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Transsphenoidal surgery for Cushing's disease: the role of early post-operative serum cortisol measurements as a predictor of success — a prospective study.

Wczesne, pooperacyjne stężenie kortyzolu w surowicy w ocenie skuteczności przezklinowej operacji choroby Cushinga — badanie prospektywne

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Abstract

Introduction: Transsphenoidal surgery (TSS) is the treatment of choice for Cushing`s disease (CD). A proper assessment of the efficacy of surgical treatment is crucial in terms of patient prognosis. The aim of this study was to evaluate the usefulness of serum cortisol measurements performed on the first post-operative day as a predictor of TSS outcome.

Material and methods: This prospective study involved 36 patients with CD who were operated on using the same surgical protocol and followed up for at least 18 months (median: 30 months). We investigated the relationship between serum cortisol measurements performed on the first post-operative day and the hormonal assessment of the pituitary-adrenal axis performed after 18 months of follow-up. The adopted criteria for remission were: serum cortisol within the referral range, normal circadian rhythm, and the ability of serum cortisol to suppress to values ≤ 1.8 μg/dL after 1 mg of dexamethasone.

Results: The median serum cortisol on the first post-operative day was 1.98 μg/dL. 23 patients (63.9%) were regarded as cured. In all these cured cases, the post-op cortisol was ≤ 2.5. On the other hand, in the cases of the 13 patients (36.1%) for whom the remission of CD was not confirmed, the post-op cortisol was ≥ 2.5.

Conclusion: Our prospective study demonstrated that early post-operative serum cortisol measurements can serve as a simple and useful test that predicts the remission of CD. Furthermore, a post-operative cortisol ≤ 2.5 can be considered as a forecast of CD remission.
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Key words: *Cushing's disease, pituitary adenoma, cortisol, transsphenoidal surgery, corticotroph tumor*

Streszczenie

Wstęp: Leczeniem z wyboru w chorobie Cushinga jest selektywna przezklinowa adenomectomia. Ocena skuteczności operacji ma kluczowe znaczenie z punktu widzenia rokowania chorych. Celem pracy była prospektywna ocena przydatności oznaczeń kortyzolu w surowicy wykonanych w 1 dobie po operacji przezklinowej gruczolaka kortykotropowego dla oceny skuteczności leczenia operacyjnego.

Materiał i metody: Badanie prospektywne obejmujące 36 pacjentów z chorobą Cushinga operowanych według identycznego protokołu operacyjnego i obserwowanych przez co najmniej 18 miesięcy (mediana: 30 mies.). Oceniano związek pomiędzy stężeniem kortyzolu w surowicy o godzinie 6.00 w 1 dobie po operacji przezklinowej a oceną hormonalną osi przysadkowo-nadnerczowej wykonaną w 18 miesiącu obserwacji pooperacyjnej. Jako kryteria remisji przyjęto: normalizację stężenia kortyzolu w surowicy, prawidłowy rytm dobowy oraz obniżenie stężenia kortyzolu do wartości poniżej 1,8 μg/dl w teście z 1 mg deksametazonu.

Wyniki: Mediana stężenia kortyzolu w 1 dobie po operacji przysadki wyniosła 1,98 μg/dl. 23 pacjentów (63,9%) zostało uznanych za wyleczonych z choroby Cushinga. We wszystkich tych przypadkach stężenie kortyzolu w surowicy w 1 dobie po operacji było mniejsze lub równe 2,5 μg/dl. Jednocześnie u żadnego z 13 pacjentów (36,1%) ze stężeniem kortyzolu w 1 dobie po operacji powyżej 2,5 μg/dl nie potwierdzono remisji choroby Cushinga.

Wnioski: W badaniu prospektywnym wykazano, iż wczesne, pooperacyjne oznaczanie stężenia kortyzolu w surowicy jest prostym i użytecznym badaniem zapowiadającym remisję choroby Cushinga. Wykazanie stężenia kortyzolu w surowicy mniejszego lub równego 2,5 μg/dL w 1 dobie po operacji przezklinowej może być traktowane jako czynnik prognozujący remisję choroby.
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Słowa kluczowe: *choroba Cushinga, gruczolak przysadki, kortyzol, operacja przezklinowa, guz kortykotropowy*

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Introduction

Cushing's disease (CD) is one of the biggest challenges in contemporary endocrinology. It is caused by a pituitary, corticotroph adenoma producing adrenocorticotropic hormone (ACTH). Increasing corticotrophin secretion leads to cortisol excess and typical clinical symptoms [1–5]. Gradually, organ complications develop, such as arterial hypertension, impaired glucose tolerance and diabetes, decreased bone mineral density and increased risk of infections. They result in impaired quality of life and reduced life expectancy [3, 6–10]. Currently, TSS is the treatment of choice [3, 11–13]. The effectiveness of the surgical procedure in reference centres is excellent, with a success rate reaching as high as 90%. The success rate of TSS depends on several factors including the precise, pre-operative localisation of the pituitary tumour in magnetic resonance imaging (MRI) [11, 14–16], the immunohistochemical confirmation of the presence of a corticotroph tumour [15, 18, 19], the post-operative decrease in serum cortisol level [10, 20–23], and the experience of the neurosurgeon. Following TSS, patients are referred to endocrinological units to assess the efficacy of the surgical treatment and to monitor the post-operative function of the pituitary-adrenal axis. However, the laboratory procedures are not uniform and adopt different follow-up schedules depending on the site. In the literature there is still a lack of prospective studies on this important issue.

Therefore, the aim of this study was to prospectively evaluate the utility of early post-operative serum cortisol measurements performed on the first post-operative day for the remission of CD and to determine the cut-off point of the serum cortisol level that enables a prediction of remission.

Material and methods

Patient population

The study population consisted of 36 patients with CD (30 women and six men; F:M ratio: 5:1) hospitalised in the Department of Endocrinology, the Medical Centre of Postgraduate Education between 2005 and 2009. The mean age was 36.3 ± 12.9 years (range 16.8 to 57.6 years). After a confirmed diagnosis of CD, the patients were referred to the Department of Neurosurgery, Military Institute of Medicine in Warsaw. They were all operated on by the same neurosurgeon using an identical surgical protocol.

All patients were informed about the aims and methods of the study and they signed the informed consent. The study protocol was approved by the Bioethics Committee at CMKP.

Clinical course of CD and pre-operative endocrine evaluation

The diagnosis of ACTH-dependent Cushing's syndrome (CS) was made based on the clinical signs and standard hormonal criteria: increased urinary excretion of UFC (urinary free cortisol) or 17-OHCS (17-hydroxysteroids), increased serum cortisol level at 8.00am, the loss of cortisol circadian rhythm (a serum cortisol level above 7.5 μg/dL in the late night hours, increased or detectable plasma ACTH at 8.00am and the failure of serum cortisol to suppress to a level ≤ 1.8 during a low dose dexamethasone suppression test (LDDST; 0.5mg q.i.d. for 48 hours). The pituitary aetiology of CS was confirmed based on a serum cortisol and UFC or 17-OHCS suppression greater than 50% on a high dose dexamethasone suppression test (HDDST; 2mg q.i.d. for 48h) and positive MRI. In the case of equivocal results of the hormonal assessment and pituitary imaging, the diagnosis of CD was confirmed by a positive result in a stimulation test with an intravenous CRH injection (100 μg).

Pre-operative MR imaging

Prior to TSS, all patients underwent high resolution MRI of the pituitary-hypothalamic region (SIEMENS Symphony 2004; 1.5 Tesla). The MRI scans were performed before and after an intravenous injection of gadolinium (Gd-DTPA). It was determined that the presence of a hypodense lesion after contrast indicated a pituitary adenoma. A microadenoma was defined as a pituitary tumour with a diameter of less than 1 cm in any dimension, whereas a macroadenoma was defined by the presence of a tumour with at least one diameter of more than 1 cm. The MRI was qualified as equivocal if the pituitary tumour was not precisely visualised or if only indirect traits of the tumour were present, such as the deviation of the pituitary stalk or a convex upper surface of the pituitary gland.

Surgical procedure

In all cases, a microsurgical transseptal transsphenoidal approach was used for the resection of an ACTH-secreting pituitary adenoma. The sella was exposed and the H-shaped incision of the dura was made. The dura mater was opened and separated from the pituitary capsule to expose the entire anterior surface of the pituitary gland. Then the pituitary gland was carefully explored, regardless of MRI findings. Selective adenomectomy was performed in all cases of visualised pituitary adenoma on MR imaging. When the MR was equivocal, or no tumour was evident, a series of vertical and horizontal incisions of the pituitary gland was carried out and all tissue deemed to be abnormal was removed and submitted for pathological examination. In patients where no abnormal tissue could be identified

intra-operatively, hemihypophysectomy, subtotal hypophysectomy, or total hypophysectomy was performed.

Histopathological and immunohistochemical assessment

The surgical specimen was collected for histopathological analysis (standard haematoxylin and eosin staining) and immunohistochemical staining for pituitary hormones. The result was considered positive if the presence of ACTH-staining in the immunohistochemical examination was confirmed. The result was treated as negative if there was no evidence of the corticotroph adenoma and ACTH-staining was negative.

Post-operative hormonal evaluation and criteria of a cure

Blood samples for serum cortisol measurements were collected from all patients at 6.00am on the first post-operative day. Glucocorticoids were not administered in the perioperative nor in the early post-operative period. Hydrocortisone replacement therapy was started after biochemical confirmation of hypocortisolaemia or the development of clinical symptoms of adrenal insufficiency. In such cases, the hydrocortisone was given intravenously in a daily dose 50 mg t.i.d. during the first two post-operative days. Afterwards the standard dose of hydrocortisone (20 mg in the morning and 10 mg at 3.00 pm) was started and continued until the next hormonal evaluation.

Following the surgical procedure, all patients were subjected to further evaluation lasting at least 18 months (median 30 months; range 18–36 months). The first biochemical evaluation of corticotroph function was performed within seven days from TSS. Subsequent reassessments were performed at six weeks and 3, 6, 12, 18, 24 and 36 months after surgery. Patients on hydrocortisone replacement therapy had their cortisol measurements taken 48 hours after the last administered dose.

The patients were regarded as cured if they fulfilled the following criteria for remission: clinical and biochemical evidence of eucortisolaemia: morning serum cortisol within referral range, the correct circadian rhythm (the late night serum cortisol level ≤ 7.5 μg/dL), and the ability of serum cortisol to suppress to less than or equal to 1.8 μg/dL following the overnight 1 mg dexamethasone suppression test (ODST).

Hormone assay

Chemiluminescent immunometric assays (IMMULITE 2000, Siemens, Great Britain) were used to measure serum cortisol. The method sensitivity was 0.2 μg/dL (5.5 nmol/L). The normal range for cortisol was 5–25 μg/dL (138–690 nmol/L). The lowest limit of quantification for cortisol, using the IMMULITE 2000 analyser, was the measured value of 1.0 μg/dL. For the purpose of further calculations it was assumed that in these cases the post-op cortisol was 1.0. Plasma ACTH was measured using a two-step radioimmunometric assay (IRMA; coated tube technique, Brahms, Germany). Method sensitivity was 1.2 pg/mL and the referral range was 10 to 60 pg/mL.

Statistical analysis

Methods of descriptive statistics (mean, median, standard deviations, proportion) were employed in the statistical analysis. Verification of hypotheses concerning the relationship between two categorical variables were expressed as frequencies and compared using the exact chi-square test (Fisher's exact test). The significance of differences between average values of continuous variable in two groups was analysed by means of the Student`s t-test for a normal distribution and the Mann-Whitney test for small samples when its distribution was not normal. Verification of hypotheses concerning comparisons of the analysed parameters in two time points was conducted using the Wilcoxon test for small samples (for non-normal distributions).

The dynamics of analysed tests results was conducted by calculating areas under the curve (AUC) using a trapezoid method. The significance of differences between these areas was checked using the Mann-Whitney test. In order to present values of average areas under the curve, the individual values were logarithmically transformed and the standard errors for logarithm values were calculated.

The level of significance was set at $p < 0.05$. The calculations were made using the commercially available statistical software package SPSS v. 18.0.

Results

Results of clinical and epidemiological assessment

The clinical characteristics covering demographic data, complications of CD, number of operations, results of the pre-operative MRI and histopathological examination are presented in Table I.

Results of early post-operative hormone evaluation

The mean first day post-operative serum cortisol was 6.0 ± 9.02 μg/dL (median: 1.975; range: 1.0 to 37.0) v. 25.9 ± 7.35 μg/dL (median: 26.05; range:14.4 to 41.3) prior to surgical treatment ($p < 0.001$).

Detailed results of first day post-operative cortisol after TSS for CD are presented in Table II. Patients were ranked in ascending order based on the results of their first day post-op cortisol.

Table I. *Demographic data and pre-operative clinical characteristics of the study group of 36 patients with CD*
Tabela I. *Dane demograficzne oraz przedoperacyjna charakterystyka kliniczna badanej grupy 36 pacjentów z chorobą Cushinga*

Number of patients			36
Age (years)		mean ± SD (range)	36.3 ± 12.9 (16.8–57.6)
Sex	Females	n (%)	30 (83.3%)
	Males	n (%)	6 (16.7%)
Complications of CD	Hypertension	n (%)	28 (77.8%)
	Diabetes	n (%)	6 (16.7%)
	Pre-diabetes	n (%)	12 (33.3%)
	↓ BMD (osteopenia or osteoporosis)	n (%)	26 (72.2%)
	Obesity (BMI ≥ 30)	n (%)	13 (36.1%)
Surgical treatment	First	n (%)	28 (77.8%)
	Subsequent operation	n (%)	8 (22.2%)
The follow-up period (months)		mean ± SD (range)	28.5 ± 7.9 (18–36)
MRI results		Microadenoma	22 (61.1%)
		Macroadenoma	6 (16.7%)
		Equivocal MRI	8 (22.2%)
Histopathology (light microscopy)	Positive	n (%)	27 (75%)
	Negative	n (%)	9 (25%)

The 36 patients were then divided into three subgroups, depending on their first day post-operative cortisol levels. Subgroup 1 consisted of 19 subjects with very low cortisol concentrations, i.e. with post-op cortisol levels ≤ 2.0. The cut-off of 2.0 μg/dL was selected based on the median value of 1.975. Subgroup 2 consisted of five patients whose serum cortisol was higher and judged to be detectable (above median value) but still below the lower limit 5.0 μg/dL of the referral range, i.e. with cortisol levels between 2.0 and 5.0. Subgroup 3 consisted of 12 patients (33.3%) with cortisol concentrations within or above the laboratory range, i.e. with serum cortisol above 5.0 μg/dL.

Results of further post-operative hormone evaluation

The serum cortisol measurements during the follow-up period were conducted at 6.00am on the seventh post-operative day, and 1.5, 3, 6, 12, 18, 24 and 36 months after surgery. The detailed results presented as mean ± SD, median values and ranges are set out in Table III.

Based on a hormonal evaluation performed 18 months after surgery, the efficacy of the TSS was assessed for all 36 patients. The patients were regarded as surgically cured from CD if they fulfilled all of the following criteria of remission: morning serum cortisol level within the referral range (or persistent adrenal insufficiency), normal circadian rhythm of serum cortisol and serum cortisol level below 1.8 μg/dL following a 1 mg dexamethasone suppression test. It was established that the above criteria for a cure from CD were met by 23 patients (63.9%). Thirteen patients (36.1%) who failed to meet the criteria of remission were regarded as non-cured subjects. Detailed results of the final hormonal assessment are presented in Table II.

A significant difference was confirmed between cured and non-cured subjects in terms of morning serum cortisol concentrations **($p < 0.001$)** and serum cortisol levels following ODST **($p < 0.001$)**. The detailed results are presented in Table IV.

At the next stage of the study, on the basis of the hormone evaluation conducted in the course of the follow-up, the initial division of the studied group into three subgroups was carefully re-assessed. The initial allocation (which was based on the results of first day serum cortisol) was analysed from the perspective of further serum cortisol dynamics, as well as clinical and biochemical assessments performed at the end of the 18-month follow-up.

Due to the fact that the distribution of poc was not normal, the upper cut-off point for Subgroup 1 was initially assumed as the median value (1.975, rounded up to 2.0 μg/dL). It appears that this group of subjects may be regarded as cured. This is confirmed by low serum cortisol levels achieved in the course of the 18-month

Table II. *Detailed results of serum cortisol measurements on the first day, at six and 12 months after TSS, along with hormonal evaluation of the pituitary-adrenal axis conducted at the end of the follow-up period*

Tabela II. *Szczegółowe wyniki oznaczeń kortyzolu w 1. dobie oraz 6 i 12 miesięcy po operacji wraz z podsumowaniem funkcji osi przysadkowo-nadnerczowej na zakończenie 18-miesięcznej obserwacji*

Demografic data			Serum cortisol 1st day after TSS [μg/dL]	Serum cortisol 6 month after TSS [μg/dL]	Serum cortisol 12 month after TSS [μg/dL]	Final hormonal assessment at the end of follow-up (18 month)			
No.	Sex	Age (years)				Cortisol 8.00	Circadian rhytm	ODST	Remission
1	F	52.6	< 1.0	< 1.0	13.8	10.36	N	< 1.0	Yes
2	F	24.8	< 1.0	14	17.7	10.5	N	< 1.0	Yes
3	F	26.3	< 1.0	< 1.0	< 1.0	1.81	*	**	Yes
4	M	19.9	< 1.0	< 1.0	4.2	16.4	N	< 1.0	Yes
5	F	52.4	< 1.0	5.46	6.55	4.57	N	< 1.0	Yes
6	F	26.2	< 1.0	< 1.0	3.48	10.9	N	< 1.0	Yes
7	F	57.2	< 1.0	7.06	5.25	10.2	N	1.38	Yes
8	F	43.7	1.08	< 1.0	4.5	5.29	N	1.6	Yes
9	F	28.0	1.14	2.02	2.19	< 1.0	*	**	Yes
10	F	29.9	1.2	< 1.0	12.6	10.9	N	< 1.0	Yes
11	F	34.4	1.2	1.81	2.92	9.04	N	< 1.0	Yes
12	F	51.0	1.6	2.97	2.13	2.84	N	1.54	Yes
13	F	41.0	1.4	13.9	7.51	11.9	N	< 1.0	Yes
14	F	29.1	1.6	3.87	8.0	9.7	N	< 1.0	Yes
15	F	29.7	1.6	3.25	6.0	14.1	N	1.1	Yes
16	M	20.9	1.69	< 1.0	12.7	14.3	N	< 1.0	Yes
17	F	18.4	1.79	6.72	10.4	8.65	N	< 1.0	Yes
18	F	40.8	1.95	8.85	8.62	6.98	N	1.37	Yes
19	F	24.3	2.0	14.2	12.3	13.9	N	< 1.0	Yes
20	F	26.3	2.04	9.03	18.6	10.2	N	1.13	Yes
21	F	25.1	2.05	< 1.0	1.28	6.49	N	1.23	Yes
22	F	56.3	2.17	4.41	3.92	13.6	N	< 1.0	Yes
23	F	52.1	2.5	2,4	3.15	8.36	N	1.71	Yes
24	F	57.6	4.08	12.7	19.2	15.5	A	3.69	No
25	F	34.1	5.3	19.8	17.6	16.8	A	9.04	No
26	F	28.4	5.5	9.74	8.1	14.6	A	3.45	No
27	F	34.8	6.5	10.1	17.7	13.5	N	3.67	No
28	M	49.3	6,7	15.5	12.6	23.7	A	4.3	No
29	F	55.8	6.6	9.4	11.4	11.6	N	3.2	No
30	M	16.8	8.7	14.2	16.3	14.8	A	8.79	No
31	F	32.1	9.2	7.4	9.7	14.4	A	8.47	No
32	F	32.4	17.5	22.6	29.7	32.4	A	17.8	No
33	M	39.8	19.7	11.7	11.0	33.1	A	7.72	No
34	M	18.8	20.1	9.61	31.6	28.5	A	6.38	No
35	F	53.8	35.5	16.3	23.1	18.3	A	11.7	No
36	F	42.5	37.0	14.0	8.97	27.3	A	8.7	No

Abbreviations: ODST — overnight (1 mg) dexamethasone suppression test; N — normal; A — abnormal;
*due to subnormal serum cortisol concentrations the assessment of circadian rhythm was not possible (secondary adrenal insufficiency)
**due to undetectable cortisol levels, ODST was not performed

Table III. *Detailed results of serum cortisol concentration conducted before TSS, on the first day after surgical treatment, and at consecutive time points of the 18-month follow-up period, presented as mean ± SD, median and ranges. The available data for the 24th and 36th months of follow up was added*

Tabela III. *Wyniki oznaczeń kortyzolu w surowicy przed operacją przezklinową, w 1. dobie po leczeniu operacyjnym oraz w kolejnych punktach czasowych 18-miesięcznej obserwacji, przedstawione w postaci wartości średnich ± SD, median i zakresu danych (min.–max.). Przedstawiono również dostępne dane dla 24. i 36. miesiąca po leczeniu operacyjnym*

	Cortisol [μg/dL]	Postoperative serum cortisol concentration in consecutive time points [μg/dL]								
	before TSS	1st day	7th days	6 weeks	3 months	6 months	12 months	18 months	24 months	36 months
N	36	36	36	36	36	36	36	36	27	18
Mean	25.91	6	6.49	6.48	6.57	7.81	10.72	12.99	15.54	14.99
Median[1]	**26.05**	**1.975**	**1.27**	**2.15**	**4.43**	**7.23**	**9.34**	**11.75**	**13.4**	**15.3**
SD	7.35	9.02	9.36	8.08	5.88	6.08	7.59	7.54	9.38	6.69
Minimum	14.40	1.0	1.0	1.0	1.0	1.0	1.0	1.0	3.9	6.64
Maximum	41.30	37	40.9	31.3	23.8	22.6	31.6	33.1	50.0	32.1

[1]Due to non-normal serum cortisol distribution the median values were marked out

Table IV. *Serum cortisol level under basic conditions and following 1 mg dexamethasone suppression test in the cured and non-cured groups*

Tabela IV. *Porównanie stężenia kortyzolu w warunkach podstawowych i w teście hamowania 1 mg deksametazonu w grupie wyleczonej i niewyleczonej*

		All operated subjects	Cured subjects	Non-cured subjects	p
Serum cortisol 8.00 (18 months)	N	36	23	13	< 0.001
	Mean	12.99	9.2	19.65	
	SD	7.54	4.12	7.7	
	Median	11.75	10.2	15.5	
	Minimum	1.0	1.0	11.6	
	Maximum	33.1	16.4	33.1	
Serum cortisol 8.00 (after 1 mg of dexamethazon)	N	36	21*	13	< 0.001
	Mean	3.56	1.14	7.46	
	SD	3.99	0.24	4.14	
	Median	1.375	1	7.72	
	Minimum	1	1	3.2	
	Maximum	17.8	1.71	17.8	

*ODST was not performed in two cured subjects due to persistent adrenal insufficiency

follow-up, remission of clinical symptoms of hypercortisolaemia, and restoration of normal circadian cortisol rhythm, as well as further results of ODST.

Further analysis of the serum cortisol dynamics in the post-operative period, abnormal circadian rhythm, the results of ODST as well as somatic features of persistent CD did not allow a diagnosis of remission in Subgroup 3 (serum cortisol > 5.0 μg/dL). Thus, every studied subject with serum cortisol concentration within or above the limits of laboratory range may be regarded as a patient with persistent CD or at higher risk of hypercortisolaemia recurrence.

For the patients in Subgroup 2, a detailed assessment of cortisol dynamics was conducted in the 18 month period after surgery, separately for each subject studied (Table V). This was followed by a comparison of the serum cortisol levels of all three subgroups on the first day after surgical treatment and at three consecutive time points (seventh day, sixth week and third month after TSS).

Table V. *Serum cortisol concentrations on the first day after TSS and in consecutive time points of 18 months follow-up. A detailed analysis of five patients with serum cortisol on first day within the range of 2-5 μg/dL compared to median cortisol concentration levels in groups regarded as cured (cortisol level ≤ 2.0 μg/dL) and non-cured (cortisol level ≥ 5μg/dL)*

Tabela V. *Stężenia kortyzolu w 1. dobie po operacji oraz w kolejnych punktach czasowych 18-miesięcznej obserwacji. Szczegółowa analiza 5 przypadków ze stężeniami kortyzolu w 1. dobie po operacji w przedziale od 2 μg/dl do 5 μg/dl zestawiona z medianą stężenia kortyzolu dla grupy uznanej za wyleczoną (≤ 2.0 μg/dl) i niewyleczoną (≥ 5μg/dl)*

1st day serum cortisol [μg/dL]		Postoperative serum cortisol concentration (μg/dL)						
		1st day	7th days	6 weeks	3 months	6 months	12 months	18 months
≤ 2.0	N	19	19	19	19	19	19	19
	Mean	1.32	1.23	1.66	3.33	4.8	7.47	9.12
	SD	0.35	0.46	0.99	2.7	4.75	4.68	4.38
	Median	**1.2**	**< 1.0**	**< 1.0**	**3.04**	**2.97**	**6.55**	**10.2**
2.01–4.99	Pt No. 20	**2.04**	< 1.0	< 1.0	2.85	9.03	18.6	10.2
	Pt No. 21	**2.05**	< 1.0	< 1.0	< 1.0	< 1.0	1.28	6.49
	Pt No. 22	**2.17**	< 1.0	< 1.0	1.5	4.41	3.92	13.60
	Pt No.23	**2.5**	3.96	3.61	3.17	2.4	3.15	8.36
	Pt No. 24	**4.08**	5.12	10.3	10.7	12.7	19.2	15.5
≥ 5.0	N	12	12	12	12	12	12	12
	Mean	14.86	16.51	15.39	12.82	13.36	16.45	20
	SD	11.39	10.6	8.32	5.32	4.6	7.87	7.93
	Median	**8.95**	**14.4**	**14.75**	**11.7**	**12.85**	**14.45**	**15.2**

As a result of the analysis conducted, it was established that four patients (patients no. 20–23) with serum cortisol lower than or equal to 2.5 μg/dL did not differ (in terms of cortisol concentrations during follow-up) from subjects in the group regarded as cured (Table V). This was proven by very low (subnormal) serum cortisol levels on the seventh day, sixth week and third month after surgery for corticotroph adenoma. Furthermore, in these four patients, there were also identified clinical features of remission, the serum cortisol measured at the end of the follow-up was normal, the circadian rhythm was preserved, and the ability of serum cortisol to suppress to less than 1.8 μg/dL following LDDST was maintained. However, one studied subject with cortisol 4.08 μg/dL (patient no. 24) did not differ significantly from the subjects regarded as non-cured. This was proven by a rapid increase in cortisol concentration after surgery, abnormal circadian cortisol rhythm and an inability of serum cortisol to suppress to a value below 1.8 μg/dL.

The above analysis shows that a threshold value of 2.5 μg/dL for first day post-operative serum cortisol could be regarded as being predictive of remission in CD.

To summarise the results of our work, we observe that all 23 patients who met the criteria of remission of CD had serum cortisol concentration on the first day after TSS lower than or equal to 2.5 μg/dL, whereas remission of CD was not confirmed in any of the 13 patients with a post-operative cortisol concentration higher than 2.5 μg/dL.

Discussion

The aim of the neurosurgical treatment of CD is to completely remove pituitary corticotroph adenoma. Currently, the treatment of choice is transsphenoidal selective adenomectomy. The most desirable result of the procedure is selective removal of pituitary tumour in such a manner as to maintain normal thyreo- and gonadotrophic function of the anterior pituitary and to leave the posterior lobe undamaged. The high selectivity of TSS allows the gradual recovery of normal corticotroph pituitary function, which was crushed by the long-term sustained overproduction of ACTH and cortisol. Obviously, such high selectivity of surgery is not always possible. In the case of repeat TSS, or in the presence of invasive macroadenomas, the scope of performed surgery is often wider — hemihypophysectomy or (in severe, selected cases) total hypophysectomy is performed [11–13,24].

Irrespective of the scope of the TSS, complete removal of autonomous neoplastic tissue results in cortisol deficiency and sequential symptoms of secondary adrenal insufficiency, because normal pituitary corticotroph function was suppressed by high ACTH and cortisol

excess. Due to the relatively short half-life of serum cortisol (about 60 minutes) the post-operative decrease in serum cortisol is rapid. Therefore, after about four hours following TSS, serum cortisol concentration should be lowered by about 90%, and after seven hours it should be less than 1% of its initial value.

One of the relatively few prospective works that discuss this issue is the study by Rollin et al. on the dynamics of cortisol concentration at six, 12 and 24 hours after surgery, conducted on 26 patients with Cushing's disease [22]. Twelve hours after TSS, these authors found a significant difference in cortisol concentrations between the cured and the non-cured group. Twenty four hours after surgery, the mean cortisol concentration in the cured group was 4.72 ± 6.72 μg/dL. This was higher than the concentration observed in our group (mean 1.5 ± 0.47 μg/dL; median: 1.36 μg/dL). It is worth noting that the criteria for a cure adopted in the present study are more stringent. The criteria for a cure adopted by Rollin were that the serum cortisol concentration following 1 mg dexamethasone suppression test should be ≤ 3 μg/dL, clinical improvement, and the need for hydrocortisone replacement therapy.

In our study, it was assumed that following a 1 mg dexamethasone suppression test an effectively cured patient should behave like a healthy individual, and that therefore the measured serum cortisol concentration should not exceed 1.8 μg/dL as recommended by the current consensus [25]. Clinical improvement and the necessity of hydrocortisone replacement may also be misleading. They may result from a merely partial lesion removal and sequential ischaemia occurring in the course of intraoperative manipulation and they do not constitute evidence, in the strict sense, of disease cure. Thus, in the group regarded as 'cured' by Rollin, there might have been patients with persistent, so-called 'smouldering' Cushing's disease.

The cut-off point of cortisol concentration on the first day after TSS of ≤ 2.5 μg/dL achieved in our study is lower than the threshold reported in the retrospective study of Esposito et al. on the material of 40 patients with CD from two American centres. Initially, the cut-off value assumed in this study was 8 μg/dL. However, this was lowered due to the fact that none of the studied patients demonstrated a post-operative serum cortisol concentration within the range of 5–8 μg/dL [20]. In our study, the female patient (patient no. 24) with cortisol = 4.08 μg/dL demonstrated features of recurrence of hypercortisolaemia, which was later confirmed by the results of hormone dynamic testing [26].

It should be emphasised here that to obtain credible results of early cortisol measurements it is necessary to refrain from administering hydrocortisone replacement in the post-operative period until serum cortisol measurements are performed at 6.00am on the following day, or until the symptoms of adrenal insufficiency occur. Contrary to previous concerns and suggestions, which required routine administration of hydrocortisone [12, 27,28], such a procedure appears to be safe [20, 22]. This is probably associated with the cellular mechanism of cortisol action towards cytoplasmic and nuclear receptors and transcription processes, which are significantly increased with cortisol excess. Therefore, a several-hour delay in initiation of hydrocortisone replacement does not lead to the development of adrenal crisis as early as on the first post-operative day. The possible risk of developing adrenal crisis is also mitigated by the renin-angiotensin-aldosterone system, which is responsible for maintaining an appropriate volume of body fluids. Its function in secondary adrenal insufficiency is not impaired. Some significance should also be attributed to the fact that TSS is a relatively safe and minimally invasive surgical procedure [13, 29, 30].

In the 2008 consensus statement on the treatment of CD, great importance was attached to post-operative serum cortisol concentration and urinary free cortisol. There are, however, no indications as to the specific point in time at which the measurements should be performed. The consensus statement also recommended considering patients with a serum cortisol concentration of less than or equal to 5 μg/dL to be surgically cured [11]. Fomekong et al. performed post-operative cortisol concentration measurements on the second and fifth days after TSS, and they initiated hydrocortisone substitution when the serum cortisol concentration was lower than 10 μg/dL. The substitution was continued until it was confirmed that the morning serum cortisol concentration did not exceed 10 μg/dL [31]. Chen et al. showed that demonstrating a serum cortisol concentration lower than or equal to 3 μg/dL following a 1 mg dexamethasone suppression test on the third day after TSS is of prognostic value with reference to a cure for Cushing's disease [21]. However, in our study, the 1 mg dexamethasone suppression test was performed later, at the end of the 18-month follow-up period. We found it interesting that in every uncured patient it was then demonstrated, similarly as in the study by Chen et al., that the cortisol concentration after the administration of 1 mg dexamethasone exceeded 3 μg/dL.

In the present study, setting the cut-off point at the level of 2.5 μg/dL allowed us to identify a group of patients, who, after 18 months of follow-up and on the basis of the strict criteria we have adopted, could be regarded as being cured from CD. The proposed early cortisol assessment also meets the basic criterion of clinical usefulness, such as ease and availability of serum cortisol determination in basic conditions. At the same time, adoption of this threshold value does

not exclude possible remission of CD in patients with slightly higher cortisol values, although such patients were not present in the studied material.

The principle which must be observed at every stage of diagnostics and treatment of Cushing's disease is to avoid drawing conclusions based on single hormone determinations or tests, because none of them is considered to be a gold standard [3, 32–35]. Therefore, in the present study, post-operative follow-up was continued and cortisol measurements were made particularly often during the first year after TSS. The advisability of this procedure is confirmed in an interesting retrospective study by Valassi et al., which is based on the experience of American and Italian centres. The authors demonstrated that early post-operative assessment (based in the mentioned study on UFC results) may be misleading, because in 5.6% of patients with normal or even elevated UFC immediately after surgery a significant decrease in cortisol concentration was observed within the period of 5–8 weeks and remission of CD [18]. Similar observations have also been made by other authors [36, 37].

In the present study, no cases of delayed remission of CD were observed, yet the adopted schedule of cortisol assessment at seven days, six weeks, three months and later guarantees detection of possible cases of such delayed remission.

In our opinion, delayed decrease of cortisol concentration may be associated with autonomous cortisol secretion by the adrenals, possibly in connection with the sometimes observed nodular hyperplasia of the adrenal cortex induced by long-term ACTH stimulation. We cannot exclude the influence of degenerative changes in the very corticotroph pituitary adenoma, developing with delay under the influence of ischaemia induced by possible damage during surgical procedures [18, 36, 37].

The cases of delayed remission of hypercortisolaemia described in the study by Valassi et al. cast doubt on the performance of so-called 'early repeat surgery' in the course of persistent CD, which has been postulated by other authors [38,39]. Qualification for such an early repeat TSS was to be conducted on the basis of early post-operative hormone assessment. The unquestionable advantage of this procedure is an attempt to perform a second treatment during the same hospital stay and by the same operating team. Furthermore, lack of cicatrices and adhesions within the surgical access is of significant importance and facilitates treatment. The optimal time for performance of consecutive TSS is debatable; however, it is agreed that it should be performed within two weeks of the first procedure [20, 38, 39]. Observations made by Valassi et al. indicate that earlier surgical intervention may unnecessarily expose patients to the risk of perioperative complications. However, it seems that the 5% probability of delayed remission is low, and the procedure of TSS is relatively safe [13]. Thus, the surgical team should make the final decision, taking into account the specifics of each treated patient, including the results of preoperative imaging studies and the results of the histopathological examination from the first surgery.

In summary, it should be stated that early post-operative assessment of serum cortisol concentration on the first day after TSS for CD is a useful and easy diagnostic tool which allows us to make an early distinction between patients cured from CD, and those patients with persistent disease or at increased risk of recurrence. The latter group will require particularly careful post-operative follow-up and, if necessary, additional pharmacological treatment, consecutive surgery, or radiotherapy [11–13, 40].

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