Recovery of the hypothalamo-pituitary-adrenal axis following trans-sphenoidal adenomectomy

Riccardo Pofi[1-3]*, Sonali Gunatilake[1]*, Victoria Macgregor[1], Brian Shine[1], Robin Joseph[2], Ashley B Grossman[1], Andrea M Isidori[3], Simon Cudlip[1], Bahram Jafar-Mohammadi[1], Jeremy W Tomlinson[1] and Aparna Pal[1].

[1]Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM) and NIHR Oxford Biomedical Research Centre, Churchill Hospital, University of Oxford, Oxford, UK, OX37LE, United Kingdom.

[2]Department of Neuroradiology, John Radcliffe Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford, OX39DU, United Kingdom

[3]Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy, Viale Regina Elena, 324, 00161, Rome, Italy.

equal contribution

Short Title: Hypothalamo-pituitary-adrenal axis recovery after TSA.

Keywords: Recovery, Pituitary, Cortisol, Trans Sphenoidal Adenomectomy; Adrenal axis.

Address all correspondence and request for reprints:

Dr Aparna Pal,

Oxford Centre for Diabetes, Endocrinology and Metabolism

University of Oxford,

Oxford OX3 7LJ, UK

Email: aparna.pal@ouh.nhs.uk

Phone: 01865 227424

Grants or fellowships supporting the writing of the paper: This work was supported by the Exchange in Endocrinology Expertise programme (3E fellowship) of the European Union of

30 Medical Specialists (UEMS) and the European Society of Endocrinology (Short-term fellowship),
31 awarded to RP. The views expressed are those of the author(s) and not necessarily those of the
32 NHS, the NIHR, RCP or the Department of Health, UK.
33
34 **Disclosure**: The authors have nothing to disclose and there are no relevant conflicts of interest

Abstract

Context: Secondary adrenal insufficiency is a potential complication of trans-sphenoidal adenomectomy (TSA). Most centers test recovery of the hypothalamo-pituitary-adrenal (HPA) axis six weeks after TSA but there are no data predicting likelihood of recovery, or the frequency of later recovery of HPA function and hence need for re-testing.

Objective: To assess timing and predictors of hypothalamo-pituitary adrenal (HPA) axis recovery after TSA.

Design: Single-centre, retrospective analysis of consecutive pituitary surgeries performed on individuals between February 2015 and September 2018.

Patients: One hundred and nine patients were identified who had Short Synacthen Test (SST) data before and at sequential time points after TSA.

Main outcome measures: Recovery of HPA axis function at 6-weeks, 3-months, 6-months and 9 to12-months post-TSA.

Results: 29% of patients failed the 6-week SST: 16%,12%,6% subsequently recovered at 3, 6 and 9-12 months respectively. Pre-op SST 30-minute cortisol, post-op day 8 cortisol and 6-week post-op SST baseline cortisol respectively above or below 430nmol/L(15.5μg/dL, AUC ROC=0.86), 160nmol/L(5.8μg/dL, AUC ROC=0.75) and 180nmol/L(6.5μg/dL, AUC ROC=0.88) were identified as cut-offs for predicting 6 weeks HPA recovery respectively. No patients with all three cut-offs below the threshold recovered within 12 months post-TSA whereas 92% with all cut-offs above the threshold recovered HPA function within 6 weeks *(OR* 12.200,95%CI 5.268-28.255).

Conclusions: HPA axis recovery can occur as late as 9-12 months following TSA demonstrating the need for periodic reassessment of patients who initially 'fail' SST post-TSA. Cortisol levels from the SST before and after surgery can be used to guide which patients are likely to recover function and therefore avoid unnecessary lifelong glucocorticoid replacement.

Précis: Following TSA pre- and post-op SST cortisol levels can predict HPA axis recovery at 6 weeks. Recovery occurs even at 12 months after TSA demonstrating the need for periodic re-testing.

Introduction

Hypopituitarism causes increased morbidity and mortality, together with reduced quality of life (1,2). Intra-sellar and peri-sellar lesions account for the majority of cases of hypopituitarism (3-5). Although no definitive mechanism is identified, destruction of pituitary neuroendocrine cells by a direct mass effect and compression of hypothalamo-pituitary portal venous flow causing reduced releasing hormone levels reaching the pituitary gland, or focal necrosis of the pituitary cells are suggested as possible explanations for hypopituitarism caused by pituitary mass lesions (6). The prevalence of hormonal deficits differ depending on the hormonal axis involved; growth hormone deficiency is most frequent followed by gonadotrophin, TSH/ACTH and prolactin in descending order (7-9).

The primary treatment modality for pituitary lesions (excluding prolactinomas) is trans-sphenoidal adenomectomy (TSA) aimed at reducing mass effect and normalising hormonal dysfunction (hyper- and hypo-secretion). Following pituitary surgery, delayed recovery of post-operative pituitary hypofunction has been observed (10); however, the concept of recovery of pituitary hormone deficit over a period of time after surgery and the behavior of different axes in such recovery is not well recognized. There are very few studies investigating recovery of hormonal axes post-TSA, and the data differ according to the specific hormonal axis involved (6,8). Most centers re-assess pituitary hormone axes function shortly after TSA (6 weeks) but further longitudinal assessments are not routinely performed, although practice does differ between centers. Following the initial post-operative (post-op) diagnosis of hormonal deficiency, patients are usually commenced on replacement with the respective deficient hormones, and recovery rates are rarely re-tested. This is particularly relevant in hypothalamo-pituitary-adrenal axis (HPA) deficiency, where treatment with excess long-term glucocorticoids (GCs) is associated with excess morbidity and mortality (11-14). We have recently shown that 57% of patients with non-functioning pituitary adenoma (NFPA) and 44% of patients who underwent pituitary surgery eventually recovered HPA axis function as determined by the short *Synacthen (Cosyntropin)* test (SST), but also that recovery can occur some time after surgery (15). This has major potential implications for clinical practice since this percentage of recovery is significantly higher than one might expect (16). Therefore, re-testing for recovery of HPA axis post-TSA is important to minimize unnecessary

exposure to GCs treatment. The timing and frequency of any re-testing, if undertaken, is variable across centers with no published guidelines.

In addition to the paucity of data on absolute recovery rates, there are no systematic studies investigating predictors of recovery. Early post-TSA basal cortisol levels, perioperative cortisol levels, post-operative cortisol stress responses, preservation of normal pituitary gland during TSA and pre-TSA prolactin levels have been identified in some studies as predictive factors, while age and tumor size have also been shown to be associated (17-19). We therefore undertook a retrospective analysis of pre- and post-op HPA axis function to identify rates and predictive markers of potential recovery of function that would potentially enable early identification of subgroups more likely to recover, and hence prioritise for retesting.

Materials and Methods

Patient selection

We undertook an observational, retrospective analysis of consecutive patients who underwent TSA for pituitary adenoma in the John Radcliffe Hospital (Oxford University Hospital NHS Foundation Trust, Oxford, UK) between February 2015 and September 2018. All pituitary adenomectomies were performed by a single surgeon (SC) via a trans-sphenoidal approach. During this time, 264 pituitary surgeries were performed on 250 individuals. Patients with apoplexy, microadenomas, corticotroph adenomas, craniopharyngioma, meningioma, Rathke cleft cyst, metastases, redo-surgery, emergency TSA, craniotomy or pituitary radiotherapy were excluded, as the potential for recovery of pituitary function was presumed to be subject to other variables and not comparable to the rest of the cohort. For the same reason, patients using suppressive dose of glucocorticoids, long-term opioid analgesics, excessive alcohol intake and patients with psychiatric disorders were excluded: 109 patients (71 male, 38 female; mean age 56 years, median 59, range 17-82) who had undergone at least one or more re-evaluation of the HPA axis were identified. Data on age, gender, tumor size and tumor histology were collated from a review of medical records. Post-operative immunohistochemistry confirmed the clinical/hormonal classification.

Assessment of HPA axis and perioperative procedures

All patients underwent pre-operative (pre-op) SST, day 8 post-op 9am cortisol and 6-week post-op SST. All the patients received a stress dose of glucocorticoids before surgery (100 mg of hydrocortisone IV at induction of anesthesia. After surgery, oral hydrocortisone 40mg was given on days 1 and 2 (20mg on waking, 10mg midday and 10mg at 6pm) and on post-op day 3 onwards, all patients received standard treatment with hydrocortisone 20mg (10mg on waking, 5mg midday and 5mg at 6 pm) until reassessment of the HPA axis at day 8 with a basal 9am cortisol. Patients with normal day 8 cortisol (>350 nmol/L – 12.6μg/dL) were taken off hydrocortisone replacement and received only stress doses of hydrocortisone after post op D8, while others continued to be on standard 20mg hydrocortisone until reassessment of the HPA axis at 6 weeks.

The interpretation of the SST was based on the 30-minutes serum cortisol where an adequate response to *Synacthen* was defined as >430nmol/L(15.5μg/dL) in accord with our assay (20). The

incremental response to *Synacthen* was calculated as delta cortisol = [30-minute – 0-minute cortisol]. Patients who failed the 6-week assessment were reassessed with an SST at 3-months and subsequently at 6-, 9- and 12-months if they continued to 'fail' the SST.

Abbott Architect i-2000 immunoassay analyser (Abbott Diagnostics, Maidenhead, UK) was used to analyse cortisol samples across the SST, with an inter-assay imprecision of 5.6% at 72 nmol/L(2.6μg/dL) 2.2% at 433 nmol/L(15.6μg/dL) and 2.4% at 667 nmol/L(24.1μg/dL). All SSTs were performed at least 18-hours after the most recent dose of glucocorticoids. Patients on oral estrogen replacement were required to stop the treatment at least 6-weeks before the test. Blood was sampled for serum cortisol at baseline (9 AM) and 30 minutes post-intravenous injection of 250μg *Synacthen* (Alliance Pharmaceuticals, Chippenham).

Thyroid hormones

The thyroid axis evaluation included serum levels of thyroid-stimulating hormone (TSH, normal range, 0.3-4.2 mIU/L) and free T4 (normal range, 9-19 pmol/L). Secondary hypothyroidism was diagnosed with subnormal serum free T4 (FT4) associated with low or inappropriately normal TSH level. Post-op 6-weeks assessment of resolution of pre-op thyroid axis deficit (in the absence of hormone replacement) was assumed for patients with normalization of free T4 and TSH. Patients with primary hypothyroidism were excluded from the analysis.

Gonadotrophins

Gonadal axis was assessed through measurements of FSH, LH and estradiol (females) or testosterone (males) pre- and 6-weeks post-TSA. In males, hypogonadism was diagnosed when serum levels of testosterone were <12nmol/L(3.4μg/L)(20-23) in the presence of low or inappropriately normal levels of LH (normal local range 2-10 IU/L). In postmenopausal women, hypogonadism was diagnosed when serum FSH levels were inappropriately low (<40 IU/L) for age (>50 years)(24). Giving the lack of standardized protocol, hormonal analysis was taken randomly in female patients, so premenopausal women were excluded from the analysis.

MRI study

Neuroradiological studies included pre-op 1.5 T magnetic resonance imaging (mean time between

MRI and surgery 101±68 days). All the MRI images were assessed by a single expert radiologist (RJ). The tumor volume was calculated by the *Ellipsoid formula* (0.52*a*b*c, where a,b,c are respectively the longitudinal, sagittal and coronal diameters), as suggested from the literature(25).

Tumor histology

All tumor specimens from surgery were formally reported by neuropathologists according to immunohistochemical characteristics of the adenoma together with the proliferations index (MIB-1/Ki-67) and p53.

Statistical Methods

An independent-samples t-test was run to determine if there were differences between groups at baseline. A binomial logistic regression was performed on the whole cohort to ascertain the effects of selected variables on the likelihood that participants will show recovery at 6-weeks assessment. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed *via* the Box-Tidwell (1962) procedure. A Bonferroni correction was applied in the model. Based on this assessment, all continuous independent variables were found to be linearly related to the logit of the dependent variable. Data are expressed as median with 95% CI assuming a normal distribution.

Receiver-operating characteristic (ROC) curve analysis was performed using a uniform threshold for any variables (selected on the results of binomial logistic regression) according to the 95% sensitivity on the ROC analysis. Area under the curve (AUC) analysis was used to express the overall diagnostic accuracy of the index criterion. A $p<0.05$ was considered indicative of a statistically significant difference. Statistical analyses were performed using SPSS (version 24, Chicago, IL, USA) and GraphPad Prism 7.0 software package (GraphPad Software, Inc. La Jolla, CA, USA).

Results

Pre-op assessments

The baseline characteristics of the 109 patients are presented in *Table 1*. The mean age was 56 years (median 59, range 17-82), 71 (65.1%) were male, median tumour volume was 7 mL (range 0.36 to 75) and the mean maximum lesion diameter was 2.7±1.0 cm (median 2.6, range 1 to 6.2). Pre-op pituitary evaluation showed no pituitary hormone deficit in 23 (21.1%) of patients whereas 44 and 25 patients (40.4% and 22.9%) had one or more than one hormonal deficiency respectively. Pre-op gonadal axis information was not available for 17 patients. Patients with normal pre-op pituitary function were younger (mean age 49±17 *vs.* 59±15 years, mean difference -10, 95%CI -17.978, -1.964 years, *p=0.02*) and had smaller tumors (mean volume 6.0±4.9 *vs.* 9.5±11 mL, mean difference -3.4, 95%CI -6.847, -0.064 mL, *p=0.05*) compared to those with at least one affected pituitary axis.

Logistic regression modeling

The binomial logistic regression model was statistically significant at predicting the presence of at least one pituitary hormonal deficit at the 6-week assessment (χ^2 = 25.462, p<0.0001) and explained 77% of the variance in the 6-week pituitary deficit, correctly classifying 85.2% of cases. Sensitivity was 91.5%, specificity was 64.3%, positive predictive value was 90% and negative predictive value was 69%. Of the 3 variables incorporated into the model (age, gender, tumor volume), tumor volume was able to predict the presence of at least one pituitary deficit at 6-weeks (p=0.010) (Table 2a). According to the ROC analysis, a pre-op tumor volume >9mL (ROC AUC=0.68, *p=0.04*) was identified as a threshold for predicting an increased likelihood of any post-op pituitary deficit: 95% of patients over this value showed at least one pituitary deficit at 6-weeks post-TSA (*odds ratio 9*.63, 95% CI 1.162 to 79.789).

HPA recovery

Complete data sets on HPA axis were available for 109 patients. 78.9% (n=86) had a normal pre-operative adrenal reserve, and 70.6% (n=77) had normal HPA function 6-weeks after TSA. Of the 21.1% (n=23) of patients with abnormal pre-operative HPA function, 34.8% recovered 6-weeks

post-TSA. Those patients who recovered were younger (mean age 50±14 *vs.* 70±9 years, *p=0.008*) with no difference in tumor volume (mean volume 9.5±6.4 *vs.* 10.6±11.9 mL, *p=0.8*) compared to those who failed. Among the 32 patients who failed the 6-weeks assessment, 15.6%, 12.5% and 6.2% recovered at 3, 6 and 9 to 12 months respectively. Figure 1 shows the cumulative percentages of patients with normal SST at different timepoints of HPA assessments.

Surgical success rate

Successful surgery for non-functioning adenomas was defined as fulfilling all of the following criteria: >50% resection of the pituitary adenoma, decompression of the optic chiasm, no requirement for any adjuvant therapy. For functioning adenomas successful surgery was defined as normalization of biochemistry without the need for additional medical therapy or radiotherapy.

We noted that among those patients with normal HPA function at 6-weeks (n=77), 73 (94.8%) had successful surgery; among those patients with abnormal HPA function at 6-weeks (n=32), 30 (93.8%) had successful surgery (*p*=1.0).

HPA recovery - Logistic regression modelling

All information derived from the SST (baseline cortisol, 30-minute cortisol and delta cortisol) were examined for their ability to predict the recovery of the HPA axis at 6 weeks post-op. The binomial logistic regression model was statistically significant (χ^2 = 48.158, *p*<0.0001) and explained 76.3% of the variance in 6-weeks HPA recovery, correctly classifying 91.3% of cases. Sensitivity was 98.4%, specificity was 68.4%, positive predictive value was 90.9% and negative predictive value was 92.8%. Of the 5 predictor variables incorporated into the model (age, gender, tumor volume, pre-op 30-minute SST cortisol, post-op day 8 cortisol), two were statistically significant: pre-op 30-minute cortisol (p<0.001) and day 8 post-op cortisol (p=0.003). Higher 30-minute cortisol pre-operatively and day 8 cortisol levels post-operatively were associated with an increased likelihood of HPA axis recovery at 6-weeks. All the detailed coefficients for multiple regression analysis are shown in *Table 2b*.

According to the ROC analysis, a pre-op 30-minute cortisol >430nmol/L (15.5μg/dL, ROC AUC=0.86, *p<0.001*) and a day 8 post-op cortisol >160nmol/L (5.8μg/dL, AUC= 0.75, *p=0.001*) best predicted future adrenal recovery at 6-weeks.

Using these cut-offs, 80.0% of patients with a pre-op 30-minute cortisol >430nmol/L *(odds ratio* 7.556, 95% CI 2.847 to 20.055) and 80.0% of patients with post-op day 8 cortisol >160nmol/L (*odds ratio* 9.00, 95% CI 2.455 to 32.989) passed the SST at 6-weeks assessment.

Combining these two cut-offs increased the predictive value of our model. The combination best predicted the 6-weeks adrenal recovery as showed by the ROC AUC (0.89, *p<0.001*) derived from the predicted probability computed through the binary logistic regression analysis: 86.6% of patients with both these parameters above the cut-offs showed a normal HPA function at 6-weeks, whereas none of the patients with both under the cut-offs recovered at 6-weeks *(odds ratio* 7.444, 95% CI 5.054 to 13.672).

In order to improve the accuracy of the test further, we added 6-week post-op baseline SST cortisol level into the logistic model (assumed to reflect a 'random 9 AM morning cortisol' in the absence of replacement therapy for >18hours). The binomial logistic regression model increased its statistical significance (χ^2 = 59.716, p<0.0001) and a 6-week baseline SST cortisol above or below 180nmol/L (6.5μg/dL, AUC= 0.88, p<0.001) predicted 6-week adrenal recovery. All the detailed coefficients for the new multiple regression analysis are shown in *Table 2c*.

Combining this cut-off with the previous findings significantly improved the model prediction (of combined predicted probability AUC ROC=0.96, p<0.001) (Figure 2a and 2b). No patient with all of these three variables below the stated cut-offs recovered HPA axis function within 12 months. Conversely, 91.8% of patients with all variables above the thresholds showed normal adrenal function at 6-weeks *(odds ratio* 12.200, 95% CI 5.268 to 28.255) (Figure 2c). The results of the analysis were not altered when corrected for age and gender.

Discussion

We have demonstrated that the pre-TSA stimulated cortisol, day 8 post-op 9am cortisol and a 6-week 'random' 9am cortisol can be utilized to predict the likelihood of recovery of the HPA axis

after trans-sphenoidal surgery. We show, not only that recovery occurs in the HPA axis, but also this recovery can occur as late as 12 months post-surgery, hence emphasizing the importance of periodic reassessment to avoid unnecessary hydrocortisone replacement in those who could eventually regain function.

A considerable number (29.4%) of our patients failed the SST at 6-weeks post–operatively, yet 10.1% eventually recovered HPA axis function by 12-months. Specifically, of those failing at 6-weeks, 15.6%, 12.5% and 6.2% recovered respectively at 3, 6 and 9 to 12-months post-TSA suggesting a significant potential for reversibility of secondary adrenal insufficiency (AI) in these patients. This is in line with our recently published data (15), and the observations of others, where recovery was delayed in some individuals by as along as 51-months post-surgery (median 20 months, range 8-51)(26). Therefore, it would appear justified to repeat dynamic testing of the HPA axis even if patients 'fail' the SST at 6-weeks: 10.1% (n=11) of our cohort of patients would have been treated with unnecessary life-long steroid therapy if not subsequently re-tested (dark, medium and light grey bars on Figure 1). However, there is currently no universal consensus as to how and when to reassess patients with pituitary disease for recovery of HPA axis function after surgery. Indeed, the exact timing of initial post-operative HPA axis assessment shows significant variability between centers (24 hours, 48 hours, 1 to 6 weeks) (27), and repeat testing beyond 6 weeks is not regarded as routine management.

Although our data show that a significant number of patients do go on to recover HPA axis after initially failing the SST, it is probably not appropriate or feasible to advocate re-testing of the HPA axis every 3 months indefinitely in all patients who fail SST at 6-weeks. Given the constraints on healthcare resources, a strategy for prioritizing re-testing in those patients most likely to achieve HPA axis recovery is desirable. Using the algorithms that we have proposed, we estimate being able to predict those 91.8% of patients who are likely to recover HPA axis function. It would therefore appear to represent a pragmatic strategy to prioritize periodic re-testing of these patients (if they unexpectedly failed the 6-week post-op SST), as opposed to re-testing all patients who fail the SST at 6 weeks (and do not meet any of the model criteria). In our data set, no patient who failed to meet all 3 specified criteria, recovered HPA function within the 12-month post-TSA testing period. Hence, one could argue a post-operative SST is not needed in those patients meeting

none of the thresholds and should only be performed in patients if there was clinical suspicion of excess cortisol replacement.

Until definitive HPA assessment is performed, the initial decision to continue glucocorticoid replacement is usually based upon the 9 AM serum cortisol concentration in the early postoperative period (28). Due to the risks associated with secondary AI, it is generally accepted that a morning serum cortisol concentration <100 nmol/L (3.6μg/dL) is used as a threshold to instigate glucocorticoid treatment with standard replacement regimens (29-33). There is little consensus regarding glucocorticoid replacement in patients with early post-operative 9 AM serum cortisol concentrations >300 nmol/L (10.8μg/dL). It has been suggested that at this level, it may be safe to withhold routine glucocorticoid replacement (29,31), although this is not universally accepted (32,34,35). Patients with 9am serum cortisol between 100 and 300 nmol/L are considered to be ACTH deficient and treated with glucocorticoid replacement until definitive testing of the HPA axis is performed (29-31,33). In our dataset a day 8, 9am cortisol of 370nmol/L (13.35μg/dL, ROC AUC 0.79, *p<0.001*) has 100% specificity to predict normal HPA axis function at 6 weeks as tested by SST. Hence, in those patients who meet this threshold, one could consider stopping hydrocortisone at day 8 and not re-testing the HPA axis unless clinically indicated.

We also identified other factors that were important in risk-stratifying patients for developing hypopituitarism. Age and tumor volume were associated with integrity of pituitary function. The contribution of the size of the lesion on pituitary axis function and recovery remains controversial. There is evidence of increased likelihood of recovery of pituitary function in patients with tumors smaller than 2.5 cm (25,36). However, Webb *et al.* failed to find any difference in tumor size among patients who did, or who did not, recover pituitary function after surgery (25,37). However, these studies used a one-dimensional method of tumor measurement, and in order to more accurately assess the potential effect of tumour size, we adopted a three-dimensional volumetric measurement (through ellipsoid formula) to determine the potential endocrine impact as has been published previously (25). Our multivariate logistic regression analysis demonstrated tumor volume as a factor which may be associated with a greater risk of 6-weeks post-operative pituitary deficit. We show that 95% with tumour volume > 9mL will have at least one pituitary hormone deficit at 6 weeks post-TSA. Thus, 9mL may represent the optimal cut-off value associated with the future

onset of at least one pituitary hormonal deficit and this threshold may predict the necessity of long-term hormonal replacement. Although such volume thresholds need replicating in larger patient cohorts, these data may be helpful when counselling patients regarding possible post-operative complications and the risk of persistent long-term hypopituitarism.

This study does have a number of limitations. It is a retrospective analysis of clinical data obtained from electronic patient records and therefore there is the potential for selection bias in those individuals undergoing pituitary axes testing. Detailed clinical assessments, for example using questionnaires, to indicate the level of glucocorticoid replacement were not performed. However the major remit of this study was to look at recovery in the context of standard medical practice. Our data extend to 12 months of follow-up only for HPA axis evaluation and therefore it is not possible to comment on whether further recovery of the HPA axis may occur after this time point. Although we demonstrate that the SST can be used in this manner to assess (and predict) recovery, the cut-offs that we have proposed are derived from this data-set. Our findings will need replicating in larger cohorts and across centers given interassay variation in cortisol measurement and cut-off thresholds (38,39) to inform more widely applicable algorithms to predict recovery of the HPA axis. This is also a heterogeneous patient cohort with differences in tumor size, invasiveness, histology, degree of surgical intervention and severity of pre-operative hypopituitarism, all of which may have some bearing on recovery of pituitary hormone function. Moreover, recovery rates observed in this study could be affected by the fact that our hospital is a referral center for pituitary disease with an expert single neurosurgeon performing the TSA procedures. Surgical outcomes are dependent on the surgical skills of the neurosurgeons, hence the parameters that we have found to be useful in predicting recovery may not be similarly useful in other centers with lesser expertise.

In conclusion, we have shown that further dynamic testing of HPA axis function is warranted beyond a 6-week post-operative assessment. Data from pre-operative, early post-operative assessments (day 8) and 6-week 9am cortisol can be informative in helping to decide which patients are most likely to regain HPA axis function and guide further repeat testing. Importantly, these data offer the opportunity for patients who may have been given life-long replacement, to safely come off therapy and therefore avoid unnecessary glucocorticoid exposure.

360

361

Acknowledgments

A.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. This work was supported by the Exchange in Endocrinology Expertise programme of the European Union of Medical Specialists (UEMS) and the European Society of Endocrinology (ESE) (3E fellowship and Short-Term Fellowship awarded to RP). The views expressed are those of the author(s) and not necessarily those of the NHS, or the Department of Health, UK.

References

1. Bates AS, Van't Hoff W, Jones PJ, Clayton RN. The effect of hypopituitarism on life expectancy. *J Clin Endocrinol Metab*. 1996;81(3):1169-1172.
2. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, Sheppard MC, Stewart PM. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. *Lancet*. 2001;357(9254):425-431.
3. Shulman DI, Palmert MR, Kemp SF, Lawson Wilkins D, Therapeutics C. Adrenal insufficiency: still a cause of morbidity and death in childhood. *Pediatrics*. 2007;119(2):e484-494.
4. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, Husebye ES, Merke DP, Murad MH, Stratakis CA, Torpy DJ. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2016;101(2):364-389.
5. Adcock IM, Mumby S. Glucocorticoids. *Handb Exp Pharmacol*. 2017;237:171-196.
6. Paragliola RM, Papi G, Pontecorvi A, Corsello SM. Treatment with Synthetic Glucocorticoids and the Hypothalamus-Pituitary-Adrenal Axis. *Int J Mol Sci*. 2017;18(10).
7. Littley MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML. Hypopituitarism following external radiotherapy for pituitary tumours in adults. *Q J Med*. 1989;70(262):145-160.
8. Henzen C, Suter A, Lerch E, Urbinelli R, Schorno XH, Briner VA. Suppression and recovery of adrenal response after short-term, high-dose glucocorticoid treatment. *Lancet*. 2000;355(9203):542-545.
9. Krasner AS. Glucocorticoid-induced adrenal insufficiency. *JAMA*. 1999;282(7):671-676.
10. Wang TW, Wong MS, Smith JF, Howlett TA. The use of the short tetracosactrin test for the investigation of suspected pituitary hypofunction. *Ann Clin Biochem*. 1996;33 (Pt 2):112-118.
11. Mortensen EM, Kapoor WN, Chang CC, Fine MJ. Assessment of mortality after long-term follow-up of patients with community-acquired pneumonia. *Clin Infect Dis*. 2003;37(12):1617-1624.
12. Overman RA, Yeh JY, Deal CL. Prevalence of oral glucocorticoid usage in the United States: a general population perspective. *Arthritis Care Res (Hoboken)*. 2013;65(2):294-298.
13. Johannsson G, Falorni A, Skrtic S, Lennernas H, Quinkler M, Monson JP, Stewart PM. Adrenal insufficiency: review of clinical outcomes with current glucocorticoid replacement therapy. *Clin Endocrinol (Oxf)*. 2015;82(1):2-11.
14. Sherlock M, Ayuk J, Tomlinson JW, Toogood AA, Aragon-Alonso A, Sheppard MC, Bates AS, Stewart PM. Mortality in patients with pituitary disease. *Endocr Rev*. 2010;31(3):301-342.
15. Pofi R, Feliciano C, Sbardella E, Argese N, Woods CP, Grossman AB, Jafar-Mohammadi B, Gleeson H, Lenzi A, Isidori AM, Tomlinson JW. The Short Synacthen (Corticotropin) Test Can Be Used to Predict Recovery of Hypothalamo-Pituitary-Adrenal Axis Function. *J Clin Endocrinol Metab*. 2018;103(8):3050-3059.
16. Sherlock M, Stewart PM. The Short Synacthen Test and Its Utility in Assessing Recovery of Adrenal Function in Patients With Central Adrenal Insufficiency. *J Clin Endocrinol Metab*. 2019;104(1):17-20.
17. Greenman Y, Tordjman K, Kisch E, Razon N, Ouaknine G, Stern N. Relative sparing of anterior pituitary function in patients with growth hormone-secreting macroadenomas: comparison with nonfunctioning macroadenomas. *J Clin Endocrinol Metab*. 1995;80(5):1577-1583.

18. Jahangiri A, Wagner JR, Han SW, Tran MT, Miller LM, Chen R, Tom MW, Ostling LR, Kunwar S, Blevins L, Aghi MK. Improved versus worsened endocrine function after transsphenoidal surgery for nonfunctional pituitary adenomas: rate, time course, and radiological analysis. *J Neurosurg*. 2016;124(3):589-595.
19. Webb SM, Rigla M, Wagner A, Oliver B, Bartumeus F. Recovery of hypopituitarism after neurosurgical treatment of pituitary adenomas. *J Clin Endocrinol Metab*. 1999;84(10):3696-3700.
20. Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, Snyder PJ, Swerdloff RS, Wu FC, Yialamas MA. Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2018;103(5):1715-1744.
21. Isidori AM, Balercia G, Calogero AE, Corona G, Ferlin A, Francavilla S, Santi D, Maggi M. Outcomes of androgen replacement therapy in adult male hypogonadism: recommendations from the Italian society of endocrinology. *J Endocrinol Invest*. 2015;38(1):103-112.
22. Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW. Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab*. 2001;281(6):E1172-1181.
23. Mooradian AD, Korenman SG. Management of the cardinal features of andropause. *Am J Ther*. 2006;13(2):145-160.
24. Sarri G, Davies M, Lumsden MA, Guideline Development G. Diagnosis and management of menopause: summary of NICE guidance. *BMJ*. 2015;351:h5746.
25. Chuang CC, Lin SY, Pai PC, Yan JL, Toh CH, Lee ST, Wei KC, Liu ZH, Chen CM, Wang YC, Lee CC. Different Volumetric Measurement Methods for Pituitary Adenomas and Their Crucial Clinical Significance. *Sci Rep*. 2017;7:40792.
26. Munro V, Tugwell B, Doucette S, Clarke DB, Lacroix A, Imran SA. Recovery of adrenal function after chronic secondary adrenal insufficiency in patients with hypopituitarism. *Clin Endocrinol (Oxf)*. 2016;85(2):216-222.
27. Inder WJ, Hunt PJ. Glucocorticoid replacement in pituitary surgery: guidelines for perioperative assessment and management. *J Clin Endocrinol Metab*. 2002;87(6):2745-2750.
28. Hout WM, Arafah BM, Salazar R, Selman W. Evaluation of the hypothalamic-pituitary-adrenal axis immediately after pituitary adenomectomy: is perioperative steroid therapy necessary? *J Clin Endocrinol Metab*. 1988;66(6):1208-1212.
29. Auchus RJ, Shewbridge RK, Shepherd MD. Which patients benefit from provocative adrenal testing after transsphenoidal pituitary surgery? *Clin Endocrinol (Oxf)*. 1997;46(1):21-27.
30. Courtney CH, McAllister AS, McCance DR, Bell PM, Hadden DR, Leslie H, Sheridan B, Atkinson AB. 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)*. 2000;53(4):431-436.
31. Watts NB, Tindall GT. Rapid assessment of corticotropin reserve after pituitary surgery. *JAMA*. 1988;259(5):708-711.
32. Jones SL, Trainer PJ, Perry L, Wass JA, Bessser GM, Grossman A. An audit of the insulin tolerance test in adult subjects in an acute investigation unit over one year. *Clin Endocrinol (Oxf)*. 1994;41(1):123-128.
33. Pavord SR, Girach A, Price DE, Absalom SR, Falconer-Smith J, Howlett TA. A retrospective audit of the combined pituitary function test, using the insulin stress test, TRH and GnRH in a district laboratory. *Clin Endocrinol (Oxf)*. 1992;36(2):135-139.

34. Hagg E, Asplund K, Lithner F. Value of basal plasma cortisol assays in the assessment of pituitary-adrenal insufficiency. *Clin Endocrinol (Oxf)*. 1987;26(2):221-226.
35. Jayasena CN, Gadhvi KA, Gohel B, Martin NM, Mendoza N, Meeran K, Dhillo WS. Day 5 morning serum cortisol predicts hypothalamic-pituitary-adrenal function after transsphenoidal surgery for pituitary tumors. *Clin Chem*. 2009;55(5):972-977.
36. Seltzer J, Wedemeyer MA, Bonney PA, Carmichael JD, Weiss M, Zada G. Outcomes following transsphenoidal surgical management of incidental pituitary adenomas: a series of 52 patients over a 17-year period. *J Neurosurg*. 2018:1-9.
37. Yedinak C, Hameed N, Gassner M, Brzana J, McCartney S, Fleseriu M. Recovery rate of adrenal function after surgery in patients with acromegaly is higher than in those with non-functioning pituitary tumors: a large single center study. *Pituitary*. 2015;18(5):701-709.
38. El-Farhan N, Pickett A, Ducroq D, Bailey C, Mitchem K, Morgan N, Armston A, Jones L, Evans C, Rees DA. Method-specific serum cortisol responses to the adrenocorticotrophin test: comparison of gas chromatography-mass spectrometry and five automated immunoassays. *Clin Endocrinol (Oxf)*. 2013;78(5):673-680.
39. Klose M, Lange M, Rasmussen AK, Skakkebaek NE, Hilsted L, Haug E, Andersen M, Feldt-Rasmussen U. Factors influencing the adrenocorticotropin test: role of contemporary cortisol assays, body composition, and oral contraceptive agents. *J Clin Endocrinol Metab*. 2007;92(4):1326-1333.

488

489 **Table 1**: Baseline characteristics of 109 patients with pituitary adenoma undergoing to Trans
490 Sphenoidal Adenomectomy. Patients with microadenomas, apoplexy, corticotroph adenomas,
491 craniopharyngioma, meningioma, rathke cleft cyst, metastases, redo-surgery, emergency TSA,
492 craniotomy or pituitary radiotherapy were excluded. Data are expressed as mean±standard
493 deviation where appropriate. Figures in parentheses reflect % of the entire cohort. (SST: short
494 *Synacthen* (corticotropin) test).

495

	Whole cohort
n	109
Age (years)	56±17
Tumor volume (median and range, ml, *Ellipsoid formula*)	7(0.36-75)
Tumor max diameter (cm)	2.6±1.0
Sex	
Male	71 (65.1%)
Female	38 (34.9%)
Tumor Histology	
Gonadotroph adenoma	72 (66.1%)
Lactotroph adenoma	5 (4.6%)
Somatotroph adenoma	11 (10.1%)
Pluri-hormonal	18 (16.5%)
Other *(TSH-secreting, null-cell adenoma)*	3 (2.7%)

Table 2: Multiple regression models evaluating possible predictors for future 6-weeks hormonal recovery in patients (n=109) with pituitary lesion treated with trans-sphenoidal adenomectomy.

(a)

(χ^2= 25.462, p<0.001*)	**Dependent: 6-weeks any pituitary deficits**			
Independent	***B***	***95% CI lower bound***	***95% CI upper bound***	***p***
Age	0.062	1.004	1.127	0.037
Tumor Volume	0.249	1.062	1.548	**0.010***
Gender	2.271	1.514	62.034	0.027

(b)

(χ^2= 48.158, p<0.001*)	**Dependent: 6-weeks HPA recovery**			
Independent	***B***	***95% CI lower bound***	***95% CI upper bound***	***p***
Age	-0.070	0.873	0.996	0.036
Gender	2.400	1.036	117.246	0.047
Tumor Volume	0.045	0.920	1.188	0.493
Pre-op 30 minute SST cortisol	0.018	1.007	1.030	**0.001***
Day 8 – 9AM cortisol	0.022	1.008	1.037	**0.003***

(c)

(χ^2= 59.716, p<0.001*)	**Dependent: 6-weeks HPA recovery**			
Independent	***B***	***95% CI lower bound***	***95% CI upper bound***	***p***
Age	-0.061	0.872	1.015	0.115
Gender	1.092	0.144	61.824	0.481
Tumor Volume	0.086	0.939	1.265	0.259
Pre-op 30 minute SST cortisol	0.016	1.005	1.027	**0.004***
Day 8 – 9AM cortisol	0.018	1.003	1.033	**0.011***
6-weeks baseline SST cortisol	0.025	1.006	1.045	**0.010***

Figure 1. Cumulative percentage of patients with normal Hypothalamic-Pituitary-Adrenal axes among the whole cohort (n=109) assessed by short Synacthen test pre-operatively, 6-weeks, 3-months, 6-months and 12 months after trans-sphenoidal surgery. Black bars represent the percentage of patients with normal HPA function at 6-weeks. Dark grey bars represent the percentage of patients recovering at 3 months. Medium grey bars represent the percentage of patients recovering at 6 months. Light grey bars represent the percentage of patients recovering at 12 months.

Figure 2. (a) ROC curve analysis to determine the ability of the pre-op 30-minute cortisol, day 8 (9 am) cortisol and 6-weeks (9AM) cortisol to predict eventual recovery of HPA axis function 6-weeks post-TSA. (b) ROC curve analysis to determine the ability of the predicted probability coefficient (computed combining pre-op 30-minute cortisol, day 8 (9 am) cortisol and 6-weeks (9AM) cortisol) to predict eventual recovery of HPA axis function 6-weeks post-TSA. (c) Detailed cumulative percentage of recovery of hypothalamic-pituitary-adrenal axis in 109 patients assessed by short Synacthen test within 12 months after trans-sphenoidal surgery, stratified by pre-op 30 minute cortisol, day 8 (9 am) cortisol and 6-weeks (9 am) cortisol cut-offs (430nmol/L – 15.5μg/dL, 160 nmol/L – 5.8μg/dL and 180 nmol/L – 6.5μg/dL respectively).