Diagnostic performance of morning serum cortisol as an alternative to short synacthen test for the assessment of adrenal reserve; a retrospective study

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ABSTRACT

Objective The short synacthen test (SST) is widely used across the UK to assess adrenal reserve. The main objective of our study was to determine the morning serum cortisol level that will predict adrenal insufficiency (AI) thus reducing our reliance on SST.

Design This was a single centre retrospective study of 393 SST tests measuring 0 and 60 min cortisol levels after administration of 250 µg of synacthen (synthetic ACTH).

Patients and methods All the SST tests for patients suspected of primary or secondary AI between April 2016 and October 2018 were included in this study. We used serum to determine circulating cortisol by a newer generation competitive electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics). A post-ACTH cortisol response of ≥420 nmol/L at 60 min was considered adequate to rule out AI. The data were analysed to ascertain the relationship between 0 min and 60 min serum cortisol.

Results A total of 393 SST results were included in this study. Overall, a total of 332 (84.5%) subjects achieved sufficient serum cortisol level at 60 min, while 61 subjects (15.5%) showed insufficient response. Using the logistic regression, we determined that a morning basal serum cortisol level of ≥354 nmol/L was able to predict normal adrenal function with 100% sensitivity. We were unable to find a lower cut-off value below which SST will not be required. By using this proposed cut-off point, approximately 37% of the SSTs tests could be avoided.

Conclusions Basal morning serum cortisol can be safely used as a first step in the evaluation of patients with suspected AI. This will enhance the number of patients being screened for this condition.

INTRODUCTION

It is important to be able to diagnose adrenal insufficiency (AI) as quickly as possible as delay in treating these patients with steroid replacement can have devastating consequences due to their inability to mount a cortisol response in times of illness or stress. The short synacthen test (SST), also known as the cosyntropin test or ACTH test, remains the most widely used test to diagnose AI and is based on the finding of insufficient responses to adrenal stimulation with synthetic ACTH, called synacthen, in the appropriate clinical setting.[1 2] Synacthen is the trade name of tetracosactide, a synthetic peptide which consists of the first 24 of the 39 amino acids of the endogenous ACTH peptide and displays the same physiological properties as ACTH.[3] The test involves intramuscular or intravenous administration of 250 µg synacthen and collection of blood sample at 0, 30 and (sometimes) 60 min later. There is another version of SST called the low-dose SST which involves administration of more physiological 1 µg dose.[4] The commercially available synacthen is available in 250 µg/mL ampoules making the high-dose test easy to perform and is therefore the preferred method across the world.[5] It is important to emphasise that despite the widespread use of SST to diagnose AI, the insulin tolerance test (ITT) remains the 'gold standard' test to assess integrity of the hypothalamic-pituitary-adrenal (HPA) axis. However, this test is too labour intensive, relatively risky, has several contraindications and is performed only very infrequently.[6]

The SST is largely a safe test and is usually carried out by the nursing staff. However, due to a small risk of severe hypersensitivity reactions, it is recommended that the test should only be done under the supervision of appropriate senior hospital medical staff (eg, consultants).[3] It is also suggested that the test should only be performed in setting where immediate resuscitation facilities are available, thus almost precluding its use in out-of-hospital environment.[7]

Atnahs Pharma are the only licenced supplier of Synacthen ampoules in the UK. The current price of Synacthen ampoules as listed in BNF is £38 per 250 µg ampoule. There have been few occasions in the last couple of years when a shortage of Synacthen injection was reported affecting doctors' ability to perform SST.[8] The shortage also meant a temporary price rise to £60 per 250 µg ampoule which does have financial implications. As the test is performed by the nursing team under supervision of a doctor, it adds further to the overall cost of a test.

We therefore decided to explore if a single morning measurement of serum cortisol can be used to screen patients for AI thus obviating the need for SST altogether. This question has been addressed by several groups in the past[9–11] but using single morning cortisol level to confirm or exclude AI remains a contentious issue due to a variety of reasons such as timing of cortisol measurement and assay variability.

The aim of our study was to identify thresholds for basal serum cortisol that would be of use in determining which patients require SST to confirm or refute the diagnosis of AI. Because AI is a serious disease with potentially fatal outcome if left untreated, we predefined a sensitivity of more than 98% for AI so as to avoid missing anyone with true disease.

We have recently demonstrated that 30 min sample in SST does not add any diagnostic utility and should perhaps be abandoned.[12] For the purpose of current study, we therefore correlated baseline cortisol level with the additional information obtained from 60 min sample and ignored 30 min sample.

SUBJECTS AND METHODS

Subjects

This was a retrospective single-centre study conducted at Bedford Hospital NHS Trust, Bedford, UK. All data collection was retrospective and completely anonymised. It was therefore decided not to seek patient consent or ethical approval.

In view of recurrent shortage of synacthen, we decided to undertake this study as a Quality Improvement Project to determine the threshold above or below which a SST would not be required, therefore reducing our reliance on this test to diagnose AI. All SSTs were conducted in accordance with local Trust protocol and informed consent was obtained for each test.

All subjects who had the SST conducted to assess adrenal reserve between April 2016 and October 2018 were included in the study. Patients' records and basal and post-ACTH serum cortisol values were extracted from the electronic database held in the hospital.

Patients who were on steroid which had not been omitted prior to the test, those taking HRT or combined oral contraceptive pill (OCP), pregnant women, children under 16 years of age and those who had an incomplete test, that is, where serum cortisol level was not measured/available at both time points (0 and 60 min) were excluded from the study. In this study, there was no patient with hypoproteinaemic states (which results in low serum albumin concentrations) such as hepatic or renal failure. Low albumin level may lead to low total serum cortisol concentrations despite normal biologically active free cortisol concentrations.

SST protocol

All SSTs were performed in accordance with our hospital protocol that involves measuring serum total cortisol (nmol/L) at 0 min. A supra-physiological dose of 250 µg synacthen (Synacthen; Atnahs Pharma UK Ltd) was then administered intravenously or intramuscularly and further blood samples were taken at 30 and 60 min. As mentioned above, for the purpose of this study, we excluded 30 min sample.

The tests were performed in the morning between 09:00 and 11:00 hour. Although we did not ask patients to fast overnight, they were advised to refrain from eating, drinking or smoking for the 30 min before the test. They were rested in a sitting position for 15 min before the test and then for the total duration of the test. Premenopausal women were usually tested within first 7 days of their menstrual cycle unless there was an urgent indication to perform the test.

The SST has a major limitation in that it cannot detect acute pituitary failure.[13] Our hospital is not a neurosurgical centre and we have no patient that requires assessment of HPA axis in an acute post-pituitary surgery situation. In our study, there was no patient who had received pituitary radiotherapy in the preceding 2 years.

Analytical methods

There are number of assays now available to measure serum cortisol and it is now well established that differences in the assays do have a major impact on the interpretation of cortisol values in SST.[14]

In our hospital, serum cortisol is assayed by electrochemiluminescence immunoassay 'ECLIA' using a Cobas 8000 analyser (Roche Diagnostics, Mannheim, Germany). The method has been standardised against the Institute for Reference Materials and Measurements (IRMM)/IFCC 451 panel (ID GC/MS, isotope dilution-gas chromatography/mass spectrometry).[15] An adequate response to short synacthen is considered if cortisol at 30 or 60 min is ≥420 nmol/L.[14] The assay has a measuring range between 1.5 nmol/L and 1750 nmol/L. The reference range for 09:00 cortisol in non-pregnant adults is 135–550 nmol/L.

Blood samples are collected in serum separator tube, are allowed to clot and following separation one aliquot of separated serum are stored at 4°C and analysed within sample stability limits. Three internal quality control (IQC) samples (mean target concentrations 122, 464 and 729 nmol/L, respectively) are tested pre and post cortisol analysis. All IQC results are persistently within 2 SD of the mean. Cortisol assay has an average uncertainty of measurement (UM) of 5.23% and coefficients of variation ratio (CVR) of 0.62 (desirable limit <1.5).

Our Laboratory is enrolled in the UK National External Quality Assurance Scheme (NEQAS) and our cortisol assay has consistently performed well within the recommended range. Our laboratory is fully accredited by United Kingdom Accreditation Service (UKAS) and meets ISO 15 189 standard for medical laboratories.

Statistical methods

All analyses were completed using the statistical software R (version 1.2.5001). Packages used were readr, ggplot2, caret and e1071. The primary analytical method used was logistic regression, supplemented with t-tests for key comparisons.

RESULTS

A total of 393 individuals with suspected AI who had SST performed (238 female and 155 male patients) were included in the analysis. Six patients were excluded from the analysis due to incomplete data collection. The median age was 60 (IQR =44, 74), the sample was made up of 60.5% female and 40.5% male. A normal response to synacthen was defined if serum total cortisol concentration of ≥420 nmol/L was achieved at 60 min. Mean baseline cortisol level before and after the synacthen test are shown in table 1. Overall serum cortisol values at baseline and post stimulation in patients with AI were significantly lower compared to those patients who did not have AI.

We conducted a logistic regression to evaluate the predictiveness of baseline serum cortisol measurement used in the SST. We found an OR of 1.02 ($p=5.26\times10^{-9}$) indicating that patients are

Table 1 Displays the mean and 95% CI (in brackets) along with the p-value from a two-sample paired t-test. Note 2.2×10^{-16} represents the smallest number that can be produced in R, all results are highly significant (all values in nmol/L)

	Baseline cortisol level (mean ± S.D)	60 min cortisol level (mean ± S.D)	P value
Overall N=393	315.3 (0, 620.65)	630.0 (179.58, 1080.43)	2.2×10^{-16}
Patients with AI N=61	125.5 (–189.83, 440.84)	263.9 (103.97, 467.71)	2.2×10^{-16}
Patients with no AI N=332	350.2 (34.86, 665.53)	697.2 (350.22, 1044.31)	2.2×10^{-16}

2% more likely to achieve a serum cortisol ≥420 nmol/L at 60 min for every one-unit increase in baseline serum cortisol. Following the logistic regression, several potential cut-off values were calculated using the predicted probability of a normal stimulated serum cortisol measurement at 60 min of ≥420 nmol/L. These were 20%, 30%, 40%, 50%, 90%, 95% and 99% probability of a normal serum cortisol measurement at 60 min.

Table 2 displays a range probability and associated cut-off values of baseline serum cortisol measurement alongside the diagnostic evaluation. 'Specificity' is defined as the proportion of patients with a serum cortisol ≥420 nmol/L at 60 min who are predicted to have and are identified as having normal measurement from the baseline cut-off. 'Sensitivity' refers to the proportion of patients with AI at 60 min who are predicted to have and are identified by the baseline cut-off as having a serum cortisol <420 nmol/L at 60 min. 'Negative predictive value (NPV)' signifies the proportion of patients that we predict to have a serum cortisol ≥420 nmol/L at 60 min from their baseline measurement and have a normal 60 min measurement. 'Positive predictive value (PPV)' refers to the proportion of patients we predict to have subnormal 60 min measurement from their baseline measurement and have a serum cortisol <420 nmol/L at 60 min (all values in nmol/L).

Table 3 displays the probability of a normal serum cortisol at 60 min along with the counts of true positives (those who have AI), true negatives (those who do not have AI), false positives (those who do not have AI but are predicted to have AI) and false negatives (those who have AI but are predicted to have normal stimulated 60 min value). All patients with baseline serum cortisol ≥354 nmol/L have a normal measurement at 60 min.

In this study, there were 31 patients with a baseline serum cortisol <100 nmol/L, of which 77.4% were proven to have AI post ACTH while 97.9% patients with a baseline cortisol of >300 nmol/L were noted to have normal response to ACTH (table 4).

Figure 1 is a scatterplot of baseline serum cortisol against 60 min measurement. The scatterplot is split into four quadrants based on the proposed baseline cut-off (≥354 nmol/L) and existing 60 min post ACTH cut-off (≥420 nmol/L). The upper-right quadrant (green shaded area) represents the true negatives while the lower-left quadrant (red shaded area) indicates the true positives. The upper-left quadrant represents false positives and the lower-right quadrant represents false negatives (although none are identified in the present study for the proposed cut-off).

Table 2 Displays probability of a normal serum cortisol measurement, diagnostic criteria of the potential cut-off values, showing that higher serum cortisol levels are associated with reduced specificity (the ability to detect patients who do not have AI), but improved sensitivity (the ability to detect patients with AI)

	Baseline serum cortisol	Specificity	Sensitivity	NPV	PPV
20% Probability	≥69	0.987	0.213	0.872	0.765
30% Probability	≥95	0.985	0.360	0.893	0.815
40% Probability	≥116	0.976	0.508	0.915	0.795
50% Probability	≥135	0.961	0.607	0.930	0.740
90% Probability	≥240	0.786	0.901	0.978	0.437
95% Probability	≥275	0.671	0.951	0.987	0.347
99% Probability	≥354	0.440	1.00	1.00	0.247

Table 3 Displays the probability and associated baseline measurement alongside the number of true positives, true negatives, false positives and false negatives (all values in nmol/L)

	Baseline serum cortisol	True negatives	True positives	False negatives	False positives
20% Probability	≥69	328	13	48	4
30% Probability	≥95	327	22	39	5
40% Probability	≥116	324	31	30	8
50% Probability	≥135	319	37	24	13
90% Probability	≥240	261	55	6	71
95% Probability	≥275	223	58	3	109
99% Probability	≥354	146	61	0	186

Table 4 Displays bands of baseline serum cortisol with measurement outcome at 60 min (all values in nmol/L)

Baseline serum cortisol	Number of patients with cortisol <420 nmol/L at 60 min (%)	Number of patients with cortisol ≥420 nmol/L at 60 min (%)
<100	24 (77.4%)	7 (22.6%)
101 to 200	28 (43.1%)	37 (56.9%)
201 to 300	7 (7.4%)	87 (92.6%)
301 to 400	2 (2.1%)	92 (97.9%)
401 to 500	0 (0%)	67 (100%)
>500	0 (0%)	41 (100%)

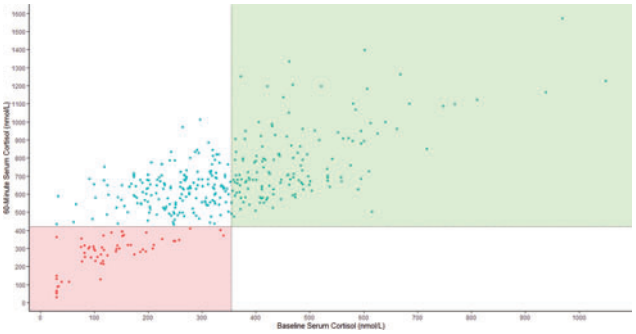

Figure 1 Displays the baseline (0 min) serum cortisol level against the 60 min measurement. Blue dots indicate normal measurements while red dotes indicate AI. The horizontal dotted line indicates a cortisol response of ≥420 at 60 min (which defines a normal response in SST), the vertical dotted line indicates the proposed baseline cut-off of ≥354 (which detects a stimulated serum cortisol of ≥420 in SST with a sensitivity of 100%). The green shaded region indicates patients that potentially do not require the SST, the red shaded region indicates patients who have AI and are correctly predicted from the baseline cut-off (all values in nmol/L).

Figure 2 is a plot of the predicted probability (calculated from the logistic regression analysis) of a normal serum cortisol across baseline measurements with the associated 95% CI.

A ROC curve was generated (figure 3) and achieved an adequate AUC of 0.783 using only the baseline serum cortisol as a predictor. This demonstrates that baseline cortisol has a 78.3% chance to distinguish between patients with and without AI.

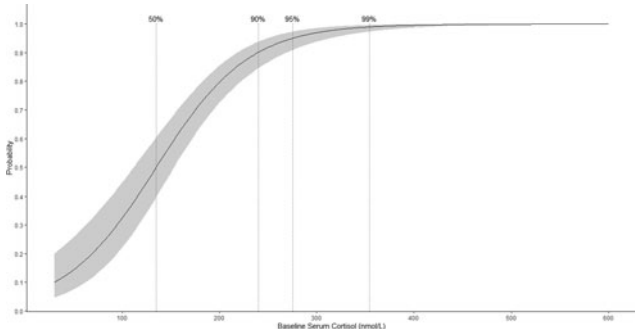

Figure 2 Displays the predicted probability of a 'normal' serum cortisol response of ≥420 nmol/L at 60 min from the baseline with a 95% confidence interval (shaded region). Vertical dotted lines indicate the baseline measurement required to achieve a 50%, 90%, 95% and 99% probability of a normal response at 60 min.

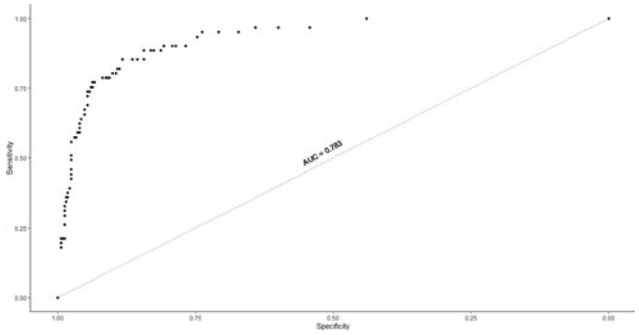

Figure 3 Displays the ROC curve of the baseline serum cortisol values, plotting the specificity on an inverse scale against the sensitivity to diagnose AI. The diagonal dotted line indicates an AUC of 0.5, a model that performs at 50% chance of differentiating between AI patients and patients with normal measurements at 60 min. The points indicate the relationship between sensitivity and specificity, highlighting that improving our ability to detect true positives reduces our ability to detect true negatives.

DISCUSSION

The SST which is widely used as a diagnostic test to exclude primary AI is generally well tolerated by majority of patients. It is important to emphasise that there remains a considerable variation in the methodology, especially with regard to sample timings with some units taking sample for serum cortisol at 0 min and 30 min while other units take sample at 0, 30 and 60 min.[16] It is pertinent to note that the only time point that has been validated against the ITT is 30 min sample,[17] which has led many to believe that 60 min sample should not be used to assess AI.[18] The Endocrine Society Clinical Practice Guideline advises that either time point can be used.[2] However, we have recently demonstrated that 30 min sample does not appear to add any diagnostics utility and should perhaps be abandoned.[12] For the purpose of current study, we therefore decided to correlate baseline cortisol level with the 60 min sample only.

Many studies have taken the peak cortisol response in SST to be 500 nmol/L.[2] This threshold is based on older immunoassays which use polyclonal antibodies to detect cortisol. The use of newer more specific monoclonal antibody immunoassays, such as Elecsys Cortisol II (Roche Diagnostic) used in the current study, provides a lower cut-off for a normal response. Due to variation among different cortisol assays, the cut-off for a normal cortisol response to synacthen must be laboratory-specific or assay-specific (ranging from 420 to 574 nmol/L).[14] A lower peak cortisol threshold of 420 nmol/L using newer generation highly specific cortisol assay is widely accepted and greatly improves sensitivity.

Our data indicate that a morning serum cortisol value which can be used reliably to exclude the diagnosis of AI with 100% sensitivity is ≥354 nmol/L. There were no patients in our study with baseline cortisol ≥354 nmol/L who demonstrated subnormal 60 min post ACTH level. It is therefore possible to avoid doing SST for these patients, thus providing an easy and convenient means of identifying patients who will require further assessment. If we are to apply this criteria this would result in the reduction in the need for SST in 146 (37.2%) of patients being tested. Reducing cut-off to 275 nmol/L would reduce sensitivity to 95%, which would mean that an additional 77 patients will not have to do the SST, 74 of which will have normal measurement at 60 min post ACTH (table 3).

AI is a life-threatening disease and can prove fatal if left undiagnosed. We therefore chose the upper cut-off level that had the 100% sensitivity to rule out AI in order to avoid any false-negative diagnoses. However, using a higher sensitivity will inevitably produce a greater number of false-positive patients who may need a formal SST (table 3). It is therefore advisable that the proposed value of ≥354 nmol/L is applied together with clinical features (pre-test probability) to form the basis for clinical decisions regarding further investigations.

We tried to identify a minimum level of cortisol below which SST will not be required and we checked several different threshold levels. In our study, 8 of the 37 patients (21.6%) with baseline serum cortisol of <116 nmol/L had a normal measurement at 60 min post ACTH. Similarly, 5 out of 27 patients (18.5%) with baseline serum cortisol of <95 nmol/L had a normal measurement at 60 min post ACTH. Four out of 17 patients (23.5%) with baseline serum cortisol of <69 nmol/L had a normal measurement at 60 min post ACTH. There does not appear to be a lower serum cortisol level that achieves 100% separation between patients with and without AI.

A number of studies in the past have postulated that basal cortisol can be used for testing the integrity of the HPA axis in adults. However, the cut-off values reported vary between 80 nmol/L and 110 nmol/L (lower cut-off values) and 250 nmol/L 494 nmol/L (upper cut-off values),[9 19–22] the reason for which are manifold such as differences in study populations and different serum cortisol assays used. It is therefore important to bear in mind that our threshold should not be applied to assays other than Roche assay that is used in our hospital and most widely in the UK (National External Quality Assessment Service (UK)) with some 45% of the laboratories using the same method.

Our cut-off value of ≥354 nmol/L to rule out AI is in line with a meta-analysis of 12 studies published recently that suggested that in outpatients with suspected HP disorder, an upper cut-off value of 365 nmol/L excluded HPA insufficiency.[23]

One of the major concern while interpreting the single cortisol measurement is diurnal rhythm of endogenous cortisol secretion. In our hospital, we perform SST in the morning only before 11:00. Scott *et al* in a recently published study have shown that the result is equally applicable if the serum cortisol is sampled in the afternoon, although the cut-off value for the afternoon sample is lower with a reduced specificity.[24]

If a single cortisol measurement can be used to screen for AI, it will not only save on time and money and will therefore be very

cost effective but it also has the potential to widen the number of patients being screened. Many GPs are reluctant to refer patients to hospital for SST due to cost and inconvenience particularly when index of suspicion is low which means there is potential to miss some patients with AI. Patients still die with untreated and undiagnosed adrenal failure. Using only one morning serum cortisol level will greatly increase the number of patients that are screened and therefore there is a possibility that more patients will be identified and treated. We therefore propose that serum basal cortisol levels should be used as the first-line test in the assessment of the HPA axis. A level of less than 354 nmol/L in the context of appropriate clinical picture should be investigated further with a formal SST.

It is important to note that our study population was a mixed one which encompassed patients with suspected primary and secondary AI. However, we had no patient in the immediate post-acute pituitary failure stage. Our patient population does reflect normal clinical practice in hospitals across the UK.

There are some obvious limitations to the present study. Although all the SSTs in our study were done in outpatient setting thus potentially avoiding the misinterpretation of cortisol that may result from the stress of being in-patient, our data collection was based on reviewing patients' notes and was therefore retrospective. It is important to interpret this data in light of the general limitations of SST (compared to gold standard ITT) in the diagnosis of AI.

CONCLUSION

Our study demonstrates that using baseline morning cortisol levels ≥354 nmol/L as a cut-off to rule out AI it is possible to avoid performing SST in a large number of patients. This test can easily be done in outpatient setting and in primary care thus potentially reducing the number of referrals received in secondary care to exclude AI. This will not only add to patients' satisfaction and increased screening but can have significant financial saving.

Main messages

- Basal morning serum cortisol can be safely used as a first step in the evaluation of patients with suspected adrenal insufficiency (AI).
- A morning serum cortisol value which can be used reliably to exclude the diagnosis of AI with 100% sensitivity is ≥354 nmol/L.
- No low serum cortisol concentration cut-off with high specificity could be identified to rule in AI with confidence.
- The result of this study has the potential to help reduce the number of referrals received in secondary care to exclude AI. This will not only add to patients' satisfaction and increased screening but can have significant financial saving.

Current research questions

- A similar study should be carried out on a paediatric population.
- To identify a lower serum cortisol level that achieves 100% separation between patients with and without AI.
- To identify an afternoon random serum cortisol level that will help reduce reliance on SST.

What is already known on the subject

- Despite lack of consensus, measurement of basal serum cortisol is often used as a screening test for adrenal insufficiency.
- Various authors have proposed different cut-off points to facilitate diagnosis of adrenal insufficiency to reduce the number of short synacthem tests.

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Patient consent for publication Not required.

Ethics approval After careful consideration, we decided not to seek patient consent or ethical approval as the data collection was retrospective and all data was completely anonymised.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All the authors of this study do commit to making the relevant anonymised patient-level data available on reasonable request.

Dissemination declaration Dissemination of study results to study participants and/or patient organisations is not applicable.

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