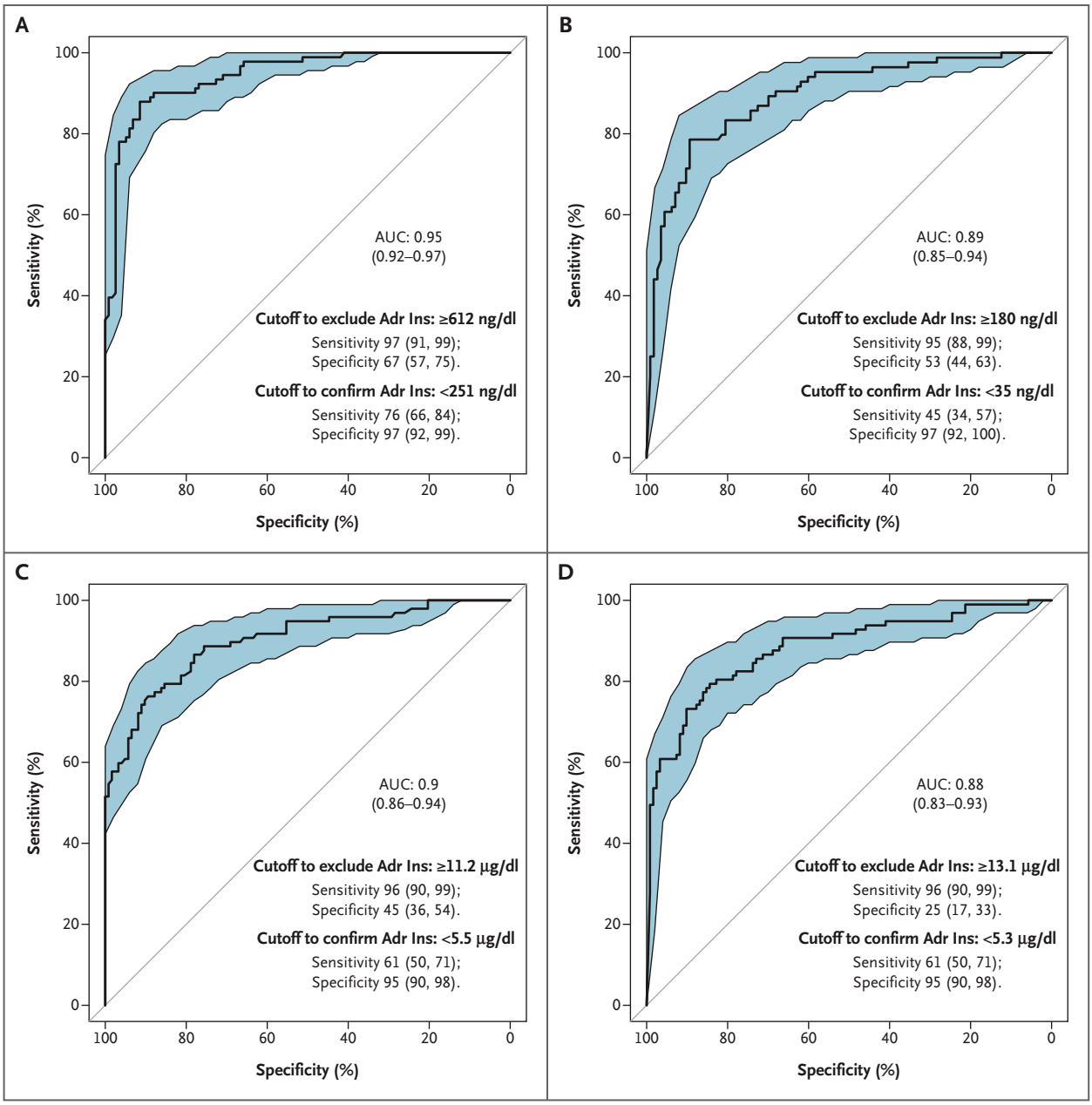

Figure 2. Receiver-Operating Curves to Assess Predictive Value for the 30-Minute Adrenocorticotropin Stimulation Test Cortisol Reference Test by the Index Tests.

Waking salivary cortisone (Panel A), waking salivary cortisol (Panel B), baseline serum cortisol by immunoassay (Panel C), and baseline serum cortisol by liquid chromatography–tandem mass spectrometry (Panel D). The 30-minute adrenocorticotropin stimulation test cortisol cutoff is 15.6 µg/dl. Area under the receiver-operating curve (AUC) indicates the strongest predictive value for waking salivary cortisone level, with lower predictive power for waking salivary cortisol and baseline serum cortisol levels. 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. Adr Ins denotes adrenal insufficiency. Values in parentheses are 95% confidence intervals.

among centers, as the criteria for diagnosis of adrenal insufficiency differ among doctors according to etiology, cortisol assay, and reference ranges applied. Commonly used cortisol immunoassays provide variable results and require individual reference ranges; therefore, LC-MS/MS assays are now considered the reference standard for measuring levels of glucocorticoids in biologic fluids.[28] Our results were validated by retesting using an LC-MS/MS assay.

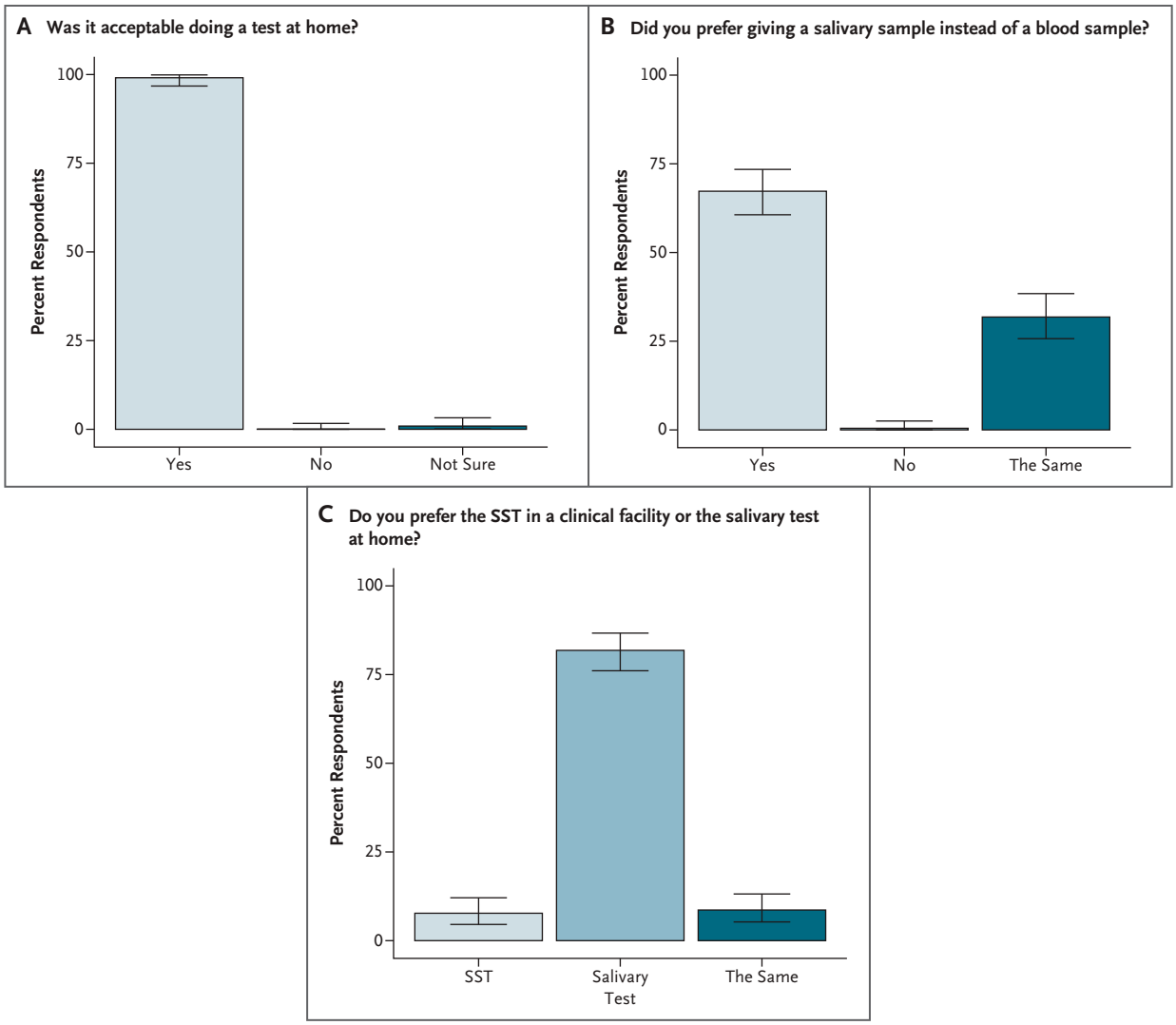

Figure 3. Selected Questionnaire Results to Assess Patient Views on ACTH Stimulation Testing and Waking Saliva Cortisone Sampling.

Bar charts indicate percent respondents (95% confidence interval) for survey results. Questions included patient views on salivary test acceptability (Panel A), test preference (Panels B and C), and response likelihood and adrenocorticotropin (ACTH) stimulation test anxiety (Fig. S4). 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 because an ACTH stimulation test can be performed in a number of different clinical venues. SST denotes short Synacthen test or ACTH stimulation test.[29]

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 on the basis of the cortisol level in response to an ACTH stimulation test and symptoms such as fatigue. Endocrine Society guidelines suggest that a peak cortisol level of <18.0 μg/dl (500 nmol/l), which is assay dependent, at 30 minutes of an ACTH stimulation test indicates adrenal insufficiency.[29] In the current study, adrenal insufficiency was defined as a peak cortisol level of <15.6 μg/dl (<430 nmol/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%. Cutoffs to ensure a minimum of 95% sensitivity and specificity gave an 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 cutoffs for waking salivary cortisone levels that defined normal cortisol secretion and diagnosed adrenal insufficiency. In a sensitivity analysis, we have shown that waking salivary cortisone levels work equally well at different cutoffs of the ACTH stimulation test, including 14.5 μg/dl (400 nmol/l) and 12.7 μg/dl (350 nmol/l), and produced similar results when serum cortisol samples were analyzed by using LC-MS/MS.

Using these identified cutoffs, ≥612 ng/dl (17 nmol/l) and <251 ng/dl (7 nmol/l), there was a small number of patients who experienced false-negative results (n=3) — that is, had a falsely high waking salivary cortisone level — or false-positive results (n=4) — that is, had a falsely low waking salivary cortisone level. We believe the false-positive and false-negative rates will have minimal overall clinical impact on the individual patient. Our false-negative waking salivary cortisone values varied between 647 and 863 ng/dl (18–24 nmol/l), which are borderline levels considering the waking salivary cortisone threshold of ≥612 ng/dl (17 nmol/l). In most settings, patients with borderline results will be given advice about corticosteroid 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; however, for secondary adrenal insufficiency, the sensitivity to diagnose adrenal insufficiency is lower compared with the insulin tolerance test.[30,31] Waking salivary cortisone directly reflects physiologic serum cortisol levels, and with increasing adoption of salivary glucocorticoid assay platforms, the ability to test may become more mainstream. If this is the case, waking salivary cortisone then has the potential to supersede the ACTH stimulation test as the diagnostic test for adrenal insufficiency. Major reference laboratories around the world report salivary cortisol measured by LC-MS/MS, the 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 performed 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 level was only a slightly weaker predictor of the ACTH stimulation test than waking salivary cortisone level (AuROC, 0.89 vs. 0.95); however, clinicians should be aware that taking oral hydrocortisone in the 24 hours before testing can give a falsely elevated salivary cortisol level as a result of oral contamination.

Five percent of patients returned an inadequate salivary sample, and additional work is required to identify the cause of this occurrence and whether better instruction could reduce this percentage. The strength of our study is that we have tested the use of waking salivary cortisone level measured by LC-MS/MS in a group of patients at high risk 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 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 analyzed patients with potentially reversible adrenal suppression from those with likely permanent adrenal suppression. This would be unlikely to alter the results as 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 that 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 of 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 that 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 level provides data that allow for a reasonably accurate prediction of whether a given patient has adrenal sufficiency or insufficiency.

Disclosures

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