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.

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>
<thead>
<tr>
<th>Table 1 Characteristics of included patients (n=207)</th>
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<tr>
<td><strong>Age (mean, range)</strong></td>
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<td><strong>Sex</strong></td>
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<td><strong>Female</strong></td>
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<tr>
<td><strong>Diagnosis</strong></td>
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<tr>
<td><strong>Suspected primary adrenal insufficiency</strong></td>
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<tr>
<td><strong>Suspected secondary adrenal insufficiency</strong></td>
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<td><strong>Autoimmune thyroid disease</strong></td>
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<tr>
<td><strong>Non-functioning macroadenoma</strong></td>
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<td><strong>Cushing’s disease (postoperative)</strong></td>
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<td><strong>Other</strong></td>
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<td><strong>Macroprolactinoma</strong></td>
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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 \( \mu \)g 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 \( \mu \)g ACTH versus 151 patients (73%) for the 250 \( \mu \)g ACTH test, when applying a cut-off value of 500 nmol/l, and the straight line shows the cut-off value of 550 nmol/l.

The proportion of patients with a normal cortisol response of 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 in both tests. In 23 patients (11%), the test results were discordant: 5 patients had a higher cortisol response for the 1 \( \mu \)g test but an abnormal cortisol response using 250 \( \mu \)g, and 18 patients had a normal cortisol response with 1 \( \mu \)g but a normal cortisol response of the 250 \( \mu \)g test. Using a cut-off value of 500 nmol/l, the 250 \( \mu \)g test showed more normal cortisol responses than the 1 \( \mu \)g 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 \( \mu \)g test but an abnormal cortisol response of the 250 \( \mu \)g test, and 11 patients (11%) had an abnormal cortisol response of the 1 \( \mu \)g test but a normal cortisol response of the 250 \( \mu \)g 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 \( \mu \)g test and an abnormal cortisol response of the 250 \( \mu \)g test; eight (7%) patients had an abnormal cortisol response of the 1 \( \mu \)g test but a normal cortisol response of the 250 \( \mu \)g test. There was no statistical difference in test performances (McNemar \( P = 1 \)).
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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