Dexamethasone Infusion Testing in the Diagnosis of Cushing's Syndrome

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Abstract. Cushing's syndrome and its various aetiologies is a markedly difficult diagnosis to make given its subtle signs, sometime cyclical nature, and the lack of a single definitive diagnostic test. Although a great variety of diagnostic tests have been developed to assist in the diagnosis, even with the best clinical acumen, biochemistry and medical imaging the diagnosis can remain elusive. The long low and high dose oral dexamethasone suppression test is cumbersome, costly and often requiring an extended inpatient stay. The utility of the dexamethasone suppression test would be greatly enhanced if it could be performed as a short outpatient procedure. In this study we sought to confirm and refine the clinical utility of the high dose 4 mg intravenous dexamethasone suppression test as an alternative diagnostic test for Cushing's syndrome. There were a total of 31 subjects: 8 patients with proven pituitary Cushing's disease, 3 with primary adrenal tumors, 10 with pseudo-Cushing's syndrome and 10 healthy controls. All subjects with pseudo-Cushing's syndrome suppress serum cortisol at +5 and at +24 hours. In subjects with pituitary Cushing's disease, 7 out of 8 (88%) had serum cortisol suppressed at +5 hours but rebounded at +24 hours to at least 70% of the original serum level. Primary adrenal tumors showed a pattern of non-suppression throughout. The 4 mg intravenous dexamethasone suppression test is excellent in ruling out pseudo-Cushing's syndrome. This test is much simpler and more convenient than the *oral* dexamethasone suppression test in confirming clinical suspicion of pituitary Cushing's disease.

Key words: Diagnostic tests, Intravenous, Dexamethasone, Cushing's syndrome

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ENDOGENOUS Cushing's syndrome (CS) has several aetiologies including pituitary-dependent Cushing's disease (PC), ectopic ACTH syndrome (EAS), primary adrenal tumour (PAT) and cyclical Cushing's (CC). Assessment is complicated by the possibility of pseudo-Cushing's syndrome (Pseudo-CS) including the effects of depression on the hypothalamic-pituitary-adrenal axis. The low and high dose oral dexamethasone suppression tests (ODST) are important procedures in the diagnostic workup but these standard four-day oral dexamethasone tests are time consuming, costly and

require an inpatient stay. Attempts to evaluate the utility of intravenous dexamethasone infusion in the diagnosis of CS scatter throughout the past few decades [1–3]. Over the years, there has been a number of evaluation of an alternative one-day outpatient procedure using intravenous dexamethasone infusion. The dosage of infusions include 1.75 mg (5 µg/kg/hr) for 5 hours [4], 4 mg (1 mg/hr) [5, 6], 7 mg (1 mg/hr) [7], 8 mg bolus infusion [8] and 10.5 mg (1.5 mg/hr for 7 hours) [9]. In this study we seek to confirm and refine the clinical utility of the dexamethasone infusion test as an alternative diagnostic test in the clarification of various causes of Cushing's syndrome.

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Patients and Methods

The test was performed in 31 consented subjects.

There were 10 *normal* subjects (6 females (F) and 4 males (M)). The mean age $\pm$ standard error of the mean (SEM) was 44.9 ± 4.4 years, range 25 to 66 years. The mean body mass index was 23.2 ± 2.6 kg/m². All normal female subjects report regular menses ranging from 24 to 34 days between cycles.

The following criteria (at least 4 out of 5) are to be met for the diagnosis of PC to be fulfilled:

- Clinical and biochemical evidence of endogenous hypercortisolaemia.
- Confirmation of pituitary adenoma by pituitary Magnetic Resonance Imaging (MRI).
- Evidence of pituitary-dependent ACTH overproduction using bilateral simultaneous inferior petrosal sinus sampling (BIPSS).
- Resolution of hypercortisolaemia after surgery requiring glucocorticoid therapy.
- (Immuno-) histological confirmation of the presence of ACTH in pituitary tissue (available in some cases only).

There were 10 (7 F : 4 M) subjects with PC satisfying the aforementioned criteria. Five female patients had oligomenorrhoea with periods ranging from 40 to 88 days between cycles. The other two patients were receiving the oral contraceptive pills.

Three patients (2 F : 1 M) with PAT were studied. All had surgically and histologically proven adrenal adenoma. The removed glands were 35, 45 and 42 grams in weight respectively. Both females in this group reported amenorrhoea in the preceding 4 months.

Ten patients (8 F : 2 M) with pseudo-CS were referred for further investigations following the finding of one or a combination of elevated morning serum cortisol, elevated 24 hour urinary free cortisol or loss of diurnal cortisol pattern. Six (F) had depressive illness, 2 (F) had elevated urinary cortisol excretion, 1 (M) suffered from alcoholism and 1 (M) had lost the diurnal cortisol rhythm. Six female patients had oligomenorrhoea with periods ranging from 38 to 98 days between cycles. Pseudo-CS was confirmed and PC, including CC, was excluded (at 4 out of 5) in all these subjects based on the following criteria:

- Low clinical suspicion of Cushing's syndrome.
- Prolonged duration of follow-ups: at 2 years at the time of this report with no clinical suspicion of hypercortisolaemia.

- The presence of co-morbidities including obesity, depression and anxiety disorders.
- Normal response to a 1 mg overnight ODS.
- Normal pituitary MRI in some cases.

Intravenous (IV) dexamethasone infusion protocol

Two intravenous (IV) cannulas were inserted for IV infusion of dexamethasone and venesection. Starting at 11:00 hours on the day of procedure, dexamethasone 4 mg was given by IV infusion at 1 mg/hr according to the Abou Samra protocol [5]. Samples were taken for serum cortisol at T-1, 0, +3, +4, +5, +22 and +24 hours. In order to refine and circumvent the need for inpatient hospitalisation, no sampling was performed in the 17 hour period between +5 and +22 hours. Previous report [8] was reassuring in that levels at +22 and +24 hours reflected accurately that which occurred in the unmonitored time gap in the various causes of CS.

Serum cortisol assay

Serum cortisol and ACTH assays were measured using the Immulite 2000 platform (Diagnostic Products Services (DPC), Los Angeles, California, USA) with the co-efficient of variations of 5.5% and 8.6% respectively. The detection limit for serum cortisol was <28 nmol/L.

Statistical analyses

Results are presented as the Mean $\pm$ Standard Error (SE) and Confidence Intervals (CI).

Statistical analyses including CI are performed with the software package Confidence Interval Analysis (Version 2.0.0) provided with reference 10.

Results

The normal control subjects (n = 10) showed an expected response to intravenous dexamethasone with continuous suppression of serum cortisol at +5, +22 and +24 hours. The 24 hour values were all suppressed to below detection capability of the assay.

In the subjects with pseudo-CS (n = 10), the range of hypercortisolaemia was broad. Similar to normal subjects however, suppression occurred at +5 hours, +22

Table 1. Shows individual cortisol concentrations at the critical time points in the various categories of CS including the Mean, SEM, 95% CI and percentage of suppression at +5, +22 and +24 hours (compared with baseline) respectively.

	TIME (HRS)				
	-1	0	+5	+22	+24
A. Normal Subjects (NS) – (n = 10)					
	<i>Serum Cortisol concentration (nmol/L)</i>				
1	421	116	41	31	28
2	368	129	82	30	32
3	228	83	55	28	28
4	556	121	72	42	28
5	481	101	66	30	28
6	391	92	51	28	28
7	310	88	48	33	32
8	288	92	57	34	28
9	411	79	60	28	28
10	251	81	62	28	28
Mean	370.5	98.2	59.4	31.2	28.8
SEM	32.7	5.6	3.8	1.4	0.53
Percentage of suppression			84%	92%	92%
95% CI	296.5 to 444.5	85.4 to 111.0	50.9 to 68.0	28.1 to 34.3	27.6 to 30.0
B. Pseudo-Cushing's syndrome (Pseudo-CS) – (n = 10)					
1*	926	652	206	107	180
2	351	151	76	28	28
3*	1136	1007	380	112	140
4	580	440	250	210	190
5	190	160	40	28	28
6	403	90	30	28	28
7	195	180	52	28	28
8	594	313	98	68	28
9	270	159	70	30	28
10	708	270	110	101	88
Mean	535.3	342.2	131.2	74	76.6
SEM	100.3	91.1	35.7	18.9	21.6
Percentage of suppression			75%	86%	86%
95% CI	308.4 to 762.2	136.1 to 548.3	50.4 to 212.0	31.2 to 116.8	27.8 to 125.4
C. Pituitary Cushing's disease (PC) – (n = 8)					
	<i>Serum cortisol concentration (nmol/L)</i>				
1	412	612	141	381	350
2	350	378	98	550	490
3	700	530	80	550	490
4	510	410	112	432	411
5*	870	811	750	898	911
6	1211	702	128	718	650
7	740	712	200	376	381
8	665	562	68	401	528
Mean	682.3	589.6	197.1	538.3	526.4
SEM	97.5	53.2	80.3	65.9	64.3
Percentage of suppression			71%	21%	22%
95% CI	451.8 to 912.7	463.8 to 715.4	7.3 to 387.0	382.4 to 694.1	374.4 to 678.4
D. Primary Adrenal Tumors (PAT) – (n = 3)					
1	301	270	510	590	600
2	1460	1370	1201	2011	1600
3	660	780	880	900	820
Mean	807	806.7	863.7	1167	1006.7
SEM	342.6	317.8	199.6	431.4	303.4

and +24 hours. Subjects number 1 and 3 (denoted * in section B, Table 1) were unusual with slight increases in cortisol levels at +24 hours compared with +22 hours rather than continued suppression. These patients however fully satisfied the aforementioned criteria for the diagnosis of pseudo-CS. Given this result, this test thus had a specificity of 80% in the assessment of Pseudo-CS.

In subjects with confirmed PC (n = 8), there was also suppression at +5 hours with rebound at +22 and +24 hours. The lowest value for serum cortisol at +22 hours was 376 nmol/L which did not overlapped with the values in the pseudo-CS group. Subject number 5 (denoted * in section C, Table 1) showed an anomalous response with no suppression at +5 hours. As this subject was satisfied the diagnostic criteria for PC, the sensitivity of this test was 90%.

In subjects with adrenal adenoma (n = 3), there was a wide range of hypercortisolaemia. No suppression of cortisol was seen at all throughout the dynamic test. There was a suggestion of a cortisol rise at +24 hours but the number was small. Serum ACTH was suppressed below the detection limit throughout the test.

All individual data at the critical time points are listed in Table 1. The broad CI's are not shown for the PAT group as the patient number is small for this analysis. Fig. 1 summarises the mean cortisol response for all categories.

Discussion

Endogenous CS presents as one of the most difficult diagnostic problems in clinical endocrinology. There is no single gold standard test and the diagnosis relies on a combination of clinical manifestation, biochemical testings and response to therapy, be it pituitary surgery or otherwise. There are vast numbers of tests used to elucidate the cause of CS after hypercortisolaemia has been confirmed biochemically [12–14]. The fact that there are numerous tests for this condition reinforces the belief that no one test is diagnostic.

These tests can be arranged into first-line screening tests which confirm or exclude the presence of hypercortisolaemia in CS. These include the 24-hour urinary free cortisol level, timed overnight cortisol/Creatinine ratio [15], the 1mg oral overnight dexamethasone suppression test and the single midnight serum cortisol level [13], all of which have sensitivities and specificities

Fig. 1. Illustrates the different responses to IV Dexamethasone infusion in the various categories of Cushing's syndrome using *mean* values at critical time points. Error bars indicate SEM. Broken lines indicate the cortisol diurnal variation pattern in normal subjects *without* Dexamethasone (adapted from reference 11).

ties ranging between 95–100% and 94–98%, respectively. Newer first-line tests include the combined oral low dose Dexamethasone suppression test with IV Corticotropin Releasing Hormone (CRH) and IV Desmopressin stimulation test which help to differentiate between Pseudo-CS and CS [12, 16, 17]. These of course are complex and require hospital admission to ensure the correct procedures are followed.

The second-line tests are more complex and are used for the diagnostic work up of the various causes of CS. Some of these include the single ACTH assay, the oral high dose dexamethasone suppression test (HDDST), CRH stimulation test with or without bilateral inferior petrosal sinus sampling (BIPSS) [12, 14]. These dynamic biochemical tests, of course, should be compli-

mented with the appropriate diagnostic imaging, most usually by MRI [13].

The most commonly used second-line test is the high dose oral dexamethasone suppression test (HDDST) where 2 mg of oral dexamethasone is given every 6 hours for 48 hours starting at 09:00 hours with serum and urinary cortisol levels taken at regular intervals. This test has a sensitivity and specificity of 81% and 65% for the diagnosis of PC respectively [18]. This test entails a complex regimen that requires the co-operation of both patient and nursing staff. Regimen confusion, patient compliance and variability in dexamethasone metabolism can confound the result [19]. Absorption and drug metabolism abnormalities are highly pertinent to tests that involve the use of oral

Fig. 2. Algorithm allocating the place of the Dexamethasone infusion test in the diagnostic work-up for CS. This test should be complemented by clinical, additional biochemical and radiological evidence.

medications. In the cases of LDDST and HDDST, both poor absorption and fast metabolisers, either inherited or induced, can lead to false non-suppression and subsequent misdiagnosis [20]. In addition, these tests are not recommended in acute illness due to heightened cortisol response [21].

In order to circumvent the confounders and complexity of oral testing, many have adopted the dexamethasone infusion test but with variable doses ranging

from 1.75 mg to 10.5 mg (with 4 mg being most commonly used) [4–7, 9] and 8 mg IV bolus injection [8]. The common pattern to all these unstandardised studies was suppression in normal subjects and PC at between +5 to 7 hours. PC cortisol levels rebounded invariably to baseline level at +24 hours. In this study we have added evidence to show that a 4 mg dexamethasone infusion test has diagnostic utility in subjects referred for the clarification of CS. These results concur with and

support previous findings in showing that the dexamethasone infusion test is useful in the differential diagnosis of CS.

In our study, the absolute level of cortisol suppression at +5 and +24 hours in normal subjects was <30 nmol/L, <83 nmol/L in the Abou Samra series [5], and <51 nmol/L in the LeClerc *et al.* series [6]. In abnormal subjects who were deemed to have pseudo-CS, the expected response would be an 80% decrease from baseline cortisol level at +5 and +24 hours. In addition, there was a statistically (though not clinically) significant difference of serum cortisol at +24 hours between NS and Pseudo-CS using 95% CI. One possibility was the presence of CC, a particularly difficult diagnosis to make as cycling cortisol levels could range between 12 hours to 85 days [22]. The dimension of time, regular follow-ups (2 years at the time of this analysis) and detailed correlation between clinical and biochemical parameters confidently delivered the extreme reassurance that CC was a remote possibility in this cohort. Another and more plausible alternative was the higher cortisol set point in pseudo-CS and relative resistance to dexamethasone [14]. This explained the difference in mean cortisol concentrations between the two groups which, despite physiological difference, retained the common pattern of response.

All patients with PC had their serum cortisol suppressed, at least partially, at +5 hours following intravenous dexamethasone. The rebound of serum cortisol the following day at +22 and +24 hours was a consistent and reliable diagnostic marker. Abou Samra [5] suggested 50% reduction from baseline value at +5 hours and rebound to >276 nmol/L at +24 hours. Van den Bogaert [7] adopted a cortisol level of more than 190 nmol/L at +7 hours to identify patients with PC. In our series, the majority of patients with PC were confirmed by histopathology, the rest by clinical and biochemical response to surgery. The observed response was more than 50% and less than 20% reduction from baseline cortisol value at +5 and +24 hours respectively. This would present a specificity of 90%.

In PAT, serum cortisol failed to suppress at any time point in response to dexamethasone infusion test and this was consistent with previous reports [5–9]. All three patients had typical clinical phenotype of cortisol excess confirmed by high urinary cortisol excretion

and undetectable ACTH. There was no adrenal carcinoma but the pattern of response was expected to be identical with non-suppression. The diagnosis of PAT would normally be straightforward based on the finding of extremely high serum cortisol but with variable set points, as shown in this study, with undetectable ACTH levels supported by positive radiological imaging. Thus the value of this dynamic test was limited, did not add to the diagnosis and probably remained of academic interest.

EAS is relatively rare and reflecting its absence in this original study. The total number of EAS cases undergoing the dexamethasone infusion test as reported in the literature in the last 24 years amounts to 18 with timed samples at either +5 to 7 hours and/or +24 hours [5–9]. The predominant response noted was non-suppression. Henceforth and based on reported literature, it is our suggestion that this test be used primarily in distinguishing PC from EAS. In this situation, a single sample at +5 hours is pivotal in separating the two conditions. The cortisol concentration here is expected to be greater than 50% reduction from baseline for PC compared with non-suppression in EAS. The +24 hour sample is unhelpful in this particular situation as both will be elevated. Normal subjects will, presumably, not be needing to proceed to this dynamic test unless first line screening tests are equivocal. The diagnosis of PAT is mostly straightforward as previously mentioned.

A possible algorithm is suggested in Fig. 2 for the differential diagnosis of CS. Although mentioned briefly, ACTH level remained crucial in the distinguishing between adrenal from non-adrenal causes of CS. Dexamethasone infusion testing can then be used to identify if PC is the source of hypercortisolaemia.

Summary

The 4 mg intravenous dexamethasone infusion test is consequently a useful diagnostic test for PC. It is useful in providing differential diagnostic information in patients with CS. It is simpler, more convenient and can be used without loss of diagnostic utility and with less consumption of valuable resources.

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