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Validated criteria for the interpretation of a single measurement of serum cortisol in the investigation of suspected adrenal insufficiency

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Abstract

Objective: The diagnostic value of a single measurement of serum cortisol as a first step in the investigation of suspected adrenal insufficiency remains unclear. Previously proposed criteria have not been validated, and little is known regarding the performance of the test outwith morning samples in outpatients. We aimed to identify and validate criteria for morning and afternoon serum cortisol which could be used to determine which individuals require dynamic testing, in both outpatient and medical inpatient settings.

Methods: We performed a retrospective analysis of 2768 patients attending endocrinology clinics and patients admitted to general medical units in two hospitals in Edinburgh, UK. In baseline samples from the short synacthen test, thresholds which identified a subnormal-stimulated serum cortisol (<430 nmol/L using the Abbott Architect assay) with 95% sensitivity were identified. Criteria drawn from data in patients attending outpatient clinics in one hospital were tested in additional outpatient and inpatient validation cohorts.

Results: A morning (8 AM-12 PM) serum cortisol of <275 nmol/L identified subnormal-stimulated cortisol with 96.2% sensitivity. For afternoon (12 PM-6 PM) samples, a cut-off of <250 nmol/L achieved 96.1% sensitivity. Sensitivity was maintained when the criteria were applied to outpatients in the validation cohort for both morning and afternoon samples. For inpatients, the test was sufficiently sensitive in morning samples only.

Conclusions: A single measurement of serum cortisol carries the potential to significantly reduce the need for dynamic testing in the investigation of adrenal insufficiency, whether this is taken in morning or afternoon outpatient clinics, or in morning samples from medical inpatients.

KEYWORDS

adrenal insufficiency, cortisol, glucocorticoids, ROC curve, synacthen stimulation test

1 | INTRODUCTION

The short synacthen test (SST), comprising measurement of serum cortisol before and typically 30 minutes after the intramuscular injection of 250 μg $ACTH_{1\text{-}24}$ (Synacthen®), is widely used in the diagnosis of adrenal insufficiency (AI). In comparison with the gold standard insulin tolerance test, the SST is less dangerous and demanding, and has been well validated.[1,2] However, it still carries a small risk of severe hypersensitivity reactions,[3] resulting in a Society for Endocrinology recommendation that the test is only performed in units where immediate resuscitation facilities are available.[4] Use of the SST as a first-line test in the investigation of suspected AI also carries significant resource implications, brought to light by well-documented periods of shortage of Synacthen in 2014 and again currently, resulting in a recent price rise to £60 in the UK per 250 μg vial.[5] The requirement for supervision by experienced staff adds a further cost to the test.

A single measurement of serum cortisol as an initial step in the investigation of AI therefore carries a number of advantages over dynamic testing. Although extensively studied,[6-18] this remains a contentious approach, and reports of its diagnostic utility, and suggested thresholds, vary considerably. This may reflect challenges in interpreting single measurements of serum cortisol due to its circadian and ultradian variability,[19] assay variation,[17] and the relative rarity of the condition relative to the size of the population tested, meaning a large cohort is required to draw reliable conclusions. Limitations of the existing literature also include the lack of validation of proposed criteria in additional cohorts. Furthermore, existing studies allow conclusions to be drawn in the relatively confined clinical setting of outpatients attending in the morning. The performance of the test in samples drawn in the afternoon has not been tested, and relatively little is known about the value of the test in the inpatient setting.[16,18]

We aimed to identify thresholds for basal serum cortisol that would be of use in determining which patients require further investigation to confirm or refute AI, and to determine the impact such a strategy on the requirement for dynamic testing. Additionally, we aimed to compare the performance of serum cortisol measured in morning and afternoon samples. Finally, our study aimed to validate proposed criteria using a second outpatient cohort, and a third cohort comprising general medical inpatients.

2 | SUBJECTS AND METHODS

As this was a retrospective study of de-identified patient data, ethics approval was not required. Analysis of 1624 SSTs, performed between 2011 and 2014, was undertaken in 1222 patients attending general endocrine clinics at the Western General Hospital, Edinburgh, UK (derivation cohort). Demographic data and information on the indication for the SST were collected by review of electronic case records. Serum cortisol was measured before and 30 minutes after intramuscular administration of $ACTH_{1\text{-}24}$ (Synacthen®) 250 μg. For individuals prescribed oral glucocorticoid replacement, this was withheld on the day of testing. Criteria were proposed to define upper cut-offs for morning (8 AM-12 PM) and afternoon (12 PM-6 PM) baseline serum cortisol which were >95% sensitive for the detection of a subnormal-stimulated cortisol. Individuals with a basal cortisol <50 nmol/L were assumed to have a subnormal-stimulated cortisol in keeping with AI, and the accuracy of this assumption was tested. The proportion of individuals lying between these two thresholds, and thus requiring confirmatory testing, was used to assess the impact of such a strategy on the number of SSTs required.

To assess the external validity of the proposed cut-offs, we tested these criteria in two validation cohorts. The criteria were applied retrospectively to 873 SSTs undertaken in 770 individuals attending

TABLE 1 Cohort characteristics

	Derivation cohort			Outpatient validation cohort			Inpatient validation cohort		
N (total patients)	1222			770			776		
N (total SSTs)	1624			873			804		
N (AM SSTs)	820			326			389		
N (PM SSTs)	804			547			415		
Male/female n (%)	770/854 (47.4/52.6)			380/493 (43.5/56.5)			351/453 (43.7/56.3)		
Age (mean ± SD)	49.2 ± 17.0			48.7 ± 17.4			69.2 ± 17.2		
	Total	**AM**	**PM**	**Total**	**AM**	**PM**	**Total**	**AM**	**PM**
Serum cortisol nM (median, IQR)									
Baseline	240 ± 128	265 ± 140	213 ± 113	240 ± 136	256 ± 158	232 ± 145	340 ± 202	351 ± 181	322 ± 217
30 min	551 ± 152	544 ± 161	555 ± 129	577 ± 150	573 ± 181	579 ± 133	653 ± 221	639 ± 255	664 ± 239
Proportion failing SST, n (%)	233 (14.3)	156 (19.0)	77 (9.6)	127 (14.5)	69 (21.2)	58 (10.6)	76 (9.5)	39 (10.0)	37 (8.9)
Time of baseline sample (mean ± SD)	12:41 ± 152 min	10:22 ± 50 min	15:03 ± 69 min	13:12 ± 162 min	10:06 ± 56 min	15:02 ± 97 min	12:25 ± 159 min	10:09 ± 85 min	14:31 ± 96 min

general endocrine clinics at the Royal Infirmary, Edinburgh, UK, over the same time period (outpatient validation cohort). The third cohort (inpatient validation cohort; n = 804 SSTs in 776 individuals) comprised patients admitted to acute medical units and general medical wards in both hospitals over the period 2011-2015. For inpatients, data were included where the baseline sample from the SST was taken between 6 AM and 6 PM.

Analysis was undertaken to: (a) compare the performance of the test in the morning to that in the afternoon; and (b) assess whether performance of the test in the outpatient and inpatient validation cohorts matched that of the derivation cohort, across both morning and afternoon periods.

Serum cortisol was measured using the same methodology across all cohorts (Abbott Architect® i2000 immunoassay system), according to the manufacturer's protocol. The assay consistently demonstrated an inter-assay precision below the manufacturer stated CV of 10%. An adequate response to Synacthen was defined as a 30 minute cortisol of ≥430 nmol/L.[20]

2.1 | Statistical methods

Sensitivity was defined as the probability that a patient failing the SST is below a given threshold for basal cortisol, and specificity the probability that a patient passing the SST is above that threshold. Positive predictive value (PPV) denotes the proportion of patients below the threshold who fail the SST and negative predictive value (NPV) the proportion of patients above the threshold who pass the SST.

Sensitivity, specificity, NPV and PPV were compared using Fisher's exact test. Area under curve (AUC) for ROC curve analysis and linear regression analysis were performed using SPSS version 23.

3 | RESULTS

Characteristics of the cohorts are summarised in Table 1. Supplementary data include details on clinical indications (Table S1) and basal serum cortisol vs time (Figure S1). Serum cortisol demonstrated an inverse correlation with time across all cohorts (derivation cohort r^2 = .131, P < .001; outpatient validation cohort r^2 = .038, P < .001; inpatient validation cohort r^2 = .014, P = .001; Figure S1).

3.1 | Derivation cohort

To achieve 95% sensitivity for the prediction of subnormal-stimulated cortisol, a threshold of <275 nmol/L was selected for morning cortisol and <250 nmol/L for afternoon cortisol (Figures 1 and 2). These achieved sensitivities of 96.2 and 96.1%, respectively (P > .99 for difference; Table 2).

The criteria provided a NPV 98.4% and 98.9% in morning vs afternoon samples, respectively (P = .74 for difference). Overall performance of the test, as assessed by AUC (ROC) was not significantly different in morning vs afternoon samples, although specificity was significantly poorer in the afternoon (am 55.1% vs pm 37.7%, P < .0001), accompanied by a poorer positive predictive value (am 37.7% vs pm 14.0%, P < .0001).

Baseline serum cortisol was <50 nmol/L in 37 samples. Of these, 36 (95%) had a stimulated serum cortisol of <430 nmol/L.

If it were assumed that confirmatory SSTs were only required for individuals whose baseline serum cortisol lay between the upper and lower cut-offs, the criteria would result in a reduction in the need for a SST in 48.2% of individuals tested in the morning and 36.2% in the afternoon.

3.2 | Validation cohorts

3.2.1 | Outpatients

Application of the cut-offs identified in the derivation cohort to the outpatient validation cohort retained sensitivities of >95% for both morning (98.6%) and afternoon (100%) samples (Table 2). Sensitivities, negative predictive values and AUC (ROC) were not significantly different in comparison with the relevant time periods in the derivation cohort. For afternoon samples, specificity was greater in comparison with afternoon samples in the derivation cohort (48.9% vs 37.7%, respectively, P = .0001).

All 38 individuals with a baseline serum cortisol <50 nmol/L had a stimulated serum cortisol of <430 nmol/L. The proposed the criteria would result in a reduction in the need for a SST in 52.2% of individuals tested in the morning and 39.7% in the afternoon.

3.2.2 | Inpatients

For inpatients, a sensitivity of 95% was achieved for morning samples (97.4%) but not afternoon samples (89.2%), and therefore, afternoon baseline serum cortisol in inpatients did not meet the primary criterion for diagnostic utility.

For morning samples, sensitivity, negative predictive values and AUC (ROC) were not significantly different in comparison with the derivation cohort. Specificity was greater in comparison with morning samples in the derivation cohort (80.0% vs 37.7% respectively, P < .0001). Baseline serum cortisol was <50 nmol/L in 7 individuals (1 of whom passed the SST; see 'false positives'). If it was assumed that confirmatory SSTs were only required for individuals whose baseline serum cortisol lay between the upper and lower cut-offs, the criteria would result in a reduction in the need for a SST in 73.8% of inpatients tested in the morning.

3.3 | False negatives

Across all 3301 tests, use of the baseline serum cortisol for the detection of a subnormal-stimulated cortisol would have resulted in a total of 16 false negatives, where stimulated cortisol was subnormal despite a baseline serum cortisol above the proposed thresholds. Excluding those inpatients tested in the afternoon, where sensitivity did not meet the prespecified threshold of 95%, the overall false negative rate was 0.4% (12 patients).

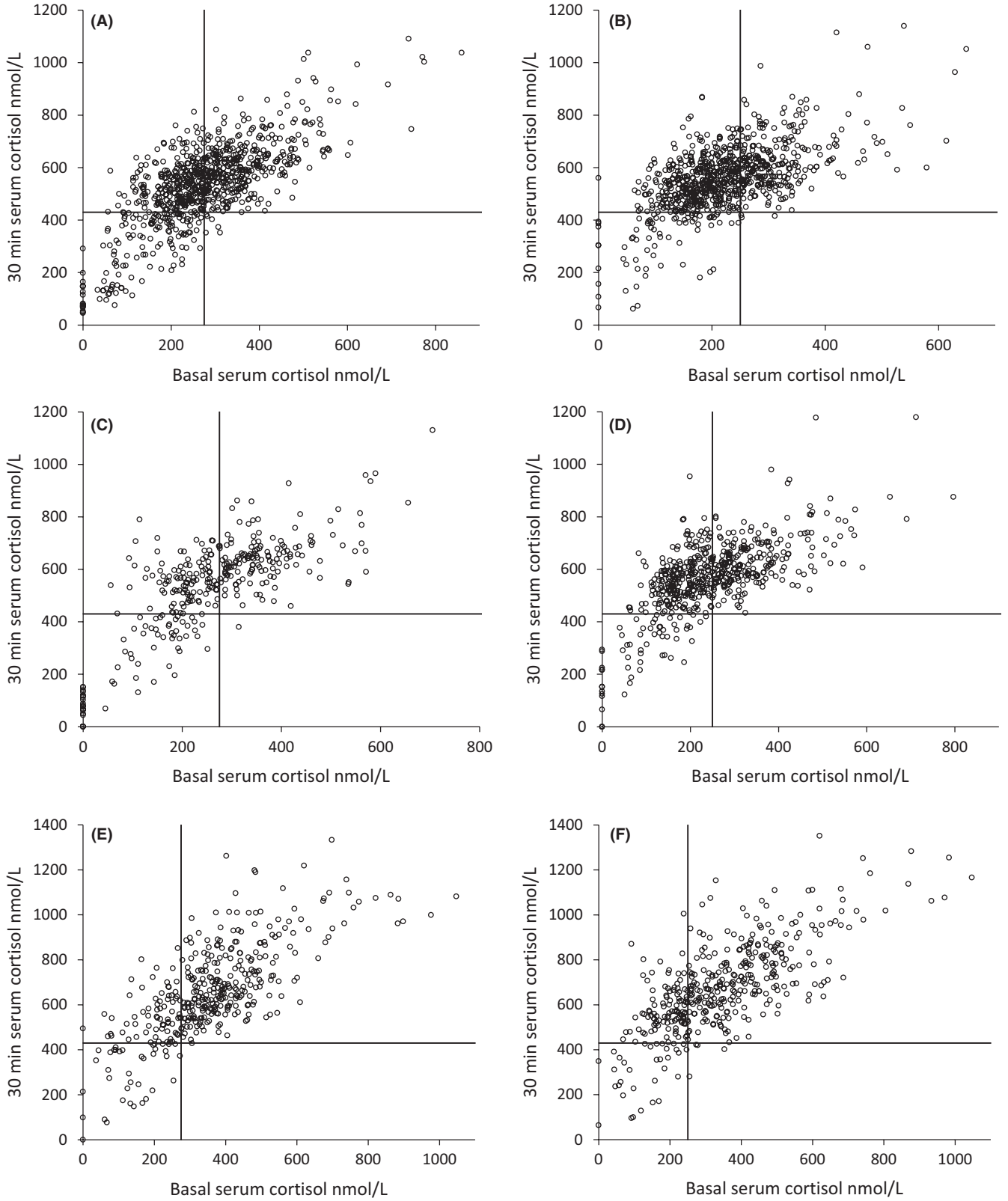

FIGURE 1 Baseline vs post synacthen serum cortisol. Serum cortisol at baseline vs 30 min post synacthen 250 μg IM in: outpatient derivation cohort (A) (B); outpatient validation cohort (C) (D) and inpatient cohort (E) (F). Baseline samples taken 06:00-12:00 (A) (C) (E) or 12:00-18:00 (B) (D) (F). Horizontal solid line refers to cut-off for post synacthen serum cortisol (430 nmol/L) that defines a normal response in the short synacthen test. Vertical solid lines refer to cut-offs for basal serum cortisol which detect a subnormal-stimulated cortisol with sensitivity > 95% in morning (<275 nmol/L) and afternoon (<250 nmol/L) samples

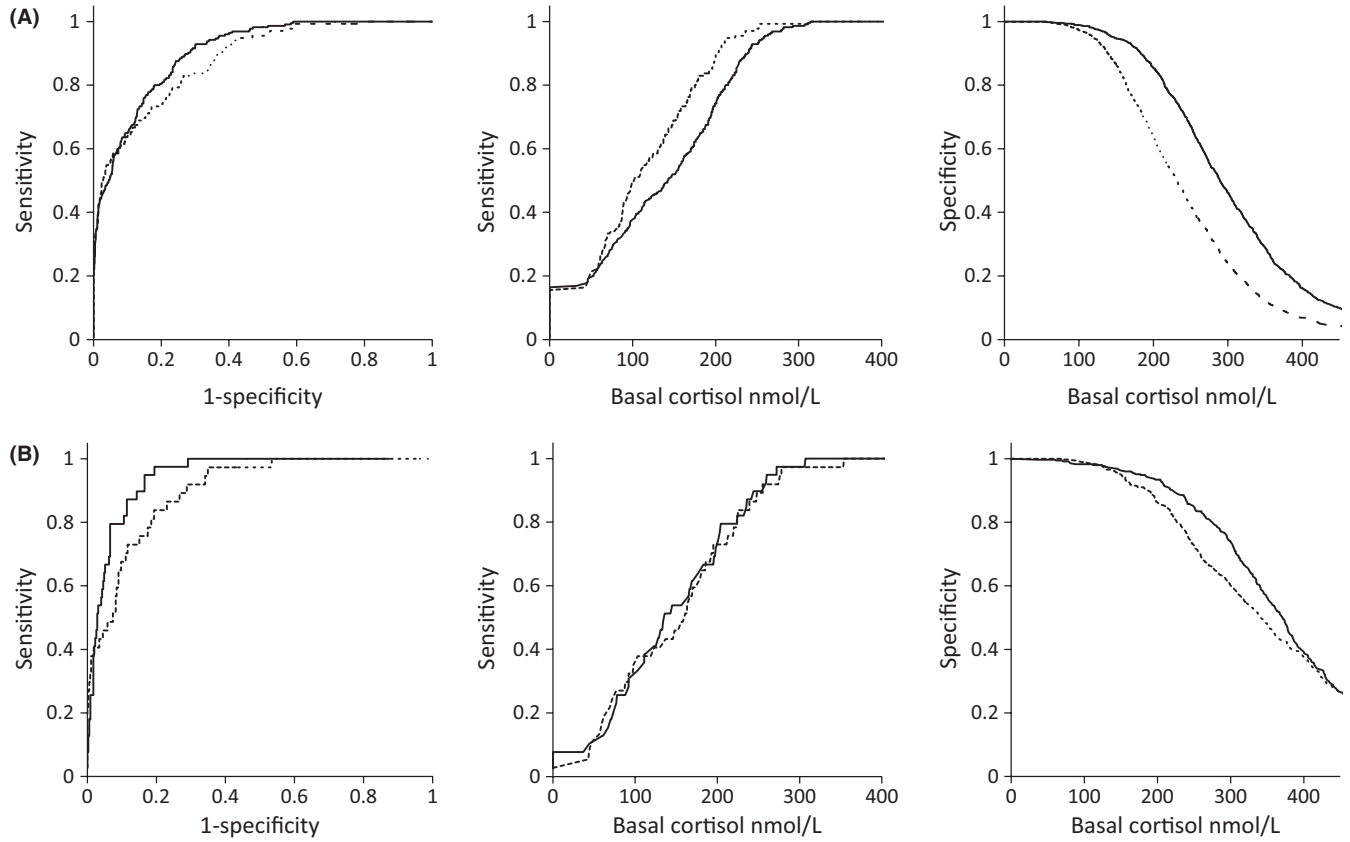

FIGURE 2 Performance of baseline serum cortisol as predictor of a subnormal-stimulated cortisol in all outpatients (A) and inpatients (B). Receiver operating characteristics and baseline cortisol vs sensitivity and specificity for the detection of a subnormal-stimulated serum cortisol (<430 nmol/L at 30 min following synacthen 250 μg IM). Solid line = baseline samples taken 06:00-12:00; Dashed line = baseline samples taken 12:00-18:00

Characteristics of these individuals are detailed in Table S2. Follow-up data are available for all but one patient and upon repeat testing the majority were subsequently found to have a normal SST. In one, the response was borderline, with a stimulated cortisol 430 nmol/L, and it was felt likely that this patient had Addison's disease on the basis of the clinical background and elevated adrenal antibodies. Only one individual failed the Synacthen test upon repeat testing, and one individual was lost to follow-up having commenced glucocorticoid replacement.

3.4 | False positives

A basal serum cortisol below 50 nmol/L predicted a subnormal-stimulated cortisol with 98% certainty. Of 88 individuals whose basal cortisol lay below this threshold, 2 were found to have a stimulated cortisol greater than 430 nmol/L and would be designated as 'false positives' under the proposed criteria: one with opioid-induced hypogonadism and one with suspected hypophysitis (see Table S3).

4 | DISCUSSION

Using what is to our knowledge the largest data set of its kind for a single assay, we have shown that using a cut-off of <275 nmol/L for samples drawn in the morning, or <250 nmol/L in the afternoon, basal serum cortisol is >95% sensitive for the detection of a subnormal-stimulated cortisol. This sensitivity is maintained when applying the criteria to a second outpatient cohort, and to a third cohort comprising inpatients, although for inpatients the test only meets the prespecified sensitivity threshold in morning samples. Although specificity and positive predictive value are low, the use of the proposed thresholds would result in a significant reduction in the number of patients requiring dynamic testing with the SST.

Previous efforts to define similar thresholds for basal serum cortisol have produced values which vary widely.[6-18] Assay variability plays a significant role in limiting the ability to define unified criteria. With this in mind, our thresholds cannot be applied to assays other than the Abbot Architect assay used in this study. A recent study by Sbardella et al compared three assays, including 449 outpatients for whom serum cortisol was measured using the Abbott Architect assay, and found a serum cortisol of <295 nmol/L achieved 95% sensitivity for the detection of AI.[17] In our study, comprising a total of over 3000 patients, this sensitivity is exceeded using the same assay and 430 nmol/L cut-off for stimulated cortisol, and receiver operating characteristics are similar.

We have targeted 95% sensitivity in order to enable comparison with recently published literature.[6,7,17] Our data demonstrate NPV is

TABLE 2 Performance of basal serum cortisol as predictor of a subnormal-stimulated cortisol

	Derivation cohort			Outpatient validation cohort				Inpatient validation cohort			
	AM (n = 820)	PM (n = 804)	*P* (AM vs PM)	AM (n = 326)	*P* (vs AM derivation cohort)	PM (n = 547)	*P* (vs PM derivation cohort)	AM (n = 389)	*P* (vs AM derivation cohort)	PM (n = 415)	*P* (vs PM derivation cohort)
Sensitivity	96.2%	96.1%	>.99	98.6%	.68	100%	.26	97.4%	>.99	89.2%	.21
Specificity	55.1%	37.7%	<.0001	56.8%	.71	48.9%	.0001	80.0%	<.0001	72.8%	<.0001
NPV	98.4%	98.9%	.74	99.3%	.68	100%	.25	99.6%	.25	98.6%	>.99
PPV	33.5%	14.0%	<.0001	38.0%	.31	18.8%	.08	35.2%	.74	24.3%	<.01
AUC ROC (95% CI)	0.893 (0.869-0.918)	0.848 (0.802-0.893)	.16	0.920 (0.889-0.952)	0.37	0.921 (0.888-0.954)	.051	0.943 (0.920-0.970)	.11	0.899 (0.856-0.943)	.13

Note: Parameters calculated using a cut-off for basal serum cortisol <275 nmol/L (morning samples) or <250 nmol/L (afternoon samples), as determined from the derivation cohort with target sensitivity >95% for the detection of a subnormal-stimulated serum cortisol (<430 nmol/L at 30 min following Synacthen 250 μg IM.

Abbreviations: AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

very high using this threshold, although because of the dependence of NPV on pretest probability, caution is required in those with a particularly high index of clinical suspicion. Although setting a higher sensitivity would come at the expense of a greater proportion of patients requiring confirmatory testing, we found cut-offs of <308 and <312 nmol/L would achieve 99% and 100% sensitivity, respectively, for morning samples. In the afternoon, a cut-off of <312 nmol/L is required to meet both 99% and 100% thresholds. Even using the lower 95% threshold however, the few individuals in the group of 'false negatives' are likely to have a degree of adrenal reserve, and in our study, the majority in this group were subsequently found to have intact adrenal function upon repeat testing.

We found a basal serum cortisol of below 50 nmol/L to be strongly predictive of a subnormal-stimulated cortisol, with only two 'false positives' across all cohorts using this approach. In one of these, a basal serum cortisol alone may have been the more appropriate test, given the suspicion of a recent pituitary insult. The second patient demonstrated a pattern previously reported in association with opioid analgesia,[21,22] and we would recommend dynamic testing as a first-line approach for this patient group.

Diurnal variability is a concern when interpreting single measurements of serum cortisol.[19] Due to this, previously published studies in outpatients have assessed morning cortisol alone. Recently, *Brown et al* produced time adjusted criteria,[7] although samples were drawn from 0700-1200 only. We have shown the test is equally sensitive when serum cortisol is sampled in the afternoon and overall performance, assessed by ROC analysis, is similar. The requirement for a slightly lower cut-off, and the reduced specificity of the test in the afternoon reflect the diurnal variation in serum cortisol. This variation is slightly smaller than reported in other studies,[23,24] perhaps reflecting a degree of stress in patients attending outpatient clinics compared with studies in healthy volunteers. Despite the reduction in specificity with afternoon testing, use of the criteria would still avoid the requirement for dynamic testing in a significant proportion of patients when tested in the afternoon.

For inpatients, comparatively little attention has been given to the use of a single measurement of serum cortisol as an initial step in the approach to suspected AI, although an approach which avoids the need for a carefully timed dynamic test in busy inpatient units is clearly advantageous. Our study addresses this question using predefined criteria and in a much larger cohort than previously published studies.[16,18] We have found morning basal serum cortisol is highly sensitive and carries the potential for a large reduction in the need for SSTs. Sensitivity was inferior in the afternoon, and while it is possible that performance of the test here would be improved by defining a higher cut-off, this would require study of a second inpatient cohort to validate separate criteria.

4.1 | Limitations

The main limitation of our study is the retrospective nature of the data collection and possible institutional referral bias for outpatients. For inpatients, interpretation of serum cortisol is

complicated by difficulty defining an 'appropriate' response to acute illness in a heterogeneous group, and the findings cannot be generalised to critically ill patients. Equally, alterations in cortisol binding in acute illness further complicate interpretation of total serum cortisol measurements[25] and we have not been able to measure free cortisol. Finally, the data need to be interpreted in light of the limitations of the SST compared with the gold standard insulin tolerance test (ITT). Although the SST is generally well validated,[1,2] it provides a less physiological stimulus than the ITT, and should be interpreted with particular caution where results are borderline. Importantly, in those with a strong preclinical probability, and particularly in those with pituitary disease,[26] a normal SST does not exclude AI.

5 | CONCLUSION

The study demonstrates a single measurement of serum cortisol carries the potential to significantly reduce the need for dynamic testing, whether in outpatients or in general medical inpatients. A single measurement of serum cortisol can also easily be done in primary care, and for outpatients, the test is of use whether samples are taken in the morning or afternoon, reducing the need for hospital referrals and further adding to the value of this strategy in the initial investigation of AI.

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Nothing to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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