CLINICAL STUDY

Comparison of the cortisol responses to testing with two doses of ACTH in patients with suspected adrenal insufficiency

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Abstract

Objectives: To compare the cortisol response of the 1 µg and the 250 µg ACTH test in a large study of patients with suspected adrenal insufficiency.

Design: Retrospective cohort study.

Methods: Single center study assessing patients tested for primary or secondary adrenal insufficiency between January 2004 and December 2007, who had both ACTH tests (1 µg and 250 µg; *n* = 207) within a time interval of 6 weeks. Test results were compared with a Bland–Altman plot and McNemar's test.

Results: The mean difference between the cortisol responses in the two ACTH tests was 26 nmol/l (95% confidence interval (CI) 13, 40), showing a marginally higher response for the 250 µg test. The diagnostic performances of the two tests were similar (*P* = 0.49) using a cut-off value for cortisol of 550 nmol/l. A normal cortisol response to the 1 µg ACTH test could be accompanied by an abnormal response to the 250 µg ACTH test, and vice versa.

Conclusion: This study shows that the 1 µg and the 250 µg ACTH tests have comparable cortisol responses in patients with suspected adrenal insufficiency. However, in individual patients, the difference in cortisol response to the two tests can be substantial, and the response in the 250 µg test is not invariably higher than the response in a 1 µg test.

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Introduction

Adrenal insufficiency is a potentially life-threatening condition characterized by decreased endogenous cortisol production. Adrenal insufficiency is associated with adrenal diseases (primary adrenal insufficiency) such as autoimmune adrenalitis, and with diseases interfering with ACTH production by the pituitary such as pituitary tumors (secondary adrenal insufficiency) (1–3). In addition, adrenal insufficiency can be caused by suppression of the hypothalamic–pituitary–adrenal (HPA) axis by prolonged use of exogenous corticosteroids.

Several tests are available for the assessment of adrenal secretory reserve and for assessment of the complete HPA axis (4). The ACTH test directly assesses cortisol reserve after administration of an i.v. bolus of exogenous ACTH. The cortisol response to ACTH can also be decreased in patients with severe secondary adrenal insufficiency (5). Currently, two doses of ACTH are used for diagnostic purposes in patients with suspected adrenal insufficiency: 1 µg and 250 µg

ACTH (3, 4, 6, 7). The 1 µg test is known to be the lowest dose to cause maximal cortisol response (7). There is, however, an ongoing debate about which dose should be used for the initial assessment of adrenal function. For instance, it is suggested that a dose of 250 µg ACTH is a supraphysiological stimulus, not sensitive enough to detect mild secondary adrenal insufficiency (8–10).

The main aim of the present study was to compare the cortisol responses of two ACTH tests (1 µg and 250 µg) in a large cohort of patients suspected of primary or secondary adrenal insufficiency.

Methods

Patient selection

This retrospective diagnostic study was performed at the outpatient clinic of the Endocrinology Department at the Leiden University Medical Center (LUMC). All consecutive patients suspected for primary or secondary

adrenal insufficiency who underwent both ACTH tests (1 µg and 250 µg) between January 2004 and December 2007 were eligible for the present analysis. For inclusion, the two ACTH tests should have been performed within a time interval of 6 weeks. We excluded patients using exogenous glucocorticoids for non-endocrine diseases ($n=5$), and females using oral contraceptives at the time of testing ($n=2$). A total of 207 patients were included in this study. The ACTH tests were part of the standard diagnostic procedures in our center for patients with suspected adrenal insufficiency. At the time the analysis was performed, no formal approval from the Ethics Committee of the LUMC was needed for retrospective data analysis. All patients were completely informed and gave oral informed consent for the diagnostic performances according to the Dutch law regarding patient care.

Test protocol of the 1 µg and the 250 µg ACTH test

Patients arrived at the hospital at 0800 h in a fasting state. An i.v. catheter was inserted for blood sampling. In all patients, endogenous ACTH and cortisol levels were measured twice with a time interval of 10 min before the administration of ACTH. For the 1 µg ACTH test, from 1 ampul tetracosactide (Synacthen 0.25 mg/ml, Novartis), a dose of 1 µg was administered as a bolus i.v. One microgram of ACTH was prepared as follows: 0.4 ml was extracted from a 1 ml ampul of tetracosactide containing 250 µg (Synacthen 0.25 mg/ml), and added to 100 cc NaCl 0.9%, resulting in an ACTH concentration of 100 µg/100 ml. Subsequently, 1 cc (= 1 µg) of this solution was extracted for injection.

For the 250 µg test, from 1 ampul tetracosactide (Synacthen 0.25 mg/ml), a dose of 250 µg was administered i.v. Thirty minutes after administration, a blood sample was drawn to measure cortisol concentrations. Monitoring of side effects was routinely performed by the nursing staff for all dynamic tests. According to test protocols in the LUMC, ACTH tests are not performed within 3 months of pituitary surgery.

Cortisol assay

Cortisol was measured by fluorescence immunoassay on a TDx (Abbott Laboratories). The interassay variation coefficient was 5–6% above 500 nmol/l and amounts to 12% under 200 nmol/l. The detection limit was 20 nmol/l. Cortisol concentrations of ≥ 550 nmol/l after ACTH stimulation were considered normal cortisol responses (11).

Statistical methods

For paired binary data, the McNemar test was used. To assess the association between the values of the two ACTH tests on a continuous scale, Pearson's correlation coefficient and the Bland–Altman plot were used.

For the Bland–Altman plot (12), the mean difference of the cortisol response including its 95% CI and the limits of agreement were calculated. STATA 10 (StataCorp Lp, College Station, TX, USA) was used for statistical analysis.

Results

Patient characteristics

We performed both a 1 µg ACTH test and a 250 µg ACTH test in 207 patients with a time interval of maximum 6 weeks between both tests. The median time interval between the two tests was 7 days. In 8% of patients only, the time interval was more than 14 days. The 1 µg ACTH test was performed before the 250 µg ACTH test in 99% of all tested persons, with intervals between both tests of at least 7 days. In three patients, the 1 µg ACTH was performed after the 250 µg ACTH test. The time interval between both tests in these three cases was 16, 18, and 24 days respectively.

The patient characteristics are shown in Table 1. Mean age of the patients was 45 years (range 17–80 years). Most patients ($n=109$, 53%) were tested for suspected secondary adrenal insufficiency, 98 patients (47%) for suspected primary adrenal insufficiency. Basal ACTH levels were higher for patients with suspected primary insufficiency (43 ng/l) than for patients with suspected secondary insufficiency (29 ng/l). None of the patients used oral contraceptives at the time of testing. Only a few patients (3%) experienced some transient (1–2 min) and non-serious side effects after the administration of the bolus injection of ACTH, such as nausea, sweating, hypotension, and headache.

Comparison of the 1 µg and the 250 µg ACTH test

The mean maximal cortisol response was 596 nmol/l (range 30, 1279) for the 1 µg test, and 622 nmol/l (range 20, 1533) for the 250 µg test (Figs 1 and 2).

Table 1 Characteristics of included patients ($n=207$).

	Number (%)
Age (mean, range)	45 (17–80)
Sex	
Male	80 (39%)
Female	127 (61%)
Diagnosis	
Suspected primary adrenal insufficiency	98 (47%)
DM	18 (9%)
Autoimmune thyroid disease	44 (21%)
Other	36 (17%)
Suspected secondary adrenal insufficiency	109 (53%)
Non-functioning macroadenoma	28 (14%)
Cushing's disease (postoperative)	20 (10%)
Macroprolactinoma	18 (9%)
GH-producing macroadenoma	15 (7%)
Other diseases affecting the pituitary	28 (14%)

Figure 1 Correlation between the cortisol responses in the 1 µg and the 250 µg ACTH stimulation tests. The dotted line shows the cut-off value of 500 nmol/l, and the straight line shows the cut-off value of 550 nmol/l.

The 30 min cortisol values of the two tests demonstrated a strong linear correlation (Pearson's correlation coefficient 0.87, $P < 0.001$). The mean difference between the responses of the two ACTH tests was 26 nmol/l (95% CI 13, 40), showing a marginally higher cortisol response for the 250 µg test. The limits of agreement were -173 to 226 nmol/l. There was no increase in difference between the two tests with increasing responses to cortisol.

Normal test responses and degree of concordance

In 146 of the 207 patients (71%), cortisol responses were normal for the 1 µg ACTH versus 151 patients (73%) for the 250 µg ACTH test, when applying a cut-off value for a normal cortisol response of ≥ 550 nmol/l. The proportion of patients with a normal cortisol response in both tests was 64% (132 patients), and both tests showed insufficient responses in 42 other patients (20%). The results of both tests were discordant in 33 patients (16%): 14 patients had a normal cortisol response of the 1 µg test, but an abnormal cortisol

response of the 250 µg test, whereas 19 patients had an abnormal cortisol response during the 1 µg test, but a normal cortisol response during the 250 µg test. There was no statistical difference in test performance (McNemar, $P = 0.49$). In patients with a normal cortisol response in the 1 µg test, but an abnormal cortisol response in the 250 µg test, the cortisol responses for the latter test ranged from 420 to 548 nmol/l. In patients with a normal cortisol response of the 250 µg test, but an abnormal cortisol response in the 1 µg test, the cortisol responses in the latter test ranged from 310 to 541 nmol/l. Of the 33 patients with discordant results, the treating physicians decided to treat nine patients with corticosteroid substitution. Five of them had a higher cortisol response for the 1 µg test, and four had a higher cortisol response of the 250 µg test.

When the cut-off value for a normal test response was lowered to a cortisol value of 500 nmol/l, the number of patients with a normal cortisol response increased to 159 (77%) for the 1 µg test, and to 172 (83%) for the 250 µg test. The proportion of patients with a normal cortisol response in both tests was 154 (74%); 30 patients (14%) had an abnormal cortisol response of both tests. In 23 patients (11%), the test results were discordant: 5 patients had a normal cortisol response of the 1 µg test but an abnormal cortisol response using 250 µg, and 18 patients had an abnormal cortisol response with 1 µg but a normal cortisol response of the 250 µg test. Using a cut-off value of 500 nmol/l, the 250 µg test showed more normal cortisol responses than the 1 µg test (McNemar, $P = 0.01$).

Test response in relation to clinical indication: suspected primary versus secondary insufficiency

In patients suspected of primary adrenal insufficiency ($n = 98$), 65 patients (66%) had a normal cortisol response in both tests and 17 patients (17%) had an abnormal cortisol response in both tests. Sixteen patients (16%) had discordant test results: 5 patients (5%) had a normal cortisol response of the 1 µg test but an abnormal cortisol response of the 250 µg test, and 11 patients (11%) had an abnormal cortisol response of the 1 µg test but a normal cortisol response of the 250 µg test (McNemar $P = 0.21$).

In those patients suspected of secondary adrenal insufficiency ($n = 109$), 67 patients (61%) had a normal cortisol response of both tests and 25 patients (23%) had an abnormal cortisol response of both tests. Seventeen patients (16%) had discordant test results: nine patients (8%) had a normal cortisol response of the 1 µg test and an abnormal cortisol response of the 250 µg test; eight (7%) patients had an abnormal cortisol response of the 1 µg test but a normal cortisol response of the 250 µg test. There was no statistical difference in test performances (McNemar $P = 1$).

Figure 2 Bland–Altman plot comparing the cortisol responses of the 1 µg and the 250 µg ACTH tests. The dotted lines represent the limits of agreement (-173 to 226 nmol/l).

Discussion

The present study analyzed the cortisol response of the 1 µg and the 250 µg ACTH tests in a large cohort of patients suspected for adrenal insufficiency. We demonstrated that the 30 min cortisol response to both tests was almost exactly the same. This is in line with findings from other studies (13). In addition, for cortisol cut-off values of 500 as well as 550 nmol/l, the diagnostic performances of both tests were similar, with a marginally higher proportion of normal test results for the 250 µg test.

The major strength of the present analysis is the large number of patients ($n=207$). This is the largest single center study directly comparing cortisol responses in high and low dose ACTH test in patients suspected for adrenal insufficiency (10). Moreover, in our study, patients tested for primary as well as secondary adrenal insufficiency were included, thereby enhancing the generalizability of the study results. In the present study, cortisol values were obtained 30 min after ACTH stimulation. The results may therefore not be generalizable to cortisol responses 60 min after ACTH tests (14). The meta-analysis comparing the 250 µg ACTH test with the 1 µg ACTH tests showed variability across studies in the optimal timing for measuring cortisol response; importantly, in no study, a statistically significant difference in diagnostic discrimination at 30 min, 60 min, or at peak response was shown (10).

A limitation of the present study is that the study does not allow to assess how these tests perform in the diagnosis of adrenal insufficiency, because the reference standard (insulin tolerance test (ITT)) was not included in the design. However, in clinical practice, ACTH tests are often performed as a first step in the diagnostic evaluation of adrenal insufficiency, and subsequent steps are based on these results in combination with the clinical picture (3). However, the results from this study are clinically relevant for two reasons. Firstly, the cortisol responses to both ACTH doses were on average the same. Secondly, the study showed that differences in cortisol response between the two tests can range from -173 nmol/l (lower response in the 250 µg test) to 226 nmol/l (higher response in the 250 µg test). This indicates that the cortisol response after a 250 µg test is not invariably higher than the response in a 1 µg test and that the differences in responses can be substantial. The imperfect sensitivity of the ACTH test for the detection of primary adrenal insufficiency (15) might be partly explained by the varying cortisol responses within patients.

Recently, a meta-analysis compared the diagnostic performances between the 1 µg ACTH and the 250 µg ACTH tests (10). That study showed a better diagnostic performance for the 1 µg ACTH test, mainly due to a better sensitivity. We showed that the cortisol responses of both tests were similar. The similarity of the results of both tests might be an argument in favor of performing a 250 µg test routinely, because of its higher ease of

administration (16). However, the finding that for a cut-off value of ≥ 500 nmol/l (1), the 250 µg test showed a slightly higher proportion of normal test results, is in line with the suggested better sensitivity of the 1 µg test (10, 17, 18).

In conclusion, this study shows that the 1 µg ACTH test and the 250 µg ACTH test have a similar cortisol response in patients with suspected adrenal insufficiency. However, in individual patients, the difference in cortisol response of the two tests can be substantial, and the response in the 250 µg test is not invariably higher than the response in a 1 µg test.

Declaration of interest

O M Dekkers, J M Timmermans, J W A Smit, and J A Romijn have nothing to declare. A M Pereira reports to have received lecture fees from Novartis and Pfizer.

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References

- 1 Arlt W & Allolio B. Adrenal insufficiency. *Lancet* 2003 **361** 1881–1893. (doi:10.1016/S0140-6736(03)13492-7)
- 2 Dekkers OM, Pereira AM, Roelfsema F, Voormolen JH, Neelis KJ, Schroijen MA, Smit JW & Romijn JA. Observation alone after transsphenoidal surgery for nonfunctioning pituitary macroadenoma. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 1796–1801. (doi:10.1210/jc.2005-2552)
- 3 Oelkers W. Adrenal insufficiency. *New England Journal of Medicine* 1996 **335** 1206–1212. (doi:10.1056/NEJM199610173351607)
- 4 Grinspoon SK & Biller BM. Clinical review 62: laboratory assessment of adrenal insufficiency. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 923–931. (doi:10.1210/jc.79.4.923)
- 5 Agha A, Tomlinson JW, Clark PM, Holder G & Stewart PM. The long-term predictive accuracy of the short synacthen (corticotropin) stimulation test for assessment of the hypothalamic–pituitary–adrenal axis. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 43–47. (doi:10.1210/jc.2005-1131)
- 6 Dickstein G, Shechner C, Nicholson WE, Rosner I, Shen-Orr Z, Adawi F & Lahav M. Adrenocorticotropin stimulation test: effects of basal cortisol level, time of day, and suggested new sensitive low dose test. *Journal of Clinical Endocrinology and Metabolism* 1991 **72** 773–778. (doi:10.1210/jcem-72-4-773)
- 7 Dickstein G, Spiegel D, Arad E & Shechner C. One microgram is the lowest ACTH dose to cause a maximal cortisol response. There is no diurnal variation of cortisol response to submaximal ACTH stimulation. *European Journal of Endocrinology* 1997 **137** 172–175. (doi:10.1530/eje.0.1370172)
- 8 Tordjman K, Jaffe A, Trostanetsky Y, Greenman Y, Limor R & Stern N. Low-dose (1 microgram) adrenocorticotrophin (ACTH) stimulation as a screening test for impaired hypothalamo-pituitary–adrenal axis function: sensitivity, specificity and accuracy in comparison with the high-dose (250 microgram) test. *Clinical Endocrinology* 2000 **52** 633–640. (doi:10.1046/j.1365-2265.2000.00984.x)
- 9 Rasmuson S, Olsson T & Hagg E. A low dose ACTH test to assess the function of the hypothalamic–pituitary–adrenal axis. *Clinical Endocrinology* 1996 **44** 151–156. (doi:10.1046/j.1365-2265.1996.600482.x)

- 10 Kazlauskaitė R, Evans AT, Villabona CV, Abdu TA, Ambrosi B, Atkinson AB, Choi CH, Clayton RN, Courtney CH, Gonc EN, Maghnie M, Rose SR, Soule SG, Tordjman K & Consortium for Evaluation of Corticotropin Test in Hypothalamic–Pituitary–Adrenal Insufficiency. Corticotropin tests for hypothalamic–pituitary–adrenal insufficiency: a metaanalysis. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4245–4253. (doi:10.1210/jc.2008-0710)
- 11 May ME & Carey RM. Rapid adrenocorticotrophic hormone test in practice. Retrospective review. *American Journal of Medicine* 1985 **79** 679–684. (doi:10.1016/0002-9343(85)90517-0)
- 12 Bland JM & Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986 **327** 307–310. (doi:10.1016/S0140-6736(86)90837-8)
- 13 Mayenknecht J, Diederich S, Bahr V, Plockinger U & Oelkers W. Comparison of low and high dose corticotropin stimulation tests in patients with pituitary disease. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1558–1562. (doi:10.1210/jc.83.5.1558)
- 14 Dekkers OM, von Elm E, Algra A, Romijn JA & Vandenbroucke JP. How to assess the external validity of therapeutic trials: a conceptual approach. *International Journal of Epidemiology* 2010 **39** 89–94. (doi:10.1093/ije/dyp174)
- 15 Suliman AM, Smith TP, Labib M, Fiad TM & McKenna TJ. The low-dose ACTH test does not provide a useful assessment of the hypothalamic–pituitary–adrenal axis in secondary adrenal insufficiency. *Clinical Endocrinology* 2002 **56** 533–539. (doi:10.1046/j.1365-2265.2002.01509.x)
- 16 Wade M, Baid S, Calis K, Raff H, Sinaii N & Nieman L. Technical details influence the diagnostic accuracy of the 1 microg ACTH stimulation test. *European Journal of Endocrinology* 2010 **162** 109–113. (doi:10.1530/EJE-09-0746)
- 17 Courtney CH, McAllister AS, Bell PM, McCance DR, Leslie H, Sheridan B & Atkinson AB. Low- and standard-dose corticotropin and insulin hypoglycemia testing in the assessment of hypothalamic–pituitary–adrenal function after pituitary surgery. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 1712–1717. (doi:10.1210/jc.2003-031577)
- 18 González J, Villabona C, Ramn J, Navarro MA, Giménez O, Ricart W & Soler J. Establishment of reference values for standard dose short synacthen test (250 microgram), low dose short synacthen test (1 microgram) and insulin tolerance test for assessment of the hypothalamo-pituitary–adrenal axis in normal subjects. *Clinical Endocrinology* 2000 **53** 199–204. (doi:10.1046/j.1365-2265.2000.01028.x)

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