0013-7227/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(5):2106–2111

doi: 10.1210/jc.2002-020969

COMMENT

Ten Years on: Safety of Short Synacthen Tests in Assessing Adrenocorticotropin Deficiency in Clinical Practice

HELENA K. GLEESON, BRIAN R. WALKER, JONATHAN R. SECKL, AND PAUL L. PADFIELD

Metabolic Unit, Western General Hospital, Edinburgh, United Kingdom EH4 2XN

Since 1988, when a retrospective study of patients attending this unit was published, we have advocated the use of the short synacthen test (SST) as the primary screening investigation to detect ACTH deficiency. However, others have published comparisons of SST and insulin tolerance tests that suggest a significant false negative rate with SST, leading to concern that some patients who pass the SST are in danger from the clinical consequences of ACTH deficiency.

To address this, we audited biochemical results and clinical outcome in 63 patients who did not have ACTH deficiency detected (*i.e.* who passed the test) by SST after pituitary surgery.

Twelve of the 63 patients who passed a SST after pituitary surgery became ACTH-deficient later as diagnosed by SST: 4 within the first year, 2 of whom had received postoperative radiotherapy (3 had symptoms of tiredness and 1 was admitted to the hospital with a viral infection); 8 in yr 3–5, 7 of whom had received postoperative radiotherapy (all had either no symptoms or symptoms of tiredness alone). Thus, the predictive value of the SST in excluding ACTH deficiency is approximately 97% (2 of 63 patients who initially passed the SST were found to be ACTH-deficient within 12 months without having received postoperative radiotherapy). Only 1 patient was ill enough to require hospital admission.

Setting the risk of false negatives with SST against the morbidity and manpower implications associated with insulin tolerance tests, SST remains the primary screening test for ACTH deficiency in our practice. However, a high index of clinical suspicion to detect false negative results must be maintained. (*J Clin Endocrinol Metab* 88: 2106–2111, 2003)

THERE HAS BEEN much debate over the last 20 yr as to which is the most appropriate screening test of ACTH deficiency. The short synacthen test (SST) is cheaper, safer, and less unpleasant for the patient than the insulin tolerance test (ITT). Consequently, the use of the SST as a screening investigation for ACTH deficiency is popular among UK endocrinologists, with two surveys, 6 yr apart, demonstrating an increase from 24% in 1988 to 50% in 1994 (1, 2).

In 1988, a study from this unit compared 70 paired SST and ITT results in patients with pituitary disease (1). We advocated the use of the SST as the primary screening investigation to detect ACTH deficiency (1). Furthermore, this retrospective audit showed that a measurement of 0900-h plasma cortisol of more than 400 nM is predictive of an intact hypothalamic-pituitary-adrenal (HPA) axis (1), and many others have confirmed this (3–8). However, different conclusions have been drawn from more recent studies that compared the SST with the ITT in the setting of ACTH deficiency, using various 'pass' levels of plasma cortisol (5, 9–14); a normal cortisol response to a SST can be found in patients with a subnormal response to ITT. This has led to concern that the SST may not demonstrate whether the patient would have an adequate response in a stressful situation, notwithstanding that even the ITT may not be 100% sensitive and specific for ACTH deficiency (10, 15).

Abbreviations: HPA, Hypothalamic-pituitary-adrenal; ITT, insulin tolerance test; SST, short synacthen test.

In the present study, we have taken a different pragmatic approach. Rather than comparing the SST with a so-called gold standard ITT in patients with known ACTH deficiency, we have examined the predictive value of postoperative SST for clinical outcome among all patients undergoing pituitary surgery, including those with and without ACTH deficiency. We have also reexamined the value of measurement of 0900-h plasma cortisol in the immediate postoperative period. This enabled us to examine whether the perceived dangers of undiagnosed ACTH deficiency translate into an unacceptable risk in the clinical scenario in which the test is commonly used.

Subjects and Methods

Patients who had pituitary surgery from March 1989 to February 1999 were traced, using the hospital diagnostic coding system (see Fig. 1). One hundred fifty-three operations in 143 patients were identified. Thirty patients, who had 33 operations, were excluded. Thus, information on 120 operations was obtained from 113 patients' case notes.

The 30 patients were excluded for the following reasons: 6 were unsuitable for the audit (5 were discharged, after neurosurgery, to other centers, 1 had Nelson's syndrome); 24 case notes were not found [18 patients were not assessed in our Metabolic Unit, 5 case notes had been destroyed (4 had died, 1 had not attended since 1993), 1 set of case notes of a patient who had died had been lost].

The recommended protocol after pituitary surgery throughout this period was that: hydrocortisone [20 mg (morning) and 10 mg (afternoon)] be administered for 48 h from the day of surgery; 0900-h plasma cortisol be measured before the hydrocortisone dose on the third postoperative day; hydrocortisone replacement therapy be continued, in the

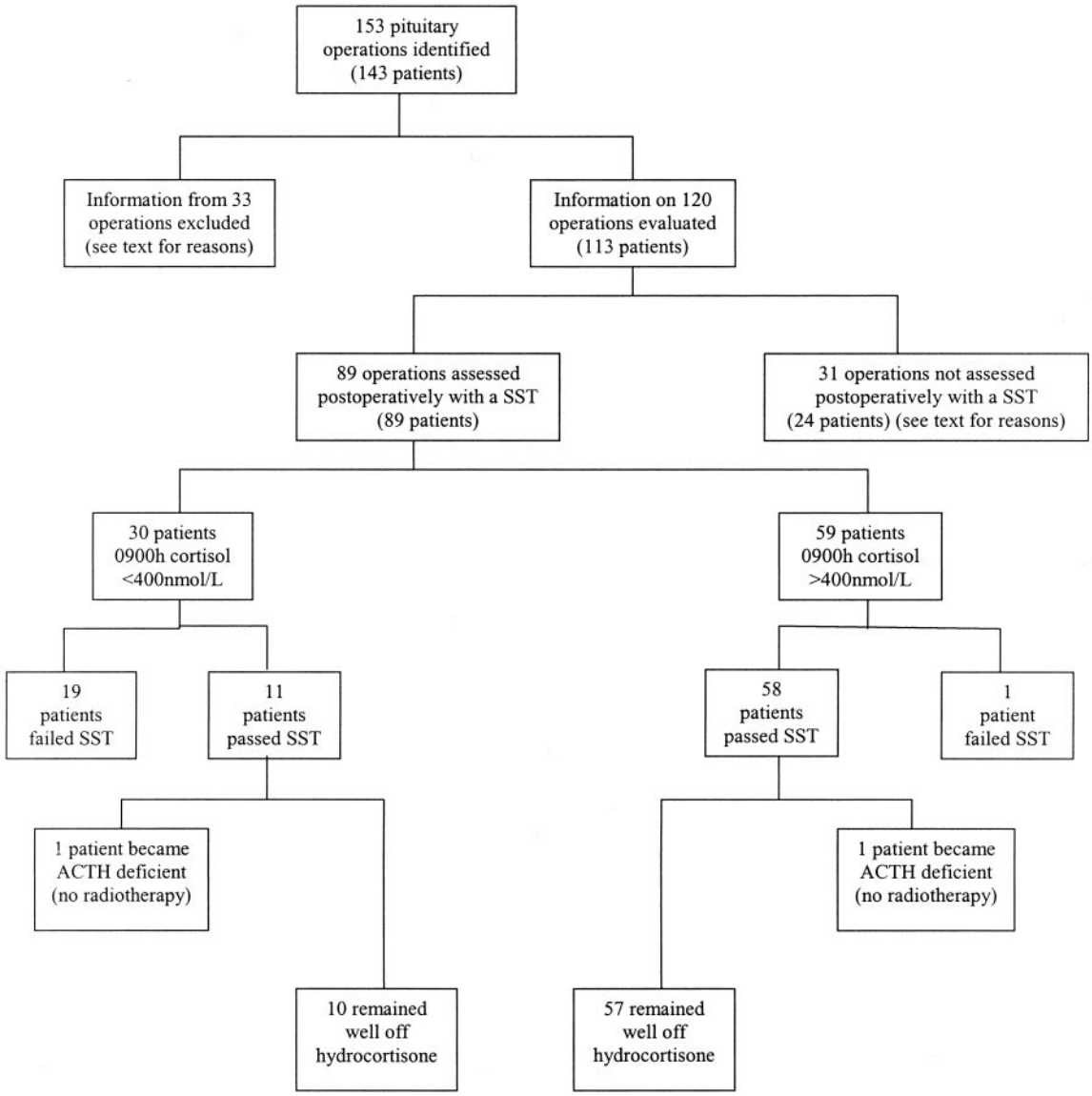

FIG. 1. Numbers of patients and operations assessed and clinical outcome, in the first postoperative year, in those patients passing SST postoperatively.

same regimen, only if 0900-h plasma cortisol was less than 400 nM; and all patients reattend for a SST 4–6 wk postoperatively.

The SST was carried out at varying times of the day by Metabolic Unit nursing staff. Hydrocortisone was omitted on the morning of the test. Blood was collected for measurement of plasma cortisol before and 30 min after im injection of 250 μg 1–24 corticotrophin. Plasma cortisol was measured as follows: from 1989 to August 1994, by a manual competitive RIA (Amerlex M; Amersham Biosciences UK Ltd., Bucks, UK); from July 1994 to May 1999, by a competitive nonisotopic, automated assay (TDX Analyser; Abbott Laboratories Ltd., Berks, UK); and from May 1999 to date, by an automated competitive magnetic separation assay (Immuno 1 Analyser, Bayer plc, Newbury, UK). The criterion for a pass cortisol level for the SST has changed over the years, as the assays have become more specific and hence have generated consistently lower values. Based on extrapolation from the assay used before 1989, for which the peak cortisol cut-off to infer ACTH deficiency was 550 nM (1), the cut-off was adjusted in 1994 to 520 nM and in 1999 to 460 nM. The incremental change after ACTH has never been used in our diagnostic criteria. Because SSTs were performed at all times of the working day, we did not evaluate predictions from the basal cortisol measurement before ACTH administration. At lower cortisol concentrations, the bias in the assay is less pronounced, so that a 0900-h plasma cortisol more than 400 nM has always been taken to indicate intact ACTH secretion 3 d after pituitary surgery.

Statistics

Data are presented as median (range). We used clinical outcome as the gold standard for ACTH deficiency. Terms denoting test outcome were defined as: a false negative result, when either 0900-h cortisol was more than 400 nM or the SST was passed in the setting of ACTH deficiency; a false positive result, when response to a test was subnormal in the setting of an intact HPA axis; the false negative rate, as the percentage of false negative results in patients with known ACTH deficiency; the false positive rate, as the percentage of false positive results in patients diagnosed as having an intact HPA axis; the predictive value of the test to exclude ACTH deficiency, as the percentage of true negative results in patients diagnosed as having an intact HPA axis by the same test; and the predictive value of the test to diagnose ACTH deficiency, as the percentage of true positive results in patients diagnosed as having ACTH deficiency by the same test.

Results

Of the 113 patients identified, 72 were male. The diagnoses of the patients are as follows: nonfunctioning pituitary adenoma (n = 70), acromegaly (n = 30), craniopharyngioma (n = 4), macroprolactinoma (n = 5), microprolactinoma (n = 1), pituitary cyst (n = 1), meningioma (n = 1), toxoplasmosis (n = 1). The median age at operation was 54 (range, 17–80) yr; 71.7% (81 of 113) of patients received radiotherapy post pituitary surgery. The patients had a total of 485 yr of follow-up after each operation, with a median of 2 (<1–10) yr.

See Fig. 1. Of the 120 operations, 89 operations in 89 patients were assessed postoperatively by the SST. SST was performed at a median of 47 (range, 10–188) d.

Of the remaining 31 operations (24 patients): there was no testing of the HPA axis after 21 operations (14 patients) because of documented long standing or preoperative ACTH deficiency, and the patients remained on hydrocortisone replacement; the HPA axis was assessed by an ITT after 3 operations (3 patients) and 0900-h plasma cortisol levels after 3 operations (3 patients); the relevant information on 4 operations (4 patients) was missing from the case notes.

0900-h Cortisol measurement

See Fig. 1 and Table 1. None of the 89 patients became unwell during the immediate postoperative period. After 30 operations, there was a third-day postoperative 0900-h plasma cortisol of less than 400 nM, and these patients were maintained on hydrocortisone until further assessment. Eleven of these patients passed the SST; however, one of these patients went on to develop signs of ACTH deficiency during the first year after the operation, requiring hydrocortisone replacement (see case 1). There were, therefore, 10 false positive results for third-day postoperative 0900-h plasma cortisol of less than 400 nM. One patient not maintained on hydrocortisone postoperatively borderline-failed the SST at 40 d (plasma cortisol, 208 nM at 0 min and 458 nM at 30 min; the SST was in 1997, so the 30-min plasma cortisol pass level was defined as 520 nM). Another patient not maintained on hydrocortisone postoperatively also passed the SST postoperatively but went on to develop signs of ACTH deficiency during the first year after the operation, requiring hydrocortisone replacement (see case 2). There were, therefore, 2 false negative results for third-day postoperative 0900-h plasma cortisol of less than 400 nM.

See Table 1. From these data, the sensitivity and specificity of postoperative 0900-h plasma cortisol of more than 400 nM in assessing ACTH deficiency is 91% and 85%, respectively, using the results of the SST postoperatively and clinical outcome after 1 yr of follow-up as diagnostic of ACTH deficiency. The predictive value of the third postoperative day 0900-h cortisol measurement to exclude ACTH deficiency was 97%; but to diagnose ACTH deficiency, was 67%.

TABLE 1. Calculated predictive value of postoperative 0900-h plasma cortisol and SST

	0900-h Cortisol >400 nM	SST
No. of patients	89	63
True negative results	57	61
False negative results	2	2
True positive results	20	
False positive results	10	
False negative rate (sensitivity)	2/22[a] (91%)	2/22[a] (91%)
False positive rate (specificity)	10/67[b] (85%)	
Predictive value to exclude ACTH deficiency	57/59 (97%)	61/63 (97%)
Predictive value to diagnose ACTH deficiency	20/30 (67%)	

[a,b] Number of patients with [a] ACTH deficiency (22) and with an [b] intact HPA axis (67) postoperatively, using the results of the SST postoperatively and clinical outcome after 1 yr of follow-up as diagnostic of ACTH deficiency.

SST

The postoperative SST was passed after 69 operations (3 of the patients had been previously ACTH-deficient) and failed after 20 operations (5 of whom were previously ACTH-deficient). Thus, new ACTH deficiency in the postoperative period occurred in 18.5%. Borderline results in the SST (with 30-min plasma cortisol levels of more than 400 nM) were achieved after 9 operations: 4 of these patients were retested and passed; the other 5 were not retested and remained on hydrocortisone.

See Tables 1 and 2. Sixty-three patients who passed the postoperative SST have undergone at least 1 yr of follow-up [median, 3 (range, 1–10) yr]. Twelve of these 63 subsequently became ACTH-deficient, as diagnosed by SST. All apart from 1 patient (see case 2) either had no symptoms or had symptoms of tiredness alone. Only 4 of the 63 patients who passed a postoperative SST developed ACTH deficiency within the first year of follow-up. However, 2 of the 4 patients who developed ACTH deficiency had received radiotherapy and may have developed ACTH deficiency as a consequence. There were, therefore, 2 false negative results for the postoperative SST.

See Table 1. The predictive value of the SST to exclude ACTH deficiency was therefore 97% (2 of 63 patients who initially passed the SST were found to be ACTH deficient within 12 months without having received postoperative radiotherapy).

These four cases represent the potentially most important group in whom the postoperative SST did not detect ACTH deficiency. Their case histories were as follows.

Case 1

The patient complained of tiredness at initial postoperative assessment at 71 d but passed a SST (plasma cortisol, 308 nM at 0 min and 554 nM at 30 min; the SST was in 1994, so the 30-min plasma cortisol pass level was defined as 520 nM). The patient was rechecked at 87 d as a precaution when he

TABLE 2. Onset of ACTH deficiency postoperatively

ACTH deficiency	0–1 yr	1–3 yr	3–4 yr	4–5 yr	5–6 yr
All patients	4	0	2	4	2
Patients receiving radiotherapy	2	0	2	4	1

failed a SST (206 nM and 476 nM, respectively). The patient had remained on hydrocortisone in the initial postoperative period.

Case 2

The patient had initial postoperative assessment at 53 d, when he passed a SST (plasma cortisol, 521 nM at 0 min and 631 nM at 30 min; the SST was in 1990, so the 30-min cortisol pass level was defined as 550 nM). At 72 d, the patient was admitted to the hospital with a chest infection, SST was repeated and passed (514 nM and 764 nM, respectively), and hydrocortisone was started as a precaution and converted to dexamethasone on discharge. SST was rechecked at 134 d, after 24 h off dexamethasone, and failed (231 nM, 395 nM); 0900-h plasma cortisol on the third postoperative day had also not detected ACTH deficiency in this patient. It should be noted that, because of the prolonged duration of HPA axis suppression of dexamethasone, 24 h may have been inadequate time to allow accurate assessment of the HPA axis.

Cases 3 and 4

Patients had initial postoperative assessment at 31 and 28 d, respectively, when they passed the SST (380 nM, 667 nM; 417 nM, 762 nM, respectively; both SSTs were performed in 1990, so the 30-min plasma cortisol pass level was defined as 550 nM). At the annual review, they complained of tiredness (318 d and 332 d, respectively), when both failed SSTs (111 nM, 358 nM, 89 nM, and 325 nM, respectively). Both cases had received radiotherapy within 5 months of pituitary surgery; 0900-h plasma cortisol on the third postoperative day had also not detected ACTH deficiency in these patients.

Discussion

The ITT has been used as the gold standard for assessing the function of the HPA axis, since its value was first demonstrated in the 1960s (16–18). It is, however, an expensive, uncomfortable, and dangerous test. The ITT is also contraindicated in certain patients (*e.g.* ischemic heart disease, cerebrovascular disease, epilepsy) (8). With chronic ACTH deficiency, the adrenal cortex undergoes atrophy and fails to respond to exogenous ACTH (19). Consequently, the SST has been used to assess the integrity of the HPA axis (1, 5, 20–22). However, despite an excellent correlation between peak plasma cortisol levels achieved in the SST and ITT, some discrepancies have been reported, with potentially serious consequences (5, 6, 9–14, 21, 23–25).

One explanation for false negative results with the SST, when compared with the ITT, is that mild adrenocortical atrophy may provide a pass cortisol level response when exposed to the supraphysiological dose of 250 μg, at least 1000 times the dose required for maximal adrenal stimulation, used in the SST (26). It has been argued that a low-dose (1 μg) synacthen test is more sensitive than the 250-μg SST in the setting of ACTH deficiency (6, 25, 27), but the low-dose test has not been subjected to a pragmatic analysis of predicting clinical outcome, as is presented here for the high-dose test. The discrepancies in sensitivity and specificity found between the ITT and SST are also partly explained by the wide variations, between studies, in the pass cortisol level. Two recent reports suggested raising the lower pass cortisol level to 600 nM to increase the sensitivity of the SST (5, 10). This would, however, risk raising the number of falsely subnormal results, resulting in unnecessary treatment with hydrocortisone (13, 28). In our audit, a lower pass cortisol level of 600 nM would increase sensitivity of the SST to detect ACTH deficiency from 91% to 96% but would decrease specificity as new ACTH deficiency postoperatively would increase from 18.5% to 28%. Unless an auxiliary test (*e.g.* an ITT) is to be performed after a screening SST, it would be more appropriate to choose the pass cortisol level on the basis of an acceptable predictive value for excluding ACTH deficiency in patients with pituitary disease. With our cut-offs, the predictive value of the SST to exclude ACTH deficiency was 97%, which we believe is sufficient to avoid using ITT routinely.

Another reason for the discrepancies between the two tests is the different interpretation of SST results, *i.e.* using peak and increment rather than the +30-min plasma cortisol level. The peak and incremental cortisol are higher in the SST than with the ITT, suggesting that +60-min plasma cortisol should not be used or at least should be evaluated with another threshold (28). Our practice is to measure plasma cortisol at +30 min. There are also reports suggesting that the ITT itself is not 100% sensitive (10, 15). Intraindividual reproducibility of cortisol response to the ITT in normal men is good (29, 30); however, in men with hypopituitarism, it can vary by 41.6% (3.5–92.7%) (30). Although these discrepancies were not sufficient to alter choice of initiation of glucocorticoid therapy, this produces concerns that the ITT itself may occasionally inadequately identify partial ACTH deficiency.

Auditing a group of patients who have recently undergone pituitary surgery, after which pituitary function may change, is a test of the safety of the SST. It has been reported that the SST may induce false negative cortisol responses shortly after pituitary surgery (11, 13, 31). However, many of the studies performed the SST within 2 wk of surgery, so that ACTH deficiency may not have been sufficiently prolonged to result in adrenal atrophy (11–13, 31). Dokemetas *et al.* (2000) also found that five patients who had subnormal cortisol responses to ITTs in the first 7 d postoperatively were not subnormal at 3 months postoperatively and suggested that this was attributable to transient dysfunction of the HPA axis post pituitary surgery (12). In our audit, SSTs were performed at 6.7 wk postoperatively (<2–27). However, despite the written protocol, three patients were tested within 2 wk of the operation. Although these patients have remained well, we do not advocate using the SST less than 4 wk after surgery. The two patients who eventually went on to fail the SST in the first year, having passed it originally, were tested at 7 and 11 wk postoperatively. It has been suggested that 0900-h plasma cortisol level may be a predictor of whether further evaluation of the HPA axis by dynamic testing is necessary (3–8). We found that a 0900-h plasma cortisol level less than 400 nM is 91% sensitive and 85% specific for diagnosing ACTH deficiency, defined by a SST and clinical outcome. This is in keeping with the original study from this unit (1) and other studies post pituitary surgery (3, 12, 13) and in more heterogeneous groups of patients with pituitary dis-

ease (7, 32). We continue to use 400 nM as our indicator of an intact HPA axis post pituitary surgery. Despite the recent recommendation that further dynamic testing of the HPA axis is not necessary if the basal plasma cortisol postoperatively is either less than 100 nM (indicative of ACTH deficiency) or more than 450 nM (indicative of an intact HPA axis) (13), we continue to use a dynamic test to further assess HPA function in all patients. This is both to reduce unnecessary long-term hydrocortisone replacement and, more importantly, to limit the number of false negative results (two in our audit).

Routine use of glucocorticoid replacement post pituitary surgery, although it can prevent adrenal insufficiency in patients with temporary or permanent corticotroph cell damage, may also cause suppression of ACTH secretion and frustrate postoperative evaluation of HPA integrity (33). This is unavoidable, given that early dynamic testing of the HPA axis is not reliable because ACTH deficiency that is demonstrated in the early postoperative period may not be permanent (12). Our protocol of continuing hydrocortisone in those with a postoperative 0900-h cortisol of less than 400 nM limits the numbers requiring glucocorticoids in the immediate postoperative period while maintaining patient safety.

The sensitivity of the SST was 91%, and the predictive value of the SST to exclude ACTH deficiency was 97%. This is in concordance with the original study, which found the SST to have a sensitivity of 90% and a predictive value of 98% for excluding ACTH deficiency (1). It is not possible, from this retrospective study, to ascertain specificity of the SST and predictive value to diagnose ACTH deficiency because of being unable to predict false positive SST results. Stewart *et al.* (1) found the predictive value of the SST to diagnose ACTH deficiency to be 50%, with a false positive rate of 15%. The development of new ACTH deficiency in our patients was 18.5%. One study reported the incidence of ACTH deficiency, post pituitary surgery, in the region of 8% (34). This suggests a significant false positive rate for the SST, resulting in inappropriate glucocorticoid replacement. The recording of additional pituitary hormone deficits would have clarified the situation. However, this suggests, as above, that our cut-offs for the SST in this clinical setting are in favor of high sensitivity at the expense of specificity. The option to perform an ITT in those that have failed the SST has been previously recommended, to reduce the number of patients remaining on long-term hydrocortisone (1).

In summary, these data confirm the use of 0900-h plasma cortisol in the immediate postoperative period and reassure that the SST is safe to use in the assessment of ACTH deficiency after pituitary surgery in clinical practice. Although the predictive value of the SST to exclude ACTH deficiency is similar to that found in the studies comparing the ITT and SST, this does not seem to expose patients to the potential devastating consequences of ACTH deficiency if reasonable clinical vigilance is maintained. However, it is essential that clinical awareness of symptoms of hypocortisolemia remains, to minimize the impact of occasional false negatives.

Acknowledgments

Received June 25, 2002. Accepted February 2, 2003.

Address all correspondence and requests for reprints to: Dr. P. L. Padfield, Department of Endocrinology, Western General Hospital, Edinburgh, United Kingdom EH4 2XN. E-mail: paul.padfield@luht.scot.nhs.uk.

References

1. **Stewart PM, Corrie J, Seckl JR, Edwards CR, Padfield PL** 1988 A rational approach for assessing the hypothalamo-pituitary-adrenal axis. Lancet 1:1208–1210
2. **Davies MJ, Howlett TA** 1996 A survey of the current methods used in the UK to assess pituitary function. J R Soc Med 89:159P–164P
3. **Watts NB, Tindall GT** 1988 Rapid assessment of corticotropin reserve after pituitary surgery. JAMA 259:708–711
4. **Auchus RJ, Shewbridge RK, Shepherd MD** 1997 Which patients benefit from provocative adrenal testing after transsphenoidal pituitary surgery? Clin Endocrinol (Oxf) 46:21–27
5. **Hurel SJ, Thompson CJ, Watson MJ, Harris MM, Baylis PH, Kendall-Taylor P** 1996 The short Synacthen and insulin stress tests in the assessment of the hypothalamic-pituitary-adrenal axis. Clin Endocrinol (Oxf) 44:141–146
6. **Rasmuson S, Olsson T, Hagg E** 1996 A low dose ACTH test to assess the function of the hypothalamic-pituitary-adrenal axis. Clin Endocrinol (Oxf) 44:151–156
7. **Hagg E, Asplund K, Lithner F** 1987 Value of basal plasma cortisol assays in the assessment of pituitary-adrenal insufficiency. Clin Endocrinol (Oxf) 26: 221–226
8. **Jones SL, Trainer PJ, Perry L, Wass JA, Bessser GM, Grossman A** 1994 An audit of the insulin tolerance test in adult subjects in an acute investigation unit over one year. Clin Endocrinol (Oxf) 41:123–128
9. **Ammari F, Issa BG, Millward E, Scanion MF** 1996 A comparison between short ACTH and insulin stress tests for assessing hypothalamo-pituitary-adrenal function. Clin Endocrinol (Oxf) 44:473–476
10. **Abdu TA, Elhadd TA, Neary R, Clayton RN** 1999 Comparison of the low-dose SST (1 microg), the conventional-dose SST (250 microg), and the insulin tolerance test for assessment of the hypothalamo-pituitary-adrenal axis in patients with pituitary disease. J Clin Endocrinol Metab 84:838–843
11. **Mukherjee JJ, de Castro JJ, Kaltsas G, Afshar F, Grossman AB, Wass JA, Besser GM** 1997 A comparison of the insulin tolerance/glucagon test with the short ACTH stimulation test in the assessment of the hypothalamo-pituitary-adrenal axis in the early post-operative period after hypophysectomy. Clin Endocrinol (Oxf) 47:51–60
12. **Dokmetas HS, Colak R, Kelestimur F, Selcuklu A, Unluhizarci K, Bayram F** 2000 A comparison between the 1-microg adrenocorticotropin (ACTH) test, the short ACTH (250 microg) test, and the insulin tolerance test in the assessment of hypothalamo-pituitary-adrenal axis immediately after pituitary surgery. J Clin Endocrinol Metab 85:3713–3719
13. **Courtney CH, McAllister AS, McCance DR, Bell PM, Hadden DR, Leslie H, Sheridan B, Atkinson AB** 2000 Comparison of one week 0900 h serum cortisol, low- and standard-dose synacthen tests with a 4 to 6 week insulin hypoglycaemia test after pituitary surgery in assessing HPA axis. Clin Endocrinol (Oxf) 53:431–436
14. **Orme SM, Peacey SR, Barth JH, Belchetz PE** 1996 Comparison of tests of stress-released cortisol secretion in pituitary disease. Clin Endocrinol (Oxf) 45:135–140
15. **Tsatsoulis A, Shalet SM, Harrison J, Ratcliffe WA, Beardwell CG, Robinson EL** 1988 Adrenocorticotrophin (ACTH) deficiency undetected by standard dynamic tests of the hypothalamic-pituitary-adrenal axis. Clin Endocrinol (Oxf) 28:225–232
16. **Jacobs HS, Nabarro JD** 1969 Tests of hypothalamic-pituitary-adrenal function in man. Q J Med 38:475–491
17. **Greenwood FC, Landon J, Stamp TC** 1966 The plasma sugar, free fatty acid, cortisol, and growth hormone response to insulin. I. In control subjects. J Clin Invest 45:429–436
18. **Plumpton FS, Besser GM** 1969 The adrenocortical response to surgery and insulin-induced hypoglycaemia in corticosteroid-treated and normal subjects. Br J Surg 56:216–219
19. **Hjortrup A, Kehlet H, Lindholm J, Stentoft P** 1983 Value of the 30-minute adrenocorticotropin (ACTH) test in demonstrating hypothalamic-pituitary-adrenocortical insufficiency after acute ACTH deprivation. J Clin Endocrinol Metab 57:668–670
20. **Lindholm J, Kehlet H** 1987 Re-evaluation of the clinical value of the 30 min ACTH test in assessing the hypothalamic-pituitary-adrenocortical function. Clin Endocrinol (Oxf) 26:53–59
21. **Lindholm J, Kehlet H, Blichert-Toft M, Dinesen B, Riishede J** 1978 Reliability of the 30-minute ACTH test in assessing hypothalamic-pituitary-adrenal function. J Clin Endocrinol Metab 47:272–274
22. **Clayton RN** 1996 Short synacthen test versus insulin stress test for assessment of the hypothalamo pituitary–adrenal axis: controversy revisited. Clin Endocrinol (Oxf) 44:147–149
23. **Borst GC, Michenfelder HJ, O'Brian JT** 1982 Discordant cortisol response to

exogenous ACTH and insulin-induced hypoglycemia in patients with pituitary disease. N Engl J Med 306:1462–1464
24. **Soule SG, Fahie-Wilson M, Tomlinson S** 1996 Failure of the short ACTH test to unequivocally diagnose long-standing symptomatic secondary hypoadrenalism. Clin Endocrinol (Oxf) 44:137–140
25. **Tordjman K, Jaffe A, Grazas N, Apter C, Stern N** 1995 The role of the low dose (1 microgram) adrenocorticotropin test in the evaluation of patients with pituitary diseases. J Clin Endocrinol Metab 80:1301–1305
26. **Landon J, James VH, Wharton MJ, Friedman M** 1967 Threshold adrenocortical sensitivity in man and its possible application to corticotrophin bioassay. Lancet 2:697–700
27. **Dickstein G, Shechner C, Nicholson WE, Rosner L, Shen-Orr Z, Adam F, Lahan M** 1991 Adrenocorticotropin stimulation test: effects of basal cortisol level, time of day, and suggested new sensitive low dose test. J Clin Endocrinol Metab 72:773–778
28. **Gonzalbez J, Villabona C, Ramon J, Navarro MA, Gimenez O, Ricart W, Soler J** 2000 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. Clin Endocrinol (Oxf) 53:199–204
29. **Vestergaard P, Hoeck HC, Jakobsen PE, Laurberg P** 1997 Reproducibility of growth hormone and cortisol responses to the insulin tolerance test and the short ACTH test in normal adults. Horm Metab Res 29:106–110
30. **Pfeifer M, Kanc K, Verhovec R, Kocijancic A** 2001 Reproducibility of the insulin tolerance test (ITT) for assessment of growth hormone and cortisol secretion in normal and hypopituitary adult men. Clin Endocrinol (Oxf) 54: 17–22
31. **Jackson RS, Carter GD, Wise PH, Alaghband-Zadeh J** 1994 Comparison of paired short Synacthen and insulin tolerance tests soon after pituitary surgery. Ann Clin Biochem 31:46–49
32. **Pavord SR, Girach A, Price DE, Absalom SR, Falconer-Smith J, Howlett TA** 1992 A retrospective audit of the combined pituitary function test, using the insulin stress test, TRH and GnRH in a district laboratory. Clin Endocrinol (Oxf) 36:135–139
33. **Grinspoon SK, Biller BM** 1994 Clinical review 62: laboratory assessment of adrenal insufficiency. J Clin Endocrinol Metab 79:923–931
34. **Comtois R, Beauregard H, Somma M, Serri O, Aris-Jilwan N, Hardy J** 1991 The clinical and endocrine outcome to trans-sphenoidal microsurgery of non-secreting pituitary adenomas. Cancer 68:860–866