



Research article

The use of baseline cortisol level in predicting the outcome of 1 µg Synacthen tests in an outpatient endocrinology unit

Najoua Lassoued^{*}, Baha Zantour, Wafa Alaya, Mohamed Hbib Sfar

Endocrinology Department, Taher Sfar University Hospital, Mahdia, 5100, Tunisia

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ABSTRACT

Introduction: The clinical requirements and the indication of the Synacthen test are increasing. The objective of our study is to determine a baseline cortisol level that reliably predicts the response to Synacthen test in a low-risk group of patients.

Materials and methods: We performed a cross-sectional analysis of all Synacthen tests conducted between January 2017 and June 2018. The diagnostic accuracy of basal cortisol levels as a predictor of an adequate response to Synacthen test was evaluated by ROC curve analysis.

Results: One hundred and fifty-three patients were included. A baseline cortisol level <40 ng/mL had a sensitivity of 100% but a specificity of 5.8% for the failure of the Synacthen test, while a baseline cortisol level > 147.5 ng/mL showed a specificity of 100% but a sensitivity of 1.2% for an adequate response to the Synacthen test. According to the ROC curve, the optimal baseline cortisol level for predicting an adequate response to the Synacthen test was 85 ng/mL with an AUC of 0.808 (95% CI [0.738–0.877]).

Conclusion: We propose a basal cortisol level assay as a first step in the evaluation of patients with suspected adrenal insufficiency.

1. Introduction

Suspected adrenal insufficiency (AI) is a frequent reason for outpatient consultation in Endocrinology. Latent AI may progress for months with non-specific symptoms such as asthenia, dizziness or hypoglycemic discomfort. In addition, the frequent long-term use of corticotherapy for various pathologies increases the risk of AI during glucocorticoid withdrawal. Hence, the number of patients referred to the endocrinologist to indicate hormone replacement therapy increases.

The insulin tolerance test (ITT) is considered to be the gold standard for the diagnosis of AI. However, this test requires hospitalization in a specialized unit with strict medical supervision given the risk of fatal hypoglycaemia. Moreover it is contraindicated in several situations such as a history of epilepsy or cardiac rhythm disorders. In addition, several studies have proved the correlation between the Synacthen test and ITT and various endocrinology societies have currently indicated its realization for the diagnosis of AI [1, 2]. But in recent years, given the shortage of Synacthen in many hospital structures, we have faced very long waiting lists of patients suspected of having an AI with a high risk of decompensation in stressful situations. This leads us, in most cases, to substitute these patients with hydrocortisone even before performing the test.

Basic cortisol levels are available and could be requested in an outpatient setting. Therefore, the objective of our study was to determine a baseline cortisol level that reliably predicts the response to the Synacthen test.

2. Methods

All patients presented between 8 am and 9 am. Informed consent was obtained from all patients before the synacthen test was performed. The 1 µg Synacthen test was prepared by adding one milliliter of Synacthen 250 µg to 9 ml of normal saline solution, which is thoroughly mixed. A 100 IU insulin syringe is then used to administer one microgram of Synacthen intravenously which corresponds to 4 IU of the preparation. The blood samples used to determine cortisol were made before the injection of Synacthen (basal cortisol level) and then at 30 and 60 min (t30 and t60). The cortisol assay was performed by electrochemiluminescence (ECLIA). A cortisol level at 30 min >180 ng/mL was considered an adequate response to the test.

2.1. Study design

This study is a cross-sectional analysis of all ancient Synacthen tests performed at the Endocrine Day Unit of Taher Sfar University Hospital in

^{*} Corresponding author.

E-mail address: najwalassoued@yahoo.fr (N. Lassoued).

Mahdia between January 2017 and June 2018. Therefore we haven't applied for an Ethical Approval because we have just analysed archived data. The indication of Synacthen test was obtained from the outpatient unit files.

2.2. Inclusion and exclusion criteria

Patients were divided into two groups based on the probability of AI before the test:

Outpatients who did not have a history of pituitary pathology (pituitary adenoma, Empty sella syndrome, etc.) or radiotherapy or surgery of the pituitary area were considered at low risk.

Patients who were hospitalized for acute adrenal crisis or severe hypoglycemia or who had a history of radiotherapy or pituitary surgery were considered at high risk.

Only low-risk patients were included. Pregnant women and women on hormonal contraception were excluded. Patients followed in nephrology or gastroenterology for any hepatopathy or nephropathy were also excluded.

2.3. Statistical analysis

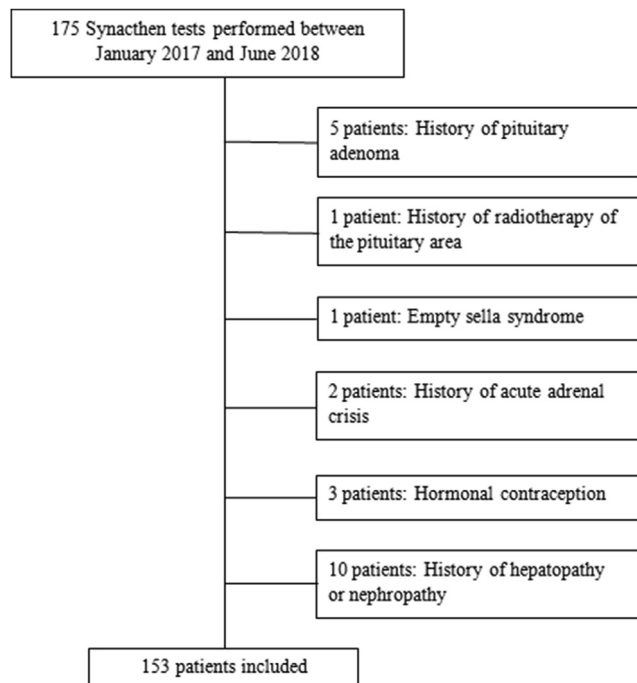
The diagnostic accuracy of basal cortisol levels as a predictor of an adequate response to 1 µg Synacthen test was assessed by the analysis of the ROC curve. An area under the ROC curve (AUC) of 0.5 indicates the absence of discrimination, while an AUC of 1.0 indicates a perfect discrimination.

Initially, the optimal threshold value of basal cortisol was established by determining the inflection point of the curve. This point corresponds to the Cut off that optimizes both sensitivity and specificity. We then examined other threshold values in order to increase the specificity to 95%.

All statistical analysis was undertaken using SPSS v.20.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Characteristics of the studied population



One hundred and fifty-three patients were included. The mean age was 49.46 years with extremes of 19 and 84 years. A clear female

predominance was found with a sex ratio of 2.18. Twenty-one patients had a history of autoimmune disease: 14 patients were followed for Hashimoto's thyroiditis, 4 for hyperthyroidism, 2 for type 1 diabetes and one patient had vitiligo. Fifty-five patients were followed for type 2 diabetes. The Synacthen test indications are presented in Tables 1 and 2.

3.2. Results of the synacthen test and determination of basal cortisol cut-off

The maximum value of cortisol was reached at 30 min in 75% of cases and at 60 min in 25% (with a cortisol peak >180 ng/mL at 30 min and >200 ng/mL at 60 min for these last ones).

Eighty-four patients (54.9%) achieved an adequate response to the Synacthen test with a cortisol peak > 180 ng/mL. Sixty nine patients (45.1%) had a diagnosis of AI.

There is an excellent correlation between basal cortisol levels and cortisol levels at 30 min of the test (r = 0.559, p < 0.0001) (Figure 1). A baseline cortisol level <40 ng/mL has a sensitivity of 100% but a specificity of 5.8% to predict the failure of the Synacthen test. A baseline cortisol level > 147.5 ng/mL shows a specificity of 100% but a sensitivity of 1.2% for an adequate response to the Synacthen test.

According to the ROC curve, the optimal baseline cortisol level for predicting an adequate response to the Synacthen test is 85 ng/mL with an AUC of 0.808 (95% CI [0.738–0.877]) (Figure 2).

Using a baseline cortisol level greater than 85 ng/mL as a predictor of an adequate response to the Synacthen test, 83 patients (52.24%) would be classified as having a sufficient response and 70 patients (44.75%) as having an AI. Therefore, a baseline cortisol level greater than 85 ng/mL has a sensitivity of 72% and a specificity of 75% to predict an adequate response to the Synacthen test (Figure 3).

To increase specificity to 95%, a basal cortisol Cut off greater than 114.5 ng/mL is required, but at the expense of a much lower sensitivity of 30% (Table 3).

Table 1. Indications for the Synacthen Test in the 153 Patients who Consulted at the Mahdia Endocrinology Outpatient Unit and Test Results.

Indication of the Synacthen test	n	%	Adrenal Insufficiency confirmed by the test n (%)
Presence of functional and/or physical signs of suspicion of Adrenal Insufficiency	133	86.93	58 (43.6)
Withdrawal of long-term corticosteroid therapy	16	10.46	10 (62.5)
Hirsutism*	1	0.65	0
Bilateral Adrenal Incidentaloma	1	0.65	0
Adrenal surgery**	2	1.3	1 (50)

* Suspicion of late-onset congenital adrenal hyperplasia.

** Unilateral adrenalectomy for an adrenocortical carcinoma in one of the 2 patients and for a pheochromocytoma for the other.

Table 2. Clinical signs suggestive of AI in 153 patients who visited the Mahdia Endocrinology Outpatient Unit and had a Synacthen test.

	n	%	
Functional signs	Asthenia	153	100
	Anorexia	16	10.5
	Hypoglycemic discomfort	78	51
	Ramadan fasting poorly tolerated	31	20.3
	Dizziness	49	32
	Tanning	21	13.7
Physical signs	Orthostatic hypotension	30	19.6
	Weight loss	48	31.4
	Melanodermia	0	0

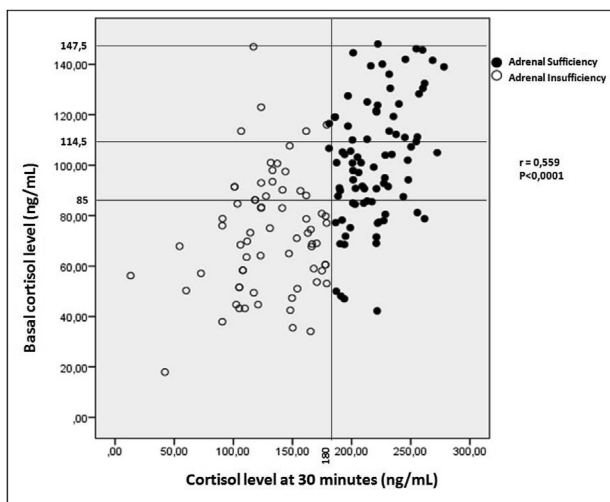


Figure 1. Correlation between basal cortisol levels and cortisol levels at 30 min of the Synacthen test.

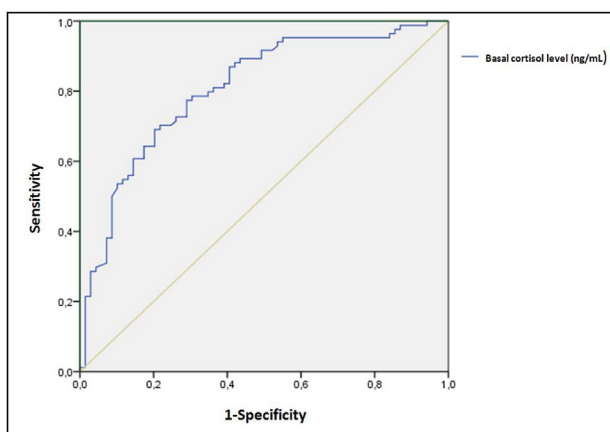


Figure 2. ROC Curve for Cortisol level >180 ng/mL at 30 min of the Synacthen Test using basal Cortisol level as a Predictive Factor.

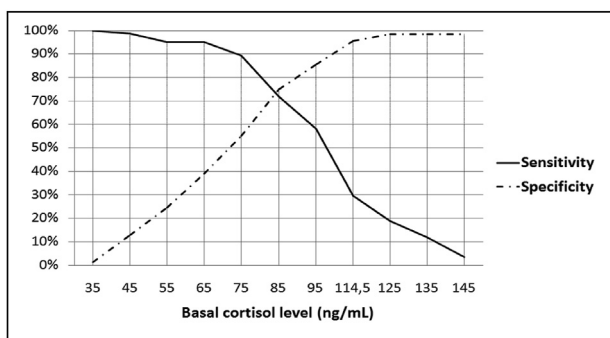


Figure 3. Sensitivity and specificity of different optimal threshold values for basal cortisol levels between 35 and 145 ng/mL.

4. Discussion

In our study, all patients underwent 1 µg Synacthen test instead of 250 µg Synacthen test because of the non-availability of the Synthetic ACTH, thing that was predicted by the Endocrine Society guidelines [1].

The choice of the most appropriate test to evaluate the hypothalamic–pituitary–adrenal axis (HPA axis) has been the subject of controversy over the last 20 years. The insulin tolerance test (ITT) has

Table 3. Performance of baseline cortisol level at 85 and 114.5 ng/mL in the prediction of Synacthen response.

	Basal cortisol level (ng/mL)			
	>85	≤85	>114.5	≤114.5
>180	63 (75.9%)	21 (30%)	25 (89.3%)	59 (47.2%)
≤180	20 (24.1%)	49 (70%)	3 (10.7%)	66 (52.8%)
Sensitivity	72%		29.7%	
Specificity	75%		95.6%	
Positive predictive value	0.70		0.53	
Negative predictive value	0.75		0.892	

traditionally been referred to as the Gold Standard. The ITT, however, has several limitations. First, it requires medical supervision. Second, it can be dangerous, especially in young children and adults with epilepsy or heart disease that contraindicated this test. Third, adequate hypoglycemia is not obtained in some cases. Several alternative tests for the evaluation of the HPA axis have been proposed over the years, but only the Synacthen (corticotropin) test has demonstrated good sensitivity and specificity compared to the ITT [3]. The usual dose of 250 µg results in a supraphysiological plasma concentration of corticotropin. A meta-analysis showed that the low-dose ACTH test appears to perform better than the standard dose test [4].

There are controversies about optimal thresholds for cortisol response to dynamic tests. Several studies have suggested a cortisol response higher than or equal to 180 ng/mL as an adequate response to the low-dose ACTH stimulation test [5, 6, 7]. This maximal response is obtained within 30 min of the test [4, 5, 6, 7, 8, 9]. We found that the peak cortisol values were achieved at 30 min in 75% of cases and at 60 min in 25% of cases in our study. For this reason, we chose cortisol values at 30 min as a criterion for interpreting the test.

It has been suggested that morning cortisol levels may predict the need for further evaluation of the HPA axis by dynamic tests [10, 11, 12, 13, 14, 15, 16]. A study of patients hospitalized for a non-critical illness (pneumonia, urinary tract infection, gastroenteritis, etc.) showed that basal cortisol level >51.5 ng/mL could predict normal adrenal function with a specificity of 100% [12]. In outpatients, a basal cortisol level less than 36 or more than 181 ng/mL may negate the need for a Synacthen test [13].

Yo et al [14] and Varadhan et al [15] conducted 2 studies with 505 patients and 346 patients respectively. A basal cortisol cut off >136 ng/mL in the Yo study had a specificity of 95% and therefore greater reliability to rule out AI, but with a sensitivity of only 43% to predict normal adrenal function. Yo study did not distinguish between the low-risk group and the high-risk group.

In the Varadhan study, a basal cortisol cut off >145 ng/mL had a specificity of 100% to exclude an AI in a low-risk group. These results are quite close to our results. A basal cortisol level of 114.5 ng/mL had a specificity of 95% to eliminate AI but a sensitivity of only 29.7%. The ROC curve showed that no threshold value of baseline cortisol was both sensitive and specific enough to predict response to Synacthen test despite good predictive value (area under the curve AUC at 0.808; 95% confidence interval [0.738–0.877]).

Using basal cortisol levels as the first step and using a Cut off of 114.5 ng/mL to rule out AI, 16.34% of Synacthen tests could have been avoided; saving health costs. Forty three point thirteen percent of patients would have been put on hormone replacement therapy right away, avoiding the time to perform the stimulation test and the risk of acute adrenal crisis if these patients are not substituted in time.

The threshold value in our study is the lowest compared to the threshold values found in the other studies [11, 12, 13, 14, 15](Table 4). This could be explained by the fact that we have studied a low-risk group of patients who consulted in an outpatient setting. The criteria defined by the various endocrine societies are very strict as they are intended for a population of high-risk patients. The guidelines of the Japanese Society

Table 4. Comparison of different Studies that tried to determine a Cut off of basal Cortisol level that predicts the response to the Synacthen Test.

	Number of patients	Population	Cut off of basal cortisol level to rule out AI (ng/mL)	Sensitivity (%)	Specificity (%)
Our study	153	Low risk group, outpatient unit	114.5	30	95
Karaca et al*	64	High risk group, hospital unit	180	47	100
Kadiyala et al*	166	Low risk group, hospital unit	152	100	54
Yo et al*	505	High risk group and low risk group	136	43	95
Varadhan et al*	346	Low risk group, outpatient unit	145	43	100

* These studies were conducted by comparing baseline cortisol levels with the 250 µg Synacthen test.

of Endocrinology, for instance, retain a basal cortisol level higher than 180 ng/mL to rule out AI [17].

In addition, all of the studies listed above compared basal cortisol levels to the 250 µg Synacthen test. Only our study compared basal cortisol levels to the 1 µg Synacthen test.

About 80% of plasma cortisol is bound to cortisol-binding globulin (CBG); the measured cortisol level is therefore a function of CBG [12]. High estrogen states, such as being pregnant or using oral contraceptive pills, increase CBG and thus total plasma cortisol level. In contrast, chronic liver disease, multiple myeloma and nephrotic syndrome result in lower levels of CBG.

One of the strengths of our study is the fact that the Synacthen test application forms specified the patient's history and current medications, which allowed us to exclude all cases with potentially confounding medication or co-morbidities.

The cortisol level vary according to the methodology used. Sbardella et al [10] compared the three most commonly used cortisol immunoassays in 3571 patients and concluded that baseline cortisol is a valuable tool for the initial evaluation of adrenal function and could avoid the need for dynamic tests in a significant number of patients. The cut off >145 ng/mL used in our study is consistent with the values proposed by Sbardella et al.

In this study we aim to assess if baseline morning cortisol could be useful to predict adrenal sufficiency and determine a baseline cortisol level that predicts the response to the Synacthen test in low risk patients. Our results should be compared to a control group in subsequent work.

5. Conclusion

Based on our findings, we propose a basal cortisol level assay as a first step in the evaluation of patients with suspected AI. Patients with basal cortisol level below 40 ng/mL should start hormone replacement therapy. A basal cortisol level > 147.5 ng/mL is highly predictive of normal adrenal function and a dynamic test is not required especially in this low risk group. For patients with basal cortisol level between 40 to 85 ng/mL, a Synacthen test should be performed. In the range of basal cortisol levels between 85 and 147.5 ng/mL, baseline cortisol level measurements should be repeated and the Synacthen test should be considered if several clinical risk factors for AI are present.

However, even though most patients will be diagnosed with high accuracy using these basal cortisol levels, clinical judgment is essential in deciding whether to fully rely on these values. It is up to the clinician to judge, on case-by-case basis, the need for dynamic tests and whether to initiate or not a hormone replacement therapy in stressful situations.

Declarations

Author contribution statement

Najoua Lassoued: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Baha Zantour, Wafa Alaya & Mohamed Habib Sfar: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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