Lessons from rare diseases: pathophysiology of stress-related diseases and organization and evaluation of care for patients with Cushing's syndrome

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Pathophysiology of stress-related diseases and organization and evaluation of care for patients with Cushing's syndrome

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CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE

As reaction to undesirable, difficult or challenging circumstances, called stressors, a psychophysiological response is generated to enable an adequate response to these stressors: the stress response.[1-3] Biologically, both the endocrine system as well as the autonomic nervous system play either individually or in close collaboration, a key role in regulating levels of stress.[4, 5] Activation of the hypothalamus-pituitary-adrenal (HPA)-axis leads to secretion of the stress hormone cortisol from the adrenal cortex. Cortisol is the key mediator of the stress response and is required for cognition, behaviour, metabolism, and immunological functioning.[5] Although stress responses are essential for survival and have evolved as adaptive processes, already back in 1956 it was observed that severe, prolonged stress might lead to tissue damage and disease.[6] Persistent (chronic) stress is characterized by prolonged exposure to cortisol, affecting vital organs such as the brain, liver, muscle, cardiovascular system, gastrointestinal system, endocrine system, and immune system.[1]

Cushing's syndrome (CS) is characterized by glucocorticoid excess, most commonly caused by medically prescribed synthetic corticosteroids. Endogenous CS is caused by either adrenocorticotropic hormone (ACTH)-independent adrenal overproduction of cortisol (e.g. an adrenal adenoma, hyperplasia, or carcinoma), or by excess ACTH-secretion from a pituitary adenoma (i.e. Cushing's disease (CD)) or an ectopic ACTH-producing tumor (most often bronchial carcinoids). Endogenous CS is a rare condition, with a reported incidence of only 2-3/million per year, although in selected patient populations such as poorly controlled diabetics and young patients with osteoporosis (particularly men) or hypertension the incidence is higher. The incidence of CD, the most common cause of endogenous CS, is estimated to be 1.2-2.4/million per year.[7, 8] The prolonged tissue exposure to increased levels of glucocorticoids induces changes in body composition (central obesity, osteoporosis, and sarcopenia), an adverse metabolic profile (insulin resistance and diabetes mellitus, hypercoagulability leading to venous and arterial thrombosis, dyslipidemia), hypertension, neuropsychiatric disorders, and also ophthalmological abnormalities may occur.[9-11] Patients with CS may present with typical clinical findings such as facial rounding ('moon face'), plethora, truncal obesity, purple striae, easy bruising, muscle weakness, and a dorsal fat pad, however the clinical manifestation of the syndrome may vary, and the diagnosis can be challenging in mild cases.[12, 13] Associated comorbidities as well as CS itself lead to a decreased quality of life.[14] If left untreated, the prognosis of clinically manifest CS is poor, with an estimated 5-year survival of only 50%. [15] This increased mortality is mainly caused by macrovascular disease (e.g. stroke and myocardial infarction), but also infections, a hypercoagulable state, and poorly controlled diabetes mellitus may play a role.[16]

Resection of primary lesion(s) underlying CS is the treatment modality of first choice, aiming at normalization of cortisol exposure to eliminate the associated symptoms, signs, and comorbidities, reducing mortality and improving prognosis, and to improve quality of life.[17, 18] In case of a pituitary corticotropic adenoma, selective adenomectomy by transsphenoidal resection is the preferred treatment.[19] Second-line therapeutic options include re-operation, medical therapy, radiotherapy, and bilateral adrenalectomy in case of noncurative pituitary surgery.[17] In case of adrenal CS or an ectopic tumor, adrenalectomy, respectively removal of the ACTH-producing tumor, will be performed if possible, or bilateral adrenalectomy will be considered in case of metastatic ectopic

ACTH secretion.[17] Surgical outcomes traditionally focus on biochemical remission, however thereby neglecting life-long complications such as hypocortisolism or hypopituitarism, which also impact quality of life, and are therefore important from a patient perspective.[20-22] Because of the (often prolonged) hypercortisolemic state of patients with CS prior to diagnosis and treatment, this rare condition can be considered a model for the effects of chronic and severe stress and corticosteroid excess on the human body and brain.

An example of a disease in which stress and cortisol are believed to play a role in the pathophysiology is central serous chorioretinopathy (CSC). CSC is a specific chorioretinal eye disease often affecting the macula, in which thickening, hyperpermeability and choroidal congestion damage the retinal pigment epithelium. This process induces serous subretinal fluid accumulation and subsequently detachment of the neuroretina.[23-25] If left untreated, irreversible loss of vision may occur. Although the pathogenesis of CSC is yet to be elucidated, an association of CSC with both psychosocial stress as well as biochemical stress in the form of exogenous corticosteroid use and endogenous hypercortisolism has been reported.[11, 23, 26-28] Therefore, in this thesis CSC is used as a model for stress vulnerability of the eye. Although acute CSC often spontaneously resolves within a few months, in chronic CSC the prolonged presence of subretinal fluid may result in permanent photoreceptor damage, resulting in metamorphopsia and variable deterioration in visual acuity, color vison, and contrast leading to a decreased quality of life.[29, 30] The two most commonly used treatment modalities are high-density subthreshold micropulse laser and photodynamic therapy, of which the latter has been shown to be superior regarding complete resolution of subretinal fluid.[31, 32]

This thesis is consists of two parts. Part one addresses the pathophysiology of stress related diseases, in which the rare stress- and cortisol-related diseases mentioned above serve as a model for stress vulnerability of the retina and the brain. In the second part of this thesis, specific features of the organization of thromboprophylaxis managment, outcome evaluation, and quality of care of patients treated for endogenous Cushing's syndrome are described.

PART ONE

Up to 5% of patients with CS have been reported to have had visual complaints during the active phase of the disease likely due to (one or more episodes of) CSC.[33] CSC has even been described as the presenting symptom of CS.[11] It has been hypothesized that endogenous glucocorticoid excess increases the risk of CSC by altering choroidal coagulation, systemic blood pressure, capillary fragility, and fibroblastic activity.[24, 34] In patients with active endogenous CS, an increased choroidal thickness as a predisposing factor for CSC has been observed, as well as retinal abnormalities.[35, 36] In the study described in **chapter 2**, an ophthalmological screening with multimodal imaging including optical coherence tomography was performed in a series of consecutive patients with active CS without visual complaints, in order to evaluate abnormalities within the CSC spectrum and the potential need for standardized ophthalmological evaluation of all CS patients.

The other way around, in consideration of the suspected relationship between CSC and endogenous hypercortisolism/overactivity of the HPA-axis, it is important to consider whether CSC patients should be screened for CS. To assess the prevalence of CS in patients with chronic CSC and to assess whether chronic CSC is associated with hyperactivity of the HPA-axis, a systematic

screening for the presence of CS in a large cohort of chronic CSC patients was conducted, using a detailed clinical and biochemical evaluation. In addition, perceived stress was evaluated using validated questionnaires. Results of the patients with CSC were compared to the results of gender-matched healthy subjects (**chapter 3**).

The activity of the HPA-axis as a proxy of exposure to stress and cortisol can be measured with a number of different tests. All tests reflect different aspects of exposure to cortisol. To estimate long-term cortisol exposure, concentrations of cortisol in scalp hair can be measured.[37, 38] Increased hair cortisol concentrations have previously been associated with psychopathology, cardiovascular disease, obesity and metabolic syndrome, and the presence of active Cushing's syndrome.[39-41] Also in patients with progressive keratoconus, another ophthalmological disease, elevated levels of hair cortisol have been reported.[42] In order to investigate the suspected relationship between cortisol and chronic CSC, hair cortisol concentrations in a large cohort of chronic CSC patients were evaluated in **chapter 4**. Data of the patients were compared with data of hair cortisol concentrations obtained from the general population of adult control subjects.

In addition to biochemical stress, psychosocial stress and 'type A' behavioural characteristics have traditionally been associated with CSC.[27, 28, 43-45] It has been hypothesized that type A behaviour might induce increased levels of catecholamines and cortisol,[46-48] leading to a predisposition to CSC. In previous studies, however, a characteristic personality profile could not be identified in patients with CSC.[49] Personality also effects coping behaviour.[50] Several studies with a limited number of subjects have reported an association between severe psychosocial stress and the onset of CSC, especially in CSC patients with poor coping mechanisms.[27, 51, 52] Coping behaviour may be subject to self-management training, however, also psychological morbidity such as apathy and irritability may be a potential point of engagement. **Chapter 5** describes a cross-sectional study aiming to assess maladaptive personality traits (i.e. traits related to type A behavioural pattern), apathy and irritability, and coping strategies in patients with chronic CSC, in order to identify potentially modifiable psychosocial factors in support to the current standard treatment.

PART TWO

Mortality in patients with CD is increased compared to the general population.[16, 53] Whether mortality is also increased in patients that have been considered cured after initial therapy because they were in long-term remission of cortisol excess is vital for proper patient management, both from the physician and patient's perspective. The question whether mortality remains increased after initial cure is also important for risk stratification in order to devise strategies for follow-up, including treatment of comorbidities. Furthermore, data on factors predictive of mortality in the cured CD patient population are rare. **Chapter 6** provides a systematic review and meta-analysis aiming at answering this important question.

Patients with remitted CD often report residual psychopathology and persisting impairments in cognitive and executive functioning, [54, 55] and also the reduction in quality of life is reported to persist despite curation.[56] A cognitive function essential to lead a functional life is the cognitive skill of planning. Cognitive planning encompasses neurological processes involved with strategy formulation, coordination, evaluation, and selection of thought sequences, followed by actions

needed to achieve your goal.[57] Reductions of these cognitive competences in remitted CD patients may lead to persisting impaired planning abilities, affecting psychological status, daily functionality, and quality of life. Whether patients with remitted Cushing's disease demonstrate altered performance and brain activity patterns with regard to cognitive planning and executive functioning compared to healthy controls, is studied in **chapter 7** using the Tower of London paradigm, a task requiring planning of intermediate, in many instances counterintuitive, steps to successfully solve a problem.[58]

The association between CS and hypercoagulability has gained growing interest in recent years. An increased risk of venous thromboembolism, both during active hypercortisolism as well as in the postoperative period and even after remission, has been reported by multiple cohort studies, and was recently confirmed in a meta-analysis.[59-61] The underlying mechanisms of, and contributing factors to the hypercoagulable state in CS patients are yet to be unraveled. The hemostatic abnormalities and coagulation profiles previously reported in CS are heterogeneously affected, with different studies reporting diverse hemostatic abnormalities, and no correlation has been found between the severity of hypercortisolism and hemostatic parameters.[59, 60] To date, no prospective studies have been conducted evaluating the effects of prophylactic anticoagulation on the occurrence of venous thromboembolism in patients with CS, and therefore evidence-based guidelines on thromboprophylaxis management in this patient category are lacking.[62] The study described in **chapter 8** aimed to map the current clinical practice for thromboprophylaxis strategies in patients with CS across reference centers of the European Reference Network on Rare Endocrine Conditions, using a survey tool.

In the pursuit of achieving remission, transsphenoidal surgery is the first line treatment in CD. Both remission and complications determine the success of the procedure, as failure to normalize cortisol levels is potentially life threatening,[15] whereas potential complications such as pituitary deficiencies are also associated with comorbidities and reduced quality of life. [20-22, 63] A complicating factor in outcome evaluations of pituitary surgery in CD is the multitude of endocrine evaluations and tests, differences in defining normal test results, and particularly the position of hypocortisolism (the way hypocortisolism is taken into account in outcome evaluations), as well as by what means multiple interventions to achieve the optimal goal are assessed and appreciated. Clinicians will need to deal with discrepant test results in many cases, and ultimately decide on the state of disease. The judgement whether remission or recurrence is present will be based on integrating the (re-)occurrence of clinical signs and symptoms, and the results of subsequent testing. These considerations are well-adapted in clinical decision making, however in registries and outcome studies the definitions of disease state are quite heterogenous and difficult to compare and interpret. Outcome Squares are developed to report multidimensional outcome measures, including both remission and complications, in a standard classification in order to unify different outcome parameters in time, while taking the delicate balance between efficacy and safety into account.[64] **Chapter 9** reports on long-term integrated postoperative follow-up measures in patients with Cushing's disease using the Outcome Squares approach.

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PART I

PATHOPHYSIOLOGY OF STRESS-RELATED DISEASES – RARE DISEASES AS A MODEL FOR STRESS VULNERABILITY

CHAPTER 2

CENTRAL SEROUS CHORIORETINOPATHY IN ACTIVE ENDOGENOUS CUSHING'S SYNDROME

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ABSTRACT

Multiple case series have provided evidence for a relatively high incidence of central serous chorioretinopathy (CSC) in patients with active Cushing's syndrome (CS). We describe the ophthalmological status in detail of consecutive patients with active endogenous CS (either de novo or recurrent active endogenous CS) in this prospective cohort study. All patients underwent complete ophthalmological examination, including multimodal imaging, which was performed shortly after establishing the diagnosis of active CS in hypercortisolemic state. Eleven CS patients (4 men, 7 women) with active hypercortisolism were included. Abnormalities reminiscent of (subclinical) CSC were found in 3 patients. Optical coherence tomography (OCT) revealed macular subretinal fluid in 1 patient, who was diagnosed as having active CSC and was successfully treated with half-dose photodynamic therapy. Two other patients showed CSC-like abnormalities: an unilateral pseudovitelliform lesion on OCT and hyperfluorescent changes on fluorescein angiography in one patient, and unilateral leakage on fluorescein angiography in the other patient. Mean subfoveal choroidal thickness on enhanced depth imaging OCT was 270 ± 40 µm (range, 178 – 357 µm). Retinal abnormalities resembling (subclinical) CSC may be more common than previously thought in patients with active CS, and may exist even in patients without visual complaints. Clinicians should have a low threshold for ophthalmological evaluation in case of a CS patient with visual symptoms since there may be therapeutic opportunities to prevent vision loss.

INTRODUCTION

Cushing's syndrome (CS) is a clinical entity that occurs after prolonged and excessive exposure to glucocorticoids. CS itself as well as comorbidities that are associated with CS result in decreased quality of life and an increased mortality risk[1,2]. CS is most often caused by exogenous use of glucocorticoids, whereas CS due to endogenous hypercortisolism is a rare condition with an estimated incidence of 1.2–2.4/million per year[2]. Patients with endogenous CS present with typical clinical findings, such as facial rounding ('moon face') and flushing (plethora), abdominal striae, muscle weakness, easy bruising, osteoporosis, hypertension, diabetes mellitus, and neuropsychiatric symptoms[3]. CS is most often dependent on pathological adrenocorticotropin (ACTH) secretion by a pituitary adenoma ('Cushing's disease'). However, CS can also be ACTH-independent in case of adrenal autonomous overproduction of cortisol, by an adrenal adenoma and seldom by an adrenal carcinoma[4].

Visual complaints in CS can occur due to central serous chorioretinopathy (CSC), characterized by a (sub) acute accumulation of serous subretinal fluid (SRF) and detachment of the neuroretina. This is presumed to be secondary to damage to the retinal pigment epithelial (RPE) outer blood-retina barrier due to congestion, thickening, and hyperpermeability of the c horoid[5–7]. Up to 5% of CS patients have been described to have had 1 or more episodes of CSC, which all occurred during a hypercortisolemic s tate[8]. As CSC can even be the principal manifestation of previously unrecognized, mildly symptomatic CS, ophthalmologists should have a high index of suspicion for systemic signs of CS that warrant referral to an endocrinologist[6]. Both exogenous glucocorticoid use, independent of the route of administration, and endogenous hypercortisolism have been described to be pronounced risk factors for CSC[6,9–13]. Although the exact pathogenetic mechanism of CSC is currently unclear, it has been hypothesized that hypercortisolism could increase the risk of developing CSC by altering capillary fragility, choroidal coagulation, systemic blood pressure, and/or fibroblastic a ctivity[11,14].

Choroidal hyperpermeability and thickening have been described to occur in both the affected and fellow eyes of CSC patients[5,15]. An increased choroidal thickness has been observed in patients with active endogenous CS, which has also been hypothesized to be a predisposing factor for CSC[16,17]. In a previous study, the assessment of the retina and choroid by means of optical coherence tomography (OCT) revealed retinal abnormalities within the CSC disease spectrum in 2 out of 11 patients[17].

The diagnosis of abnormalities within the CSC spectrum can only be established with multimodal imaging including OCT. We conducted the first study in which ophthalmological imaging was performed in a consecutive series of patients with active endogenous CS without visual complaints, in order to evaluate the potential need of ophthalmological screening of any patient with CS.

METHODS

Patient selection

Between August 2016 and November 2018, all consecutive patients diagnosed with CS on the outpatient clinic of the endocrinology department of our tertiary referral center were found eligible to participate in this study and referral to the ophthalmologist was discussed.

The diagnosis of CS was established based on clinical characteristics and corresponding abnormalities on biochemical evaluation in at least 2 out of 3 currently available screening tests, according to the Endocrine Society guideline[4]. Abnormal test results were defined as: insufficient suppression of cortisol secretion in the morning after 1 mg of dexamethasone the evening before (cortisol > 50 nmol/L), increased midnight saliva cortisol (> 5.7 nmol/L), increased 24-h urinary free cortisol excretion (> 150 nmol/L). Depending on the ACTH status of the patients, the etiology of CS was assessed either by magnetic resonance imaging scan of the brain or computed tomography scan of the adrenals/thorax. Eleven patients could be included in the current study, all with active (either as a first episode of CS or a new episode of CS in case of recurrence of disease), endogenous CS and without visual complaints at the moment of establishing the diagnosis. Five other patients met the inclusion criteria for this study, but could not be evaluated: 3 of these patients could not receive multimodal ophthalmological imaging before surgery due to logistical issues and the need for urgent pituitary surgery, and 2 did not prefer to participate.

The study adhered to the tenets of the Declaration of Helsinki. Both the institutional review board and the Medical Ethics Committee Leiden Den Haag Delft (METC-LDD) of Leiden University Medical Center approved this study (NL50816.058.14). Written informed consent was obtained from all participants.

Ophthalmological evaluation

Complete ophthalmic examination started with an Early Treatment of Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) measurement. Pupils were dilated by using 1% tropicamide and 5% phenylephrine, after which indirect ophthalmoscopy was performed. The obtained ophthalmological imaging consisted of digital color fundus photography (Topcon Corp., Tokyo, Japan), and OCT, enhanced depth imaging (EDI-)OCT of the choroid, fundus autofluorescence (FAF), and oral fluorescein angiography (FA), with a spectral-domain OCT device (Spectralis HRA + OCT; Heidelberg Engineering, Dublin, CA, United States). Subfoveal choroidal thickness (distance from the outer part of the hyperreflective RPE layer to the hyperreflective line of the inner surface of the sclera) was measured on EDI-OCT. Two experienced retina specialists (CJFB and EVD) evaluated the imaging. For oral FA images, 10 ml of 20% fluorescein was administered after a fasting period of at least 3 h, and photos were taken at 10, 15, 20, 25, and 30 min after ingestion.

When abnormal ophthalmological findings were present in patients with CS, patients were invited to the outpatient clinic for the ophthalmological assessment once again, after remission of CS. Moreover, when treatment and/or further follow-up of the ophthalmological situation was required according to the treating ophthalmologist, this was scheduled.

RESULTS

Patient characteristics

Eleven patients (4 males, 7 females) with active CS were included in this study. The mean age of these patients was 52.6 ± 16.0 years (range, 22 – 75 years). CS was caused by Cushing's disease (pituitary adenoma) in 7 patients, a unilateral adrenal adenoma in 2 patients, bilateral adrenal hyperplasia in 1

patient, and ACTH-dependent hypercortisolism of unknown origin (invisible pituitary adenoma or ectopic ACTH production of unknown origin) in 1 other patient. No other diseases or risk factors for which a possible association with CSC has been described, were present in the CS patients.

At the time of ophthalmological imaging, 8 patients were using antihypertensive drugs, for a mean duration of 8.3 ± 9.1 years (range, 0 – 25 years). One patient with persisting active CS with proven hypercortisolism on biochemical testing was still using hydrocortisone coverage in a low, physiological dosage because of concurrent insufficient cortisol peak production following dynamic testing. One other patient reported sporadic use of intranasal corticosteroids because of hay fever, and 1 other patient received an intra-articular shoulder injection with corticosteroids for the treatment of neuropathic pain 3 months prior to the study. None of the patients reported the use of either sildenafil or tadalafil, which has been associated with CSC[18].

The mean duration between the diagnosis of active CS and ophthalmological phenotyping was 8 ± 6 weeks (range, 1 – 25 weeks). At the moment of ophthalmological phenotyping, 9 patients were already scheduled for surgery, but this had not been performed yet. Two other patients received non-surgical treatment, which had not started yet: this concerned 1 patient with a pituitary adenoma scheduled for levo-ketoconazol treatment in a trial setting and 1 patient with bilateral adrenal hyperplasia scheduled for leuporelin injections. Two patients had previously received pituitary surgery. In these patients, the active CS was considered to be a recurrence of the pituitary adenoma.

Furthermore, temporary preoperative treatment with oral medication for the inhibition of endogenous cortisol production was already started in 7 patients. Five patients were on temporary metyrapone treatment for a mean duration of 14 ± 13 days (range, 1 – 37 days) at the day of the visit to the department of Ophthalmology, and 1 other patient had received temporary ketoconazole treatment for 7 days. In another patient pasireotide treatment was started 3 days before ophthalmological evaluation. Biochemical evaluation showed a persistent hypercortisolemic state at the time of ophthalmological evaluation in all of these patients. Clinical characteristics of the patients are summarized in Table 1.

Ophthalmological characteristics

An overview of the ophthalmological characteristics is shown in Table 2. Mean ETDRS BCVA of the 22 eyes was 82.3 ± 9.7 letters (range, 50 – 96 letters), with a mean spherical equivalent of the manifest refraction of 0.8 ± 3.3 diopters (range,-7.5 to + 7 diopters). Mean subfoveal choroidal thickness on EDI-OCT was 270 ± 40 µm (range, 178 – 357 µm), and no evident pachyvessels were seen in any of the patients.

In 3 out of the 11 patients CSC or CSC-like changes reminiscent of chronic CSC were observed (Figure 1). In one of these patients, which had a history of grade 4 hypertensive retinopathy, SRF was observed on OCT in the right eye, accompanied with RPE alterations, outer retinal changes, and a flat irregular RPE detachment with midreflective accumulation beneath it, which raised the suspicion of a flat type 1 subretinal neovascularization below the RPE. In this patient, 2 parafoveal 'hot spots' of leakage were observed on FA, with corresponding hyper-fluorescence

Table 1. Clinical characteristics of patients with active Cushing's syndrome.

Patient	Age	Sex	Presenting symptoms	Urinary free cortisol at diagnosis (xULN)	Type CS	Blood pressure (mmHg)	Received treatment at first visit outpatient clinic ophthalmology	Medication first visit outpatient clinic ophthalmology
1	63	M	Central adiposity, hematomas	2–3	Pituitary adenoma	152/87	-	Clopidogrel, pravastatin
2	58	F	Central adiposity, diabetes, hematomas, hirsutism, hypertension, muscle weakness	13–16	Pituitary adenoma	160/90	Metyrapone (3 days)	Atenolol, cotrimoxazol, digoxin, losartan, metformin, metyrapone, pantoprazole, phenprocoumon, potassium chloride, spironolactone
3	59	M	Central adiposity, diabetes, fatigue, hematomas, hypertension, psychosocial complaints	Cannot be interpreted due to medication use	Pituitary adenoma (recurrence of disease)	110/75	-	Calcium carbonate, carbasalate calcium, hydrocortisone, levothyroxine, metformin, metoprolol, oxazepam, paracetamol, pravastatin, testosterone
4	41	F	Central adiposity, fatigue, hematomas, hypertension, muscle weakness, psychosocial complaints	2–4	ACTH-dependent hypercortisolism with negative pituitary surgery	145/95	Metyrapone (1 day)	Labetalol, methyldopa, metyrapone, nifedipine, omeprazol, oxazepam
5	50	M	Central adiposity, hypertension, insomnia	2–6	Pituitary adenoma (recurrence of disease)	144/94	Pasireotide (3 days)	Levothyroxine, pasireotide, testosterone
6	66	F	Central adiposity, fatigue, hypertension, insomnia	1.5	Bilateral adrenal hyperplasia	150/100	-	Amlodipin, furosemide, levothyroxine, losartan, propranolol
7	22	F	Central adiposity, hypertension, oligomenorrhoe, psychosocial complaints	26	Pituitary adenoma	130/90	Metyrapone (37 days)	Amlodipin, cotrimoxazole, metyrapone, spironolactone

Table 1. continued.

Patient	Age	Sex	Presenting symptoms	Urinary free cortisol at diagnosis (xULN)	Type CS	Blood pressure (mmHg)	Received treatment at first visit outpatient clinic ophthalmology	Medication first visit outpatient clinic ophthalmology
8	25	F	Amenorrhoe, central adiposity, fatigue, hematomas, psychosocial complaints	5–6	Pituitary adenoma	120/80	Metyrapone (19 days)	Metyrapone
9	75	F	Central adiposity, hematomas, insomnia	1.5–3.5	Pituitary adenoma	140/80	-	Alendronic acid, cholecalciferol, rivaroxaban
10	59	F	Central adiposity, fatigue, hypertension, muscle weakness, psychosocial complaints	2	Adrenal adenoma	165/87	Ketoconazole (7 days)	Amlodipin, chole-calciferol, gliclazide, hydrochlorothiazide, ketoconazole, levothyroxine, metformin, nebivolol, rosuvastatin
11	61	M	Hematomas, hypertension, muscle weakness, psychosocial complaints	2.5–3	Adrenal adenoma	150/90	Metyrapone (10 days)	Atorvastatin, cotrimoxazol, metformin, metoprolol, metyrapone, perindopril, spironolactone, tamsulosin

ACTH adrenocorticotropic hormoneadrenocorticotropic hormone, *CS* Cushing's syndrome, x*ULN* x upper limit of normal.

Table 2. Ophthalmological characteristics of patients with active Cushing's syndrome.

Patient	Steroid use	Chorioretinal disease history	BCVA OD	BCVA OS	SPH EQ OD	SPH EQ OS	OCT OD
1*	–	–	94	90	0	+ 0.50	Parafoveal pseudovitelliform lesions, RPE alterations
2*	–	–	82	82	+ 1.00	+ 1.25	–
3*	HC (2012—cur-rent)	Central retinal vein occlusion OD (2012), grade 2 hypertensive retinopathy ODS (2015)	91	91	+ 1.25	+ 1.00	–
4*	–	–	74	80	−7.50	−6.50	–
5	HC (1994—4 months before visit)	Epiretinal membrane, myelinated fibers ODS, congenital hypertrophy of RPE OD	50	83	+ 7.00	+ 5.50	Epiretinal membrane
6*	–	Grade 4 hypertensive retinopathy ODS (2017)	75	78	+ 5.75	+ 5.00	SRF, flat irregular RPE detachment with midreflective accumulation beneath it, outer retinal changes, RPE alterations
7	–	–	93	96	+ 0.50	−0.25	–
8	Intranasal (2017—current)	–	88	88	0	+ 0.25	–
9	–	Dry age-related macular degeneration	74	78	+ 2.25	+ 2.00	Age-related drusen
10	Intra-articular injection (3 months before visit)	–	85	82	+ 1.00	+ 1.00	–
11	–	–	73	83	−1.75	−1.50	–

* = 2 or more visits. *BCVA* best-corrected visual acuity, *CHRPE* congenital hypertrophy of the retinal pigment epithelium, *CRVO* central retinal vein occlusion, *CT* subfoveal choroidal thickness, *FA* fluorescein angiography, *FAF* fundus autofluorescence, *HC* hydrocortisone,

on indocyanine green angiography, which was obtained for diagnostic purposes, and showed neither a clear neovascularization nor signs of polypoidal choroidal vasculopathy (aneurysmal type 1 neovascularization) (Figure 1A–E). This patient was diagnosed with CSC and treated with half-dose photodynamic therapy (PDT), which initially resulted in a complete resolution of SRF (Figure 1F). Half-dose PDT treatment was given 5 months after the start of leuproreline injections which was prescribed to treat the CS. Approximately 3 months after halfdose PDT, intraretinal fluid occurred (Figure 1G), which resolved approximately 3 months after treatment with anti-vascular endothelial growth factor injections (Figure 1H). Interestingly, no abnormalities were seen in the left eye of this patient on any of the imaging modalities (imaging not shown). The second patient showed

CT OD (µm)	OCT OS	CT OS (µm)	FAF OD	FAF OS	FA OD	FA OS
301	–	317	HAF changes	–	HF lesions	–
271	–	178	–	–	–	–
215	RPE alterations	257	–	–	Subtle HF lesions	Subtle HF lesions
232	–	286	–	–	–	–
–	Epiretinal mem-brane	296	Myelinated fibers	Myelinated fibers	CHRPE, myelinated fibers	Myelinated fibers
357	–	335	HAF changes	–	Two para-foveal 'hot spots' of leakage	–
242	–	257	–	–	–	–
259	–	242	–	–	–	–
263	Age-related drusen	257	–	–	–	–
299	–	277	–	–	–	–
250	–	291	HAF area	–	Single 'hot spot' of leakage	–

HF hyperfluorescent, *HAF* hyperautofluorescent, *OCT* optical coherence tomography, *OD* right eye, *OS* left eye, *RPE* retinal pigment epithelium, *SPH EQ* spherical equivalent of the manifest refraction, *SRF* subretinal fluid.

RPE alterations and parafoveal pseudovitelliform lesions in the right eye with corresponding hyperfluorescent changes on FA and FAF (Figure 1I–L). For this patient 2 more follow-up visits were scheduled 3 months and 12 months after the start of levoketoconazole as treatment for CS, during which no changes were observed (Figure 1M). The third patient showed a single 'hot spot' of leakage on FA in the right eye with a corresponding hyperautofluorescent area on FAF (Figure 1N–Q). This patient preferred not to receive follow-up for this study.

In 8 out of the 11 patients, no abnormalities reminiscent of diseases within the CSC spectrum were found on multimodal imaging. One of the patients within this group was diagnosed elsewhere with central retinal vein occlusion in the right eye and grade 2 hypertensive retinopathy, 3–5 years before

ophthalmological screening in the context of the current study. This patient showed RPE alterations and subtle hyperfluorescent changes on FA, which was considered to be characteristic of chronic arterial hypertension (Figure 2A–D). These changes showed to be stable over time at a second visit within the current study. For 2 other patients within this group a second visit was scheduled as well, but also for these patients no changes were observed compared to the first visit (Table 2). Another patient showed myelinated nerve fibers and an epiretinal membrane in both eyes, together with congenital hypertrophy of the RPE in the right eye (Figure 2E–H). A patient showing drusen formation in both eyes on OCT was previously diagnosed with dry age-related macular degeneration (Figure 2I–L). Five patients within this group preferred not to receive follow-up for this study.

DISCUSSION

To the best of our knowledge, this is the first study in which complete ophthalmological examination has been performed in a consecutive series of CS patients with active disease. Ophthalmological changes could be detected in 3 of the 11 included patients without visual complaints. One of the CS patients was diagnosed with CSC based on unilateral SRF accumulation accompanied by corresponding hyperfluorescent changes on FA and indocyanine green angiography. This patient was initially successfully treated with half-dose PDT (which was performed 5 months after leuproreline injections were started to treat CS), which resulted in complete resolution of SRF. Three months after half-dose PDT, intraretinal fluid occurred, which was successfully treated with anti-vascular endothelial growth factor injections. Together with this intraretinal fluid a flat irregular RPE detachment with a midreflective accumulation beneath it was observed, suggesting that RPE detachments in patients with active CS could be rather fibrous than serous. It could, however, be hypothesized that the normalization of cortisol levels may also have contributed the successful treatment of the ocular pathology[6]. In 2 other patients hyperfluorescent changes on FA reminiscent of a 'hot spot' of leakage as typically seen in CSC patients could be observed, together with unilateral pseudovitelliform lesions in one of these subjects. Interestingly, posterior subcapsular cataract, a rare ocular manifestation of CS[19], was not observed in this study.

CSC is a poorly understood chorioretinal disease, for which corticosteroid exposure has been found to be the most pronounced risk factor[9,12,20,21]. It has been hypothesized that this exposure only leads to characteristic chorioretinal abnormalities in patients with a certain disease susceptibility[22,23]. The results of our study confirm that endogenous hypercortisolism may indeed trigger the occurrence of ophthalmological changes within the CSC spectrum. As findings within this spectrum were only observed in the minority of the patients who developed extraordinary hypercortisolaemia as observed in CS, this study emphasizes that a local ocular susceptibility for developing CSC may play a role, increasing the risk of developing CSC upon exposure to corticosteroids[24]. This is supported by findings on multimodal imaging of CSC patients, which demonstrates that choroidal abnormalities on OCT (pachychoroid and pachyvessels) and indocyanine green angiography (choroidal stasis and hyperpermeability) frequently occur bilaterally, whereas actual accumulation of SRF and active leakage through the RPE on FA occur unilaterally in most cases[14,20,25,26]. Additionally, previous reports have found that even topical use of exogenous corticosteroids can trigger CSC[9,27],

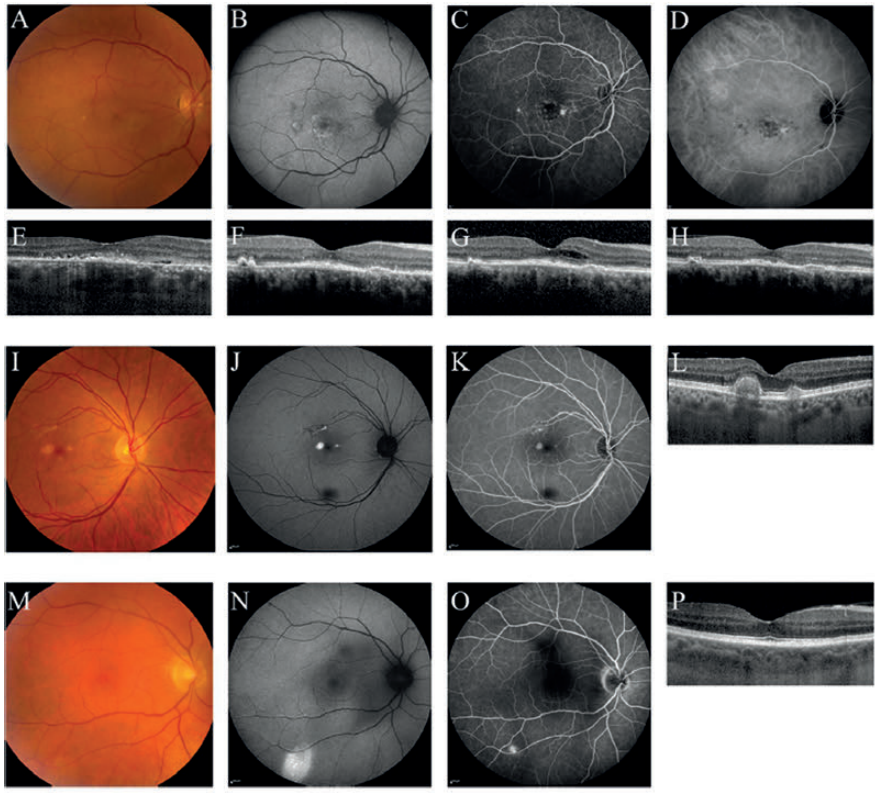

Figure 1. Multimodal imaging. (**A**–**E**) of the right eye of a 66-year-old female patient with active Cushing's syndrome. The optical coherence tomography (OCT) scan (**E**) revealed a small amount of subretinal fluid (SRF) with hyperreflective debris. Fundus autofluorescence (**B**) mainly showed hyperautofluorescent changes macularly. On the mid-phase fluorescein angiography (**C**) 2 'hot spots' of leakage were observed, nasally and temporally to the fovea. Mid-phase indocyanine green angiography (**D**) at that time did not provide evidence for a neovascularization. In the left eye of this patient, no abnormalities were detected on multimodal imaging (not shown). As SRF persisted after 6 months of leuprorelin treatment for Cushing's syndrome, half-dose photodynamic therapy was performed, after which the SRF almost completely resolved (**F**). However, 3 months later intraretinal fluid occurred (**G**), and intravitreal injections with bevacizumab were scheduled. After 3 initial injections once per month, a treat-and-extend protocol was used. Eight weeks after the last bevacizumab injection until to date, SRF and intraretinal fluid on OCT had disappeared (**H**). Unfortunately, visual acuity did not improve. Multimodal imaging of the right eye of a 63-year-old male with active Cushing's syndrome (**I**–**M**). The foveal OCT scan (**L**) showed retinal pigment epithelium alterations and parafoveal pseudovitelliform lesions. Corresponding hyperautofluorescent abnormalities were observed on fundus autofluorescence (**J**) and mid-phase fluorescein angiography (**K**). The abnormalities observed on multimodal imaging showed to be stable during follow-up, as can be seen on the OCT scan that was obtained at the last follow-up visit at 12 months after baseline (**M**). No abnormalities were observed in the left eye of this patient (imaging not shown). Multimodal imaging (**N**–**Q**) of the right eye of a 61-year-old male with active Cushing's syndrome. No abnormalities were seen on the OCT scan (**Q**). Mid-phase fluorescein angiography (**P**) showed a 'hot spot' of focal leakage, with an eccentric location outside the vascular arcade, and on fundus autofluorescence (**O**) hyperautofluorescent changes were observed in the same area. Unfortunately, this area was outside the covering area of the OCT scan, so possible presence of SRF could not be evaluated. No abnormalities were observed in the left eye of this patient (imaging not shown).

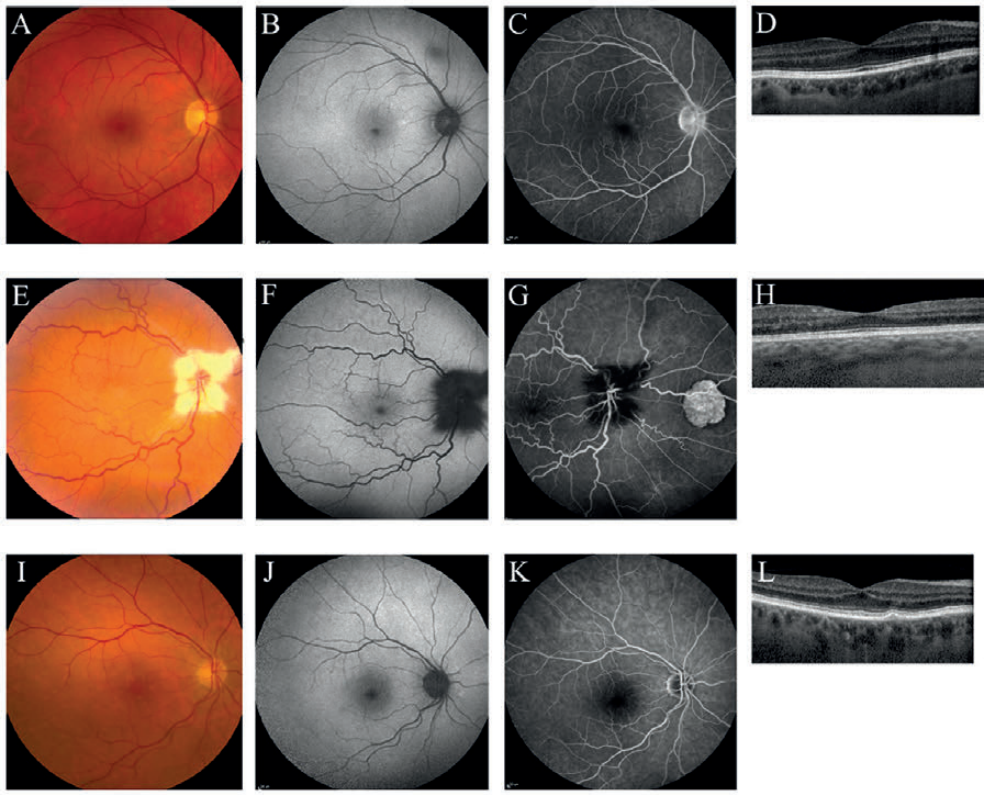

Figure 2. Multimodal imaging of ocular abnormalities not attributed to the spectrum of central serous chorioretinopathy. A 58-year-old male patient with Cushing's syndrome due to a pituitary adenoma and a history of central retinal vein occlusion in the right eye (5 years ago) and grade 2 hypertensive retinopathy (3 years ago) showed mild parafoveal hyperfluorescent changes on fluorescein angiography (**C**). No abnormalities were observed on fundus photography (**A**), fundus autofluorescence imaging (**B**), and the foveal optical coherence tomography (OCT) scan (**D**). Furthermore, no abnormalities were found in the left eye on any of the imaging modalities (imaging not shown). The observed abnormalities in the right eye were stable after 1 year of follow-up after the first visit (imaging not shown). A 50-year-old male patient with Cushing's syndrome due to a pituitary adenoma, who had a history of amblyopia in the right eye. Ophthalmological examination showed myelinated fibers which were present in both eyes (**E**–**G**). Furthermore, on fluorescein angiography (**G**) congenital hypertrophy of the retinal pigment epithelium was visible in the right eye. Due to amblyopia in the right eye, an OCT scan of sufficient quality could not be obtained. Therefore, an OCT of the left eye is shown, on which no abnormalities were observed (**H**). Multimodal imaging of the right eye a 75-year-old female with Cushing's syndrome due to an adrenal adenoma, which showed drusen on fundus photography (**I**), with only very mild changes on fundus autofluorescence imaging (**J**), mid-phase fluorescein angiography (**K**), and the foveal OCT scan (**L**). These observations were similar in the left eye (imaging not shown).

indicating 'hypersensitivity' of some patients to develop CSC in response to modest amounts of exogenous corticosteroids.

Our findings are partially in line with the findings from a cross-sectional study in which 11 patients with active CS and 12 healthy controls were included, and in which bilateral macular

changes characteristic for (subclinical) CSC on OCT were seen in 1 patient[17]. In contrast with our study, a significantly increased choroidal thickness compared to healthy subjects could be detected in these 11 patients[17]. Moreover, episodes of hypercortisolaemia in CS patients have previously been linked to CSC by Bouzas et al., who included 60 CS patients, out of whom 3 cases developed hypercortisolaemia-dependant episodes of CSC. In contrast with our study, this study also included patients that had no active disease, which might explain the fact that we have found a higher percentage of patients with abnormalities within the CSC spectrum[8].

In our study, we only included patients with active CS and performed multimodal ophthalmological imaging in all patients, which is a particular strength of the study. Additionally, 5 of the 11 patients visited the outpatient clinic at least 2 times. There are several limitations in our study. Importantly, our sample size is limited, which is mostly due to the rare incidence of CS. Another limitation of our study might be that 6 patients were already receiving treatment at the moment of ophthalmological phenotyping in an attempt to control endogenous cortisol production. Due to logistics with scheduling visits to the outpatient clinics, patients were on medical pretreatment for varying periods of time. Because of the seriousness of the disease, it was considered not ethical to withhold this treatment before the ophthalmological examinations. However, only in 3 patients this treatment was prescribed for more than 7 days, and in all patients biochemical analysis still showed hypercortisolism at the time of ophthalmological evaluation.. This is in line with a previous study showing that cortisol lowering agents most often do not lead to complete normalization of h ypercortisolaemia[28]. We therefore assume that it is unlikely that the medical pretreatment has influenced the outcome of our study. Albeit, if it had any effect, it could only have lowered the incidence of CSC(-like) abnormalities found in this study. Furthermore, our study included CS patients with a mixed origin of hypercortisolaemia. However, we expect that this will not have influenced the outcome of our study, since hypercortisolaemia, which is considered to be the most important risk factor for developing CSC, was present in all included patients. Notwithstanding, although there is a common trunk of hypercortisolism in CS, it is very interesting to better understand the origin of CS on CSC by studying a larger cohort – probably multicenter. This would also allow for a more comprehensive analysis of risk factors associated with CSC such as gender, or manifestations of CS such as neuropsychiatric symptoms or biochemical (hormonal) abnormalities such as A CTH[29].

The relationship between CS and CSC is relatively well-known[12]. CSC is a relatively rare disease, for which an incidence of 9.9 per 100,000 for males and 1.7 per 100,000 for females has been described in a retrospective cohort study[30]. Of note, our study included 4 male CS patients and 7 female CS patients and we observed CSC(like) abnormalities in 2 males and 1 female, despite the fact that CS occurs 5 times more often in f emales[29]. The incidence of CSC(-like) ophthalmological changes, which occurred in 3 out of the 11 CS patients in our study, may suggest that (subclinical) CSC is more likely to occur in CS patients compared to the general population. Furthermore, CSC has previously been shown to occasionally be the presenting symptom of CS[6]. Moreover, CS surgery has been observed to result in complete and persistent resolution of SRF[6]. However, in a systematic cross-sectional study that recently evaluated 86 CSC patients for presence of CS, we previously did not find any cases of (subclinical) C S[31]. Collectively, it might be of clinical importance to screen CS patients

for pathology within the CSC spectrum on a regular basis. In the current study, we found active CSC with unilateral SRF in 1 patient without visual complaints, who required treatment. Moreover, abnormalities reminiscent of (subclinical) CSC were observed in 2 other patients. Patients with active CS should therefore be actively questioned about the presence of ophthalmological symptoms and referred to an ophthalmologist if such symptoms are present. Further studies are needed to assess whether all patients presenting with CS should be referred for an ophthalmological screening.

In conclusion, findings suggestive of (subclinical) CSC and other ocular fundus abnormalities were detected in a noteworthy percentage of patients with active CS without visual complaints. Ophthalmological screening including multimodal imaging may be indicated in recently diagnosed patients with (active) CS. However, additional studies on the prevalence of subclinical CSC in CS and the natural course of these abnormalities (also after CS treatment) are required to determine whether standard ophthalmological screening of newly diagnosed CS patients should be incorporated in the clinical work-up of patients diagnosed with CS.

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CHAPTER 3

CUSHING'S SYNDROME AND HYPOTHALAMIC–PITUITARY–ADRENAL AXIS HYPERACTIVITY IN CHRONIC CENTRAL SEROUS CHORIORETINOPATHY

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ABSTRACT

Objective

Central serous chorioretinopathy (CSC), a specific form of macular degeneration, has been reported as presenting manifestation of Cushing's syndrome. Furthermore, CSC has been associated with both exogenous hypercortisolism and endogenous Cushing's syndrome. It is important to know whether CSC patients should be screened for Cushing's syndrome. Although hypothalamic-pituitary-adrenal (HPA) axis hyperactivity in CSC has been suggested, no detailed evaluation of the HPA axis has been performed in a large cohort of CSC patients. This study aimed to investigate whether Cushing's syndrome prevalence is increased among chronic CSC (cCSC) patients and whether detailed endocrinological phenotyping indicates hyperactivity of the HPA axis.

Design

Cross-sectional study.

Patients

86 cCSC patients and 24 controls.

Measurements

Prevalence of Cushing's syndrome, HPA axis activity.

Results

None of the cCSC patients met the clinical or biochemical criteria of Cushing's syndrome. However, compared to controls, HPA axis activity was increased in cCSC patients, reflected by higher 24 h urinary free cortisol, and accompanying higher waist circumference and diastolic blood pressure, whereas circadian cortisol rhythm and feedback were not different. Chronic CSC patients did not report more stress or stress-related problems on questionnaires.

Conclusion

No case of Cushing's syndrome was revealed in a large cohort of cCSC patients. Therefore, we advise against screening for Cushing's syndrome in CSC patients, unless additional clinical features are present. However, our results indicate that cCSC is associated with hyperactivity of the HPA axis, albeit not accompanied with perception of more psychosocial stress.

INTRODUCTION

Cushing's syndrome is a rare disease characterized by excessive exposure to cortisol and is associated with both metabolic and behavioral abnormalities. The clinical manifestation may vary, and in addition to well-known features like facial rounding, truncal obesity, and dorsal fat pad [1], ophthalmological abnormalities also occur. We recently reported patients who developed visual symptoms caused by chronic central serous chorioretinopathy (cCSC) as presenting manifestation of Cushing's syndrome [2].

Central serous chorioretinopathy (CSC) is a relatively common eye disease often affecting the macula, in which choroidal congestion, thickening, and hyperpermeability lead to retinal pigment epithelial damage and cause serous subretinal fluid accumulation. Persistent neuroretinal detachments in untreated cCSC may result in irreversible photoreceptor damage, which may lead to permanent visual loss and decreased quality of life [3, 4].

The association of CSC with both exogenous steroids and endogenous hypercortisolism has been reported [2, 5, 6]. Although no data are available on the prevalence of CSC in patients treated with corticosteroids, up to 52% of CSC patients in different cohorts reported to use steroids during the active phase of disease [6, 7]. Higher endogenous cortisol levels were reported in 30 patients with acute CSC [8], and 24 h urinary free cortisol (UFC) was higher among 16 patients with chronic CSC compared to controls [9]. However, clinical characteristics, circadian tests, and cortisol feedback were not included in these studies, making it impossible to conclude on the prevalence of Cushing's syndrome.

In addition, psychosocial stress has been described in relation to CSC. Different studies reported associations between psychosocial stressful events and CSC, especially in patients with poor coping mechanisms [10]. People with type A personality characteristics have been suggested to be at higher risk for the development of CSC [11].

In view of the suspected relationship between overactivity of the hypothalamic–pituitary–adrenal (HPA) axis and CSC, a relevant question is whether CSC patients should be screened for Cushing's syndrome. Therefore, we conducted a systematic screening for the presence of Cushing's syndrome in a large cohort of cCSC patients, using detailed clinical and biochemical evaluation of the HPA axis, and compared the latter to a control group. Furthermore, perceived stress was evaluated using validated questionnaires.

MATERIALS AND METHODS

Study Design

Cross-sectional study with the following key objectives: to assess the prevalence of Cushing's syndrome in cCSC patients and to assess whether cCSC is associated with hyperactivity of the HPA axis. If this second aim was confirmed, we aimed to explore the association between HPA axis hyperactivity and psychosocial stress in cCSC.

Study Population

Eighty-six consecutive cCSC patients, who were followed at the Department of Ophthalmology at our tertiary referral center, were screened. The cCSC diagnosis had been confirmed by fundoscopy, digital color fundus photography (Topcon Corp., Tokyo, Japan), fundus autofluorescence (Spectralis HRA + OCT; Heidelberg Engineering, Heidelberg, Germany), spectral-domain optical coherence tomography (Spectralis HRA + OCT), fluorescein angiography (Spectralis HRA + OCT), and indocyanine green angiography (Spectralis HRA + OCT), according to current standard [5, 12–16]. Patients diagnosed with acute CSC (focal leakage spot or a smokestack pattern on fluorescein angiography) were excluded [5, 12–16]. No evidence of other retinal diagnoses had to be present.

Other exclusion criteria possibly affecting the evaluation of the HPA axis were use of corticosteroids/sleep medication prior to the development or during the time course of cCSC, excessive alcohol intake (>21 U/week), nightshift work, or traveling from another time zone in the 6 weeks before evaluation.

We also performed tests for hypercortisolism in a set of gender-matched controls. Thirty-eight healthy subjects responded to advertisements. Fourteen were excluded based on criteria described below. A total of 24 healthy gender-matched control subjects were eligible for inclusion (inclusion period: September 2015 to December 2016). Exclusion criteria were (familial) history of eye diseases/visual problems, psychiatric diseases, or chronic physical diseases possibly influencing endocrinological screening, corticosteroids/antidepressants/sleep medication use, excessive alcohol intake (>21 U/week), recent weight loss/gain of >10%, and working nightshifts or traveling from another time zone in the 6 weeks before evaluation.

Written informed consent was obtained from all participants and approval of the institutional review board and the ethics committee was obtained (NL50816.058.14).

Endocrinological Evaluation

Screening was performed including a detailed medical history, complete physical examination, and biochemical analysis. The physical examination consisted among others of the evaluation of clinical Cushing stigmata and was performed by two physicians (FH/MB).

For evaluating HPA axis activity, all three commonly available screening tests were performed: UFC in two 24 h urine samples, midnight salivary cortisol (mSC), and 1 mg dexamethasone overnight suppression test. Healthy controls were subjected to one 24 h urine and one midnight saliva collection only. In case of deviant test results, participants were re-tested to exclude relevant pathology. The first test results were included in the analysis. UFC (82 patients and all controls) was analyzed using an in-house LC-tandem MS method, calibrated using Cerilliant certified reference material C-106, cortisol 1 mg/ml in methanol. The analytical variation was between 6.5 and 5% for urine cortisol levels between 50 and 900 nmol/L. Cortisol levels below 150 nmol/24 h were considered normal. Serum (81 patients) and salivary cortisol (82 patients and 23 controls) were analyzed using a Roche ECLIA Cortisol assay (second generation) on a Modular E170 immunoanalyser (Roche Holding AG, Basel, Switzerland). Analytical variation ranged between 10.1 and 1.9% for serum cortisol levels between 3.6 and 1,660 nmol/L and between 14.2 and 2.5% for saliva cortisol levels between

2.6 and 78 nmol/L. Cortisol levels below 1.5 nmol/L could not be determined. In midnight saliva, cortisol levels below 5.7 nmol/L were considered normal. The cutoff limit for the dexamethasone suppression test was 50 nmol/L [17].

Questionnaires

Perceived Stress Scale (PSS)

The PSS developed by Cohen et al. was designed to measure the intensity of perceived stress and considers the degree to which individuals experience their lives as unpredictable, uncontrollable, and overloading [18]. The original scale contained 14 items, but its creators refined it to 10 items, of which four are positively and six are negatively phrased [19]. Items are coded from 0 to 4 and summed to compute a total score. Higher scores indicate greater perceived stress. Scores around 13 on the PSS are considered average, whereas high stress groups have reported scores of approximately 20 points [19].

Stress Thermometer

A visual analog scale was designed by the authors to measure the amount of stress experienced in the week before evaluation. Individuals rate their amount of stress on a scale from 0 to 10, with 0 indicating "no stress at all" and 10 indicating "the highest possible amount of stress."

Insomnia Severity Index

This seven-item scale assesses self-reports of insomnia symptoms over the last 2 weeks. The items are scored on a scale from 0 to 4. Total scores of 0 to 7 are categorized as "no insomnia," scores from 8 to 14 are considered to indicate "sub-threshold insomnia," scores from 15 to 21 are indicative of "moderate insomnia," and scores from 22 to 28 are considered "severe insomnia" [20].

Brugha Questionnaire on Life Events

This list to assess the occurrence of stressful events includes 12 life events that were found to have long-term negative effects on most people who experience them. Participants indicate whether certain events have occurred to them during the past year or earlier in their lives [21].

Statistical Analysis

Based on data derived from a recent study by Aranda and colleagues [22], a power calculation was performed on the difference in 24 h UFC deemed relevant to detect (20 nmol/24 h). To detect such a difference (with power 80% and alpha 0.05), a sample size of 54 cCSC patients and 18 healthy controls would suffice.

Data were analyzed using SPSS Statistics (version 23; IBM Corp., Armonk, NY, USA). Data were presented as mean and SD, unless mentioned otherwise. The primary analyses comprised: (1) prevalence of Cushing's syndrome in cCSC and (2) comparison of biochemical results between cCSC patients and healthy controls. Because the majority of cCSC patients were males (in line with other

cohorts described in literature), a male-only sensitivity analysis was performed. Mean and SD scores for each questionnaire were calculated.

Normality of data was tested using the Shapiro–Wilk test. All normally distributed data were analyzed using independent sample t-tests. Data with a non-normal distribution were analyzed by means of nonparametric independent sample tests. The two groups were compared using a general linear model, correcting for potential confounders such as age, waist–hip ratio, and waist circumference. Associations were assessed using linear regression analyzes. The level of significance was set at P = 0.05. For the analysis of the questionnaires, the level of significance was set at P = 0.01 to correct for multiple testing.

After reassessment of the retinal imaging by two independent ophthalmologists, five patients considered to have less typical cCSC findings on imaging were excluded from analysis. Moreover, an analysis excluding outliers (n = 1) was performed. All results are described below.

RESULTS

Baseline Characteristics

Eighty-six cCSC patients (77 males) and 24 healthy controls (19 males) were included (Table 1). The gender distribution was in line with available literature [5, 15]. The mean duration of disease at the time of evaluation was 3.86 years (range 0.17–37.06). Fifty-eight patients had active CSC (presence of subretinal fluid) at the moment of screening.

There was no difference in gender distribution or body mass index between the two groups. Patients were 7.5 years older than controls.

Clinical Evaluation

None of the cCSC patients presented with a combination of clinical signs and symptoms typical for Cushing's syndrome. Hypertension was reported by 27% of patients and one control (4%, P = 0.023). In addition, cCSC patients had a higher prevalence of other comorbidities, e.g., dyslipidemia and psychiatric disorders (see Table 1). Waist circumference, waist–hip ratio, and diastolic blood pressure were higher in patients compared to controls, despite a higher prevalence of ongoing antihypertensive medication use in the patient group. These differences remained significant after adjustment for age. Characteristic Cushing features were rare among cCSC patients.

Hormonal Evaluation

Clinical Evaluation of Patients

None of the cCSC patients had Cushing's syndrome, but several patients demonstrated an abnormally high cortisol value in one or more of the screening tests (Table 2). Increased UFC (>150 nmol/24 h, average of two portions) was present in seven patients, in whom repeated testing revealed normal values. Increased mSC levels (>5.7 nmol/L, average of two portions) was observed in three patients, which normalized upon retesting in two and persisted to be slightly elevated in one patient. Insufficient suppression after dexamethasone was observed in four patients. Retesting revealed normal test results in one patient. In the absence of other biochemical and clinical features

Table 1. Clinical characteristics of participants.

	cCSC patients *n*=86	Controls *n*=24	*P* value
Age, yr.	48.74 (10.84)	41.08 (13.08)	0.004
Sex, male / female	77 / 9	19 / 5	0.182
Duration of cCSC disease, yr. (range)	3.86 (0.17 - 37.06)	-	-
History of hypertension, n (%)	23 (26.7%)	1 (4.2%)	0.023[a]
History of diabetes mellitus, n (%)	6 (7.0%)	0 (0.0%)	0.336
History of dyslipidemia, n (%)	18 (20.9%)	1 (4.2%)	0.068
History of psychiatric disorders[b], n (%)	16 (18.6%)	1 (4.2%)	0.113
History of thromboembolic events, n (%)	0 (0%)	0 (0%)	-
History of cardiac events[c], n (%)	5 (5.9%)	2 (8.3%)	0.648
History of sexual disorders[d], n (%)	19 (22.1%)	1 (4.2%)	0.069
Systolic blood pressure, mmHg	135.41 (16.64)	129.75 (12.41)	0.143
Diastolic blood pressure, mmHg	82.94 (10.30)	77.29 (12.36)	0.006
Body mass index	26.15 (3.59)	24.92 (3.14)	0.096
Waist circumference, cm	92.74 (11.07)	86.42 (9.28)	0.011
Waist-hip ratio	0.95 (0.07)	0.90 (0.06)	0.003
Moon face, n (%)	1 (1.2%)	0 (0.0%)	1.000
Dorsal fat pad, n (%)	1 (1.2%)	0 (0.0%)	1.000
Purple striae, n (%)	0 (0.0%)	0 (0.0%)	-
Muscle weakness, n (%)	3 (3.5%)	0 (0.0%)	1.000
Active skin infections, n (%)	2 (2.3%)	0 (0.0%)	1.000
Hematomas, n (%)	3 (3.5%)	1 (4.2%)	1.000
Ankle oedema, n (%)	2 (2.3%)	0 (0.0%)	1.000

Data are presented as mean (SD) or as numbers, unless specified otherwise.

[a]Not statistically significant after correction for age.

[b]Consisting of depression, anxiety or panic disorder, posttraumatic stress disorder, burn-out, alcohol abuse, and schizophrenia.

[c]Consisting of myocardial infarction, endocarditis, and atrial fibrillation.

[d]Consisting of impotence, hirsutism, menstrual cycle disorders, and loss of libido.

cCSC, chronic central serous chorioretinopathy.

of Cushing's syndrome, we concluded that the abnormal test results in the other patients were likely due to intervening medication (antidepressants, gonadotropin-releasing hormone analogs, covert stimulant use). Furthermore, normal lipid profiles, no elevated inflammation parameters, and no hypokalemia were detected (data not shown).

Comparison with Healthy Controls

Mean UFC levels were higher in cCSC patients, compared to controls (Figure 1). This difference remained after correction for age (P = 0.001), age, and waist–hip ratio (P = 0.002), age and comorbidities (e.g., psychiatric disorders, diabetes mellitus, hypertension, obesity; P = 0.011), and when males were evaluated solely (P = 0.001). Nonparametric analysis revealed that non-detectable mSC was present in 76% of cCSC patients compared to 39% of healthy controls (P = 0.002), with a similar difference in a male-only analysis (72 versus 33%, P = 0.001). Three patients (4%) showed

abnormally elevated mSC, compared to two controls (9%). The other participants' mSC levels were between 1.5 and 5.7 nmol/L.

HPA Axis at Different cCSC Disease Stages

Hypothalamic–pituitary–adrenal axis activity was not different between patients with active and inactive cCSC. Mean UFC levels were 78.44 (SD 38.63) versus 95.30 (SD 64.76), respectively (P = 0.524, Figure 2), and mSC was detectable in 27% of patients with active disease versus 19% of patients with inactive disease (P = 0.386).

The exclusion of five atypical cCSC patients or the exclusion of one outlier in UFC did not affect any of the described results. Clustered analysis did also not significantly change the aforementioned results (data not shown).

Questionnaire Analysis

Perceived Stress Scale

Chronic CSC patients (n = 81, 94%) reported a mean total score of 12.95 (SD 5.82, range 0–30). After correction for multiple testing, no significant difference in PSS score was found between cCSC patients with active disease compared to inactive patients (mean 11.89 versus 15.07, P = 0.019). Furthermore, no association was found between UFC level and the total score in patients (β = 2.04, P = 0.032, R2 = 0.06).

Stress Thermometer

Eighty-three cCSC patients (96%) scored their amount of experienced stress in the week prior to evaluation, reporting a mean score of 4.4 (range 0–10). In addition, no differences were found when active patients were compared to patients with inactive disease, and no association between UFC level and score on this scale was found (β = 0.71, P = 0.742, R2 = 0.001).

Insomnia Severity Index

Total scores were calculated for 83 cCSC patients (mean 6.54, range 0–24). The mean score was categorized as "no clinical significant insomnia." When insomnia was scored as a "yes or no" variable, 11% of patients scored either moderate or severe insomnia. There was no difference in presumed insomnia between active patients and inactive patients, and no association between UFC levels and Insomnia Severity Index scores was found (β = −0.97, P = 0.378, R2 = 0.010).

Brugha Questionnaire on Life Events

Thirty out of 83 patients (36%) reported serious life events in the past year. Twenty-four hour UFCs were not higher in these patients compared to the patients with no serious life event in the preceding year. Disease activity did not affect the report of serious life events in the past year (P = 1.000). The type of life events experienced, however, was different, with active patients reporting more experiences with serious illness or violence of a near relative, whereas inactive patients more often reported the same experiences earlier in live.

Table 2. Biochemical characteristics of participants.

	cCSC patients	Controls	*P* value
Urinary free cortisol, nmol/24 hours[a]	83.99 (49.04)	51.55 (28.49)	0.000
Detectable midnight salivary cortisol[a,b], %	24.4	60.9	0.002
Serum cortisol after 1mg Dexa, mol/L[a]	0.032 (0.047)	-	-
	cCSC patients with active disease	**cCSC patients with inactive disease**	
Urinary free cortisol, nmol/24 hours[a]	78.44 (38.63)	95.30. (64.76)	0.524
Detectable midnight salivary cortisol[a], %	27.3	18.5	0.428
Serum cortisol after 1mg Dexa, mol/L[a]	0.028 (0.045)	0.031 (0.041)	0.855

Data are presented as mean (SD) or as numbers, unless specified otherwise.

[a]Number of participants:

Urinary free cortisol: 82 cCSC patients (55 active cCSC patients, 27 inactive CSC patients) and 24 controls

Midnight salivary cortisol: 82 cCSC patients (55 active cCSC patients, 27 inactive CSC patients) and 23 controls

Serum cortisol after 1mg dexamethasone: 55 active cCSC patients, 26 inactive CSC patients

[b] > 1.5 nmol/L

Abbreviations: cCSC, chronic central serous chorioretinopathy; Dexa, dexamethasone; SD, Standard deviation.

Associations of Questionnaire Outcomes and Cortisol

Analyses performed with exclusion of the five atypical cCSC patients and analyses with exclusion of the UFC outlier did not significantly change the aforementioned results.

DISCUSSION

This is the first study that systematically evaluated various aspects of the activity of the HPA axis in a large cohort of cCSC patients. Whereas we did not find any case of Cushing's syndrome, the activity of the HPA axis appeared to be increased in cCSC patients, without disruption of circadian rhythm. This was reflected by significantly higher 24 h UFCs, increased waist circumference, and diastolic blood pressure, but normal mSC levels. Our study demonstrates that systematic screening of all cCSC patients for the presence of Cushing's syndrome is not indicated.

In the present study, we have screened a large patient cohort in detail, combining all currently available biochemical screening tests with a detailed clinical phenotyping. We found significantly higher 24 h UFC levels in cCSC patients, albeit within the normal reference range, with preservation of normal diurnal rhythmicity. Elevated UFC levels have been reported previously in a small cohort of acute CSC patients during hospital admission, when compared to patients with acute retinal detachment [8], and in a small cohort of cCSC patients that were compared to age- and sex-matched controls [9], though data on demographics of these participants were lacking.

In contrast to earlier studies suggesting an association between cCSC and psychosocial stress, we did not find a clear relationship between cCSC activity and stress, based on the results of four stress questionnaires. In addition, no association was found between HPA axis activity and psychosocial stress. A critical evaluation of the available literature does not support a clear association between

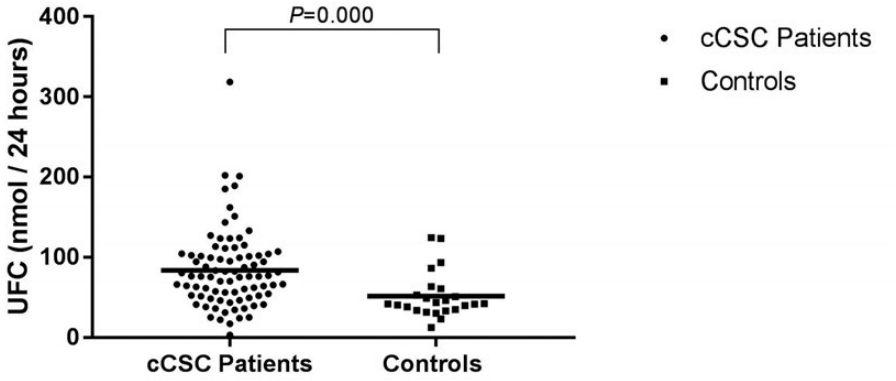

Figure 1. UFC levels in cCSC patients and healthy controls. Data presented as individual values and mean. Patients n = 82; controls n = 24. Abbreviations: UFC, urinary free cortisol; cCSC, chronic central serous chorioretinopathy.

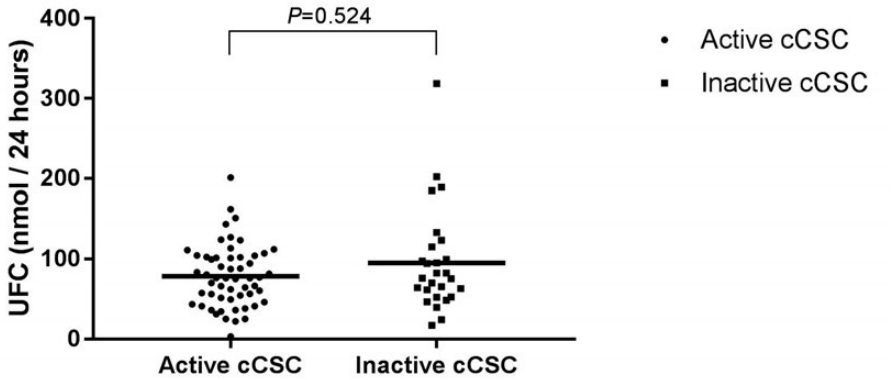

Figure 2. UFC levels in active cCSC patients and inactive cCSC patients.Data presented as individual values and mean. Active cCSC patients n = 55; inactive cCSC patients n = 27. Abbreviations: UFC, urinary free cortisol; cCSC, chronic central serous chorioretinopathy.

cCSC and stress: Conrad et al. demonstrated no increased exposure to critical life events in 30 CSC patients and reported other findings suggestive of difficulties in emotional regulation [23]. Other studies reported an association between stress, severe stressful events, and CSC, especially in patients with poor coping mechanisms [10, 24, 25], but the provided information on how stress was measured was very limited, circumstantial, or even absent. Our patients scores (on the PSS) did not differ from reported average scores and were not comparable with scores reported by high stress groups [19]. In addition, our patients with active cCSC reported no difference in experienced life events, insomnia (as an expression of stress) or perceived stress on two different scales, indicating that cCSC activity is not associated with psychosocial stress.

Both endogenous hypercortisolism and exogenous administration of corticosteroids are related to CSC [2, 5, 6, 9]. Occurrence of one or more episodes of CSC has previously been described in 5% of 60 patients with active endogenous hypercortisolism. All these CSC patients had been

diagnosed with pituitary adenoma [26]. Fundus characteristics resembling CSC have also been reported in patients with Cushing's disease [27]. Moreover, in a patient with hypercortisolism due to adrenocortical carcinoma, bilateral CSC has been found [28]. Several underlying mechanisms have been hypothesized. Endogenous hypercortisolism increases platelet aggregation leading to microthrombi and increased blood viscosity, which could be of importance in the pathogenesis of CSC [29]. Hypercortisolism has also been associated with choroidal fragility and hyperpermeability [30]. Moreover, increased transcription of adrenergic receptors has been correlated with CSC [31]. In addition, a role for the mineralocorticoid pathway has been suggested by recent studies in rats and by findings in CSC patients treated with mineralocorticoid receptor antagonists (eplerenone or spironolactone) [15, 32]. Both glucocorticoids and mineralocorticoids activate the mineralocorticoid receptor expressed on choroidal endothelial cells. Activation of the mineralocorticoid receptor, via upregulation of the endothelial vasodilatory calcium-dependent potassium channel KCa2.3 by hyperpolarization of these endothelial cells and of smooth muscle cells, has been suggested to lead to vasodilation [15].

Our study also has limitations. The cross-sectional character does not allow drawing conclusions on any causal relationship. Furthermore, a reversed causation (cCSC as a trigger for activation of the HPA axis) seems to be less likely in light of the currently available literature, yet is not ruled out. Also, the number of healthy control subjects recruited via advertisements was limited, and because our study was not powered for the questionnaire outcomes, we did not compare patient and control data. Only one 24 h urine sample was collected by healthy controls. Volume and creatinine level analyses confirmed adequate collection of these single samples. The fact that 24 h UFC is higher in the presence of equal results of mSC can very well be explained by an increased activity of the HPA axis with preservation of normal diurnal rhythmicity (in contrast to the "autonomous" cortisol secretion that is characterized by loss of diurnal rhythmicity). The absence of associations between UFC level and either cCSC activity or outcomes of stress questionnaires in our study may appear to be contradictory to the conclusion that the HPA axis is more activated in cCSC patients. Nonetheless, one should keep in mind that there is a wide individual variation in normal cortisol levels and in cortisol receptor activation thresholds, leading to different thresholds for the development of cortisol-related symptoms and pathology. Together, this may explain why the HPA axis could still be activated in cCSC patients despite the absence of an association between UFC and cCSC activity or questionnaire outcomes in our patient population.

Although CSC has been described to be a presenting symptom of Cushing's syndrome and these diseases are known to sporadically co-exist [2], our results argue against screening for endogenous hypercortisolism in all cCSC patients. Since the interpretation of the available biochemical screening tests in light of the clinical features is challenging and in order to minimize the risk of false positive test results, screening should be reserved for those cCSC patients in whom clinical signs or symptoms raise suspicion of Cushing's syndrome. Only then patients should be referred to an endocrinologist for evaluation of the HPA axis. In dealing with Cushing's syndrome patients, endocrinologists also need to be aware of the potential coexistence of CSC.

In conclusion, systematic screening of all patients with cCSC for Cushing's syndrome is not indicated. However, the activity of the HPA axis appears to be increased, with preservation of

circadian rhythm. Finally, in contrast to earlier ideas, we did not find obvious associations between cCSC, cCSC activity, and psychosocial stress. The observed hyperactivity of the HPA axis confirms the previously reported association between cortisol and CSC and merits further studies to unravel the underlying pathophysiological mechanisms.

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CHAPTER 4

HAIR CORTISOL CONCENTRATIONS IN CHRONIC CENTRAL SEROUS CHORIORETINOPATHY

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ABSTRACT

Purpose

Central serous chorioretinopathy (CSC), a distinct form of macular degeneration, has been associated with glucocorticoid use, and possibly also with an increased endogenous activity of the hypothalamic-pituitary-adrenal (HPA) axis. To estimate long-term glucocorticoid exposure, measurement of hair cortisol concentrations (HCC) have emerged. This cross-sectional study aimed to investigate HCC, as a reflection of chronic endogenous steroid exposure, in a cohort of chronic CSC patients (cCSC).

Methods

HCC were determined in 48 cCSC patients and 230 population-based controls (Lifelines cohort study), not using exogenous corticosteroids.

Results

Increased HCC (defined as > 10.49 pg/mg) were present in 2 (4%) cCSC patients and 13 (6%) controls. Mean HCC values were not different between patients and controls, and no difference in HCC were found between patients with active cCSC disease and patients with inactive disease. No correlation between HCC and urinary free cortisol (UFC) levels in cCSC patients was found.

Conclusions

This study shows that HCC in cCSC patients are not elevated compared to population-based controls, and no association between HCC and cCSC severity was found. This finding questions the previous suggestion that cCSC is associated with increased HPA axis activity. In line, HCC do not seem useful in monitoring cCSC disease activity.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a specific chorioretinal disease, in which choroidal hyperpermeability and retinal pigment epithelium damage occurs, leading to serous subretinal fluid accumulation.[1-3] When persistent and left untreated, irreversible loss of vision occurs, resulting in a decreased quality of life.[4, 5] Although the pathogenesis of CSC is currently unclear, biochemical stress in the form of both exogenous steroids as well as endogenous hypercortisolism have been reported in association with CSC.[1, 6, 7] Recently, we have reported an increased activity of the hypothalamic-pituitary-adrenal (HPA) axis based on increased 24 hour urinary free cortisol (UFC) excretion, albeit still within the normal cortisol range, and without disruption of circadian rhythm.[8] Although some of our cases of Cushing's syndrome have presented with CSC,[6] in this consecutive series we did not diagnose a single new case of Cushing's syndrome during screening of a large cohort of chronic CSC (cCSC) patients.[8]

The activity of the HPA axis as a proxy of endogenous exposure to stress can be evaluated with a number of tests, all reflecting different aspects and periods of endogenous cortisol exposure: 24 hour UFC levels reflect cortisol exposure during one day, whereas plasma and salivary cortisol levels provide information on the extent of cortisol present at a certain moment in time and its diurnal variation. To estimate long-term glucocorticoid exposure, measuring cortisol concentrations in scalp hair (hair cortisol concentrations (HCC)) has emerged over the past years. Scalp hair grows approximately 1 cm a month at a relatively stable rate, and steroid hormones are shown to retain in hair,[9, 10] making hair useful for the estimation of glucocorticoid exposure over a period of months.[11] Cushing's syndrome, obesity, cardiovascular disease, metabolic syndrome, and psychopathology have previously been associated with increased HCC,[11-13] and in patients with other ophthalmological diseases such as progressive keratoconus, elevated hair cortisol levels have been reported.[14] Recently, a small pilot study including 11 patients showed increased HCC in patients with active CSC.[15]

In the present study, we evaluated HCC in a large cohort of cCSC patients. In order to further investigate the suspected relationship between cCSC and cortisol as a measure for HPA axis activity, patients data were compared to the HCC of adult controls from the general population.

MATERIALS AND METHODS

Study design

Cross-sectional study in cCSC patients. The key objective was to assess HCC as a measure for the long-term endogenous cortisol exposure in these patients. For this purpose, HCC of patients with cCSC were compared to HCC of a population-based control group. In addition, a clinical evaluation of the patients took place on the outpatient clinic of the Division of Endocrinology of the Leiden University Medical Center. The relation between HCC and UFC was evaluated in cCSC patients. Written informed consent was obtained from all participants, and approval of the institutional review board and the ethics committee was obtained (NL50816.058.14). Research was conducted following the tenets of the Declaration of Helsinki.

Study population

Patients

Of the adult patients with cCSC who were followed at our tertiary referral center, 86 consecutive patients were invited to participate. The diagnosis of cCSC had been confirmed according to current standards (i.e. fundoscopy, digital colour fundus photography (Topcon Corp., Tokyo, Japan), fundus autofluorescence (Spectralis Heidelberg retinal angiography (HRA) + optical coherence tomography (OCT); Heidelberg Engineering, Heidelberg, Germany), spectral-domain OCT (Spectralis HRA + OCT), fluorescein angiography (Spectralis HRA + OCT) and indocyanine green angiography (Spectralis HRA + OCT)).[1, 2, 16-19] For inclusion, the following characteristics had to be present on multimodal imaging within the past two years: serous subretinal fluid on OCT, and either ≥ 1 area of irregular retinal pigment epithelium window defects or multifocal diffuse leakage on fluorescein angiography. Patients were divided in subgroups of either active or nonactive cCSC at the moment of HCC evaluation, in which active disease was defined by subretinal fluid presence. Patients diagnosed with acute CSC were excluded, defined by a smoke stack pattern of a focal leakage spot on fluorescein angiography,[1, 2, 16-19] as well as patients with evidence for another retinal diagnosis.

All cCSC patients participated in the study on endocrine phenotyping of the HPA axis as mentioned above (n=86),[8] as well as in a psychological questionnaire survey (n=86, data presented elsewhere).[20] For the present study, exclusion criteria were excessive alcohol intake (>21 units/week), the use of corticosteroids (both systemic as well as local) or sleep medication prior to the development or during the time-course of cCSC, and either night shift work or travelling from another time zone in the six weeks prior to evaluation. Endocrine evaluation of the patients consisted of a detailed medical history, a complete physical examination, specifically aimed to detect subtle signs of Cushing's disease, and blood, urine, and saliva analysis (data presented elsewhere)[8]. The collection of scalp hair succeeded in 48 patients. In the other 38 patients, hair collection failed due to the absence of at least 1 cm of hair (n=33), or due to the absence of patients' permission to cut hair (n=5). After reassessment of the retinal imaging by two independent ophthalmologists, two of the cCSC patients were considered to have less typical findings on imaging, and were excluded in a sensitivity analysis.

Population-based controls

Control data were derived from Lifelines, a multi-disciplinary prospective population-based cohort study examining in a three-generation design the health and health-related behaviours of 167,729 persons living in the North of The Netherlands (www.lifelines.nl). It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics.[21]

For the present study, data on HCC collected for a previously described study were used.[22] For this preceding research, approved by the Medical Ethics Review Committee of the University Medical Center Groningen, 295 adult participants of Lifelines were included in November and

December of 2013. Written informed consent was provided by all participants. The participants came for a study site visit including measurements of vital parameters and anthropometry, a fasting venepuncture, and scalp hair collection, of which the results were presented by Wester et al.[22] HCC were successfully determined in 266 participant samples. From this cohort, participants using systemic (n=3) or local glucocorticoids (n=33) were excluded for the present analysis.

Hair processing and analysis

During the study visits, a sample of scalp hair of approximately 100-150 hairs from the posterior vertex was cut, as close to the scalp as possible. The hairs were taped to a paper, and stored at room temperature in the dark in envelopes until further processing. Hair samples, both from the cCSC patients as well as from the controls, were processed and analysed as was described previously.[10] In controls, approximately 20 mg of the proximal 3 cm (if present) of each hair sample was weighed, and cut into 1 cm segments; an average of the 3 HCC was used for analysis. In patients, only the most proximal cm of hair was used for the measurement of HCC. The samples were washed for 2 minutes in 2 mL of liquid chromatography – mass spectrometry (LC-MS) grade isopropanolol, and left to dry. The hairs were extracted for 18 hours at 25° centigrade in 1.4 mL LC-MS grade methanol and 100 µL of internal standard. Solid phase extraction was used to purify the extracted samples, and quantification of cortisol was performed by liquid chromatography – tandem mass spectrometry (LC-MS/MS) using a Xevo TQ-S system (Waters, Milford, MA, USA). Increased HCC were defined as > 10.49 pg/mg, as described by Wester et al. [22]

Statistical analysis

SPSS Statistics version 23 was used for statistical analysis (IBM Corp., Armonk, NY, USA). Data were presented as mean and standard deviation (SD), unless mentioned otherwise. Hair cortisol concentrations were logarithmically transformed to achieve a normal distribution. Data were analysed using independent sample *t*-tests. Analyses were stratified according to gender. The groups were compared using a linear regression model, correcting for potential confounders such as duration of cCSC disease and age, since hair cortisol levels were shown to increase with age.[22] A *P* value below 0.05 was considered statistically significant. A post hoc sensitivity analysis excluding the two less typical cCSC patients was performed. Moreover, a sensitivity analysis excluding outliers (n = 2 control, no patients) was completed, using an outlier test (Rosner's Extreme Studentized Deviate test) to determine significant outliers. The correlation between HCC and UFC levels was assessed using Pearson's correlation.

RESULTS

Baseline characteristics

Forty-eight cCSC patients (41 males [85%]) and 230 population-based controls (63 males [27%]) were included (Table 1). The gender distribution in cCSC patients was in line with the currently available literature.[1, 19] At the time of evaluation, the mean duration of cCSC disease since diagnosis had been established by an ophthalmologist was 3.9 years (range 0.2-33.0). Active cCSC (i.e. presence

of subretinal fluid) was present in 31 patients (65% of the patients). With a mean age of 49.2 years (range 33-72), cCSC patients were 7 years older than controls (mean age 42.2 years, range 18-85, *P*<0.01). No cases of Cushing's syndrome according to conventional tests were present.[8]

Hair cortisol concentrations (HCC)

Hair cortisol concentrations ranged from 0.6 to 20.8 pg/mg in cCSC patients and from 0.7 to 79.8 pg/mg in controls. Increased HCC, i.e. > 10.49 pg/mg, was present in 2 cCSC patients (4%) and 13 controls (6%).

Mean HCC in male cCSC patients were 3.9 (SD 3.7) compared to 4.6 (4.7) in male controls, *P*=0.32. In females, mean HCC were 4.3 (4.3) in cCSC patients and 3.5 (7.1) in controls, *P*=0.60 (Figure 1). Also, after correction for age, no significant differences in HCC between the cCSC patients and controls were found (*P*=0.14 males, *P*=0.08 females). Likewise, correction for duration of cCSC disease did not change the results (males *P*=0.72, females *P*=0.89).

Patients with active cCSC had mean HCC of 3.8 (2.9), and in patients with inactive disease mean HCC of 4.2 (5.0) were found, *P*=0.86 (Figure 2).

In 47 of the 48 cCSC patients (98%) UFC levels were measured. UFC ranged from 19 to 274 nmol/24 hour (mean 84.0 (44.2)). Figure 3 shows the absence of a correlation between HCC and UFC levels in cCSC patients (R^2=0.07, *P*=0.63).

The exclusion of two atypical cCSC patients did not affect any of the described results. Also the exclusion of the significant outliers in HCC did not change the aforementioned results (data not shown).

DISCUSSION

This study revealed that HCC in patients with cCSC were not different when compared to population-based controls. In addition, no differences in HCC were found between patients with active cCSC disease and patients with inactive disease. Hence, our study demonstrates that HCC are not useful in monitoring cCSC disease activity. No correlation between HCC and UFC was found in cCSC patients either.

To our knowledge, this is the first study evaluating HCC in a relatively large cohort of cCSC patients. The only study published to date involved a pilot study investigating HCC in a very small group of 11 patients with either active acute or chronic CSC, which showed, in contrast to

Table 1. Clinical characteristics of participants.

	cCSC patients *n=48*	**Controls** *n=230*	**p value**
Age, yrs (mean, SD)	49.2 (9.5)	42.2 (11.6)	<0.01
Sex, male/female	41/7	63/167	<0.01
Duration of cCSC disease, yrs (median, range)	0.9 (0.2–33.0)	-	-

Data are presented as mean (SD), median (range) or as numbers. cCSC = chronic central serous chorioretinopathy, yrs = years.

our study, increased HCC in these patients.[15] The pilot study showed mean age-adjusted HCC of 20.1 pg/mg in CSC patients and 11.1 pg/mg in healthy controls, compared to our 3.9 pg/mg in male cCSC patients and 4.6 pg/mg in controls. However, the size of the pilot study makes the results susceptible to sampling and selection bias, and the heterogeneity of the patients stands in the way of generalizability. Our study included 48 consecutive patients with only chronic CSC, making the results valid and generalizable to this patients category. Moreover, the pilot study used an immunoassay for the determination of HCC, whereas LC-MS/MS measurements were used in the present study. Immunoassays are shown to differ in steroid crossreactivity depending on the assay used, and are described to measure substantially higher HCC with a greater variation than the more accurate LC-MS/MS based methods.[23] The absence of a correlation between HCC and UFC in our cCSC patient population is in line with a previously published evaluation of HCC in combination with UFC in healthy controls.[24] However, in patients with Cushing's syndrome and corresponding pathological cortisol excess, strong correlations between HCC and UFC have been reported.[25, 26] The proposed relationship between cortisol, both endogenous as well as exogenous, and CSC has been widely described.[1, 6, 7] The pathophysiology, however, remains to be elucidated, although several underlying mechamisms have been hypothesized. Platelet aggregation is increased by endogenous hypercortisolism, leading to increased blood viscosity and microthrombi.[27] Hyperpermeability and choroidal fragility have also been associated with hypercortisolism,[28] and an increased expression of adrenergic receptors has been correlated with corticosteroids.[29] Previous animal studies have suggested that mineralocorticoids play a pathophysiological role,[19, 30] by activating the mineralocorticoid receptor in choroidal endothelial cells, leading to choroidal vasodilation.[19] Moreover, the possible pathogenetic effect of activating

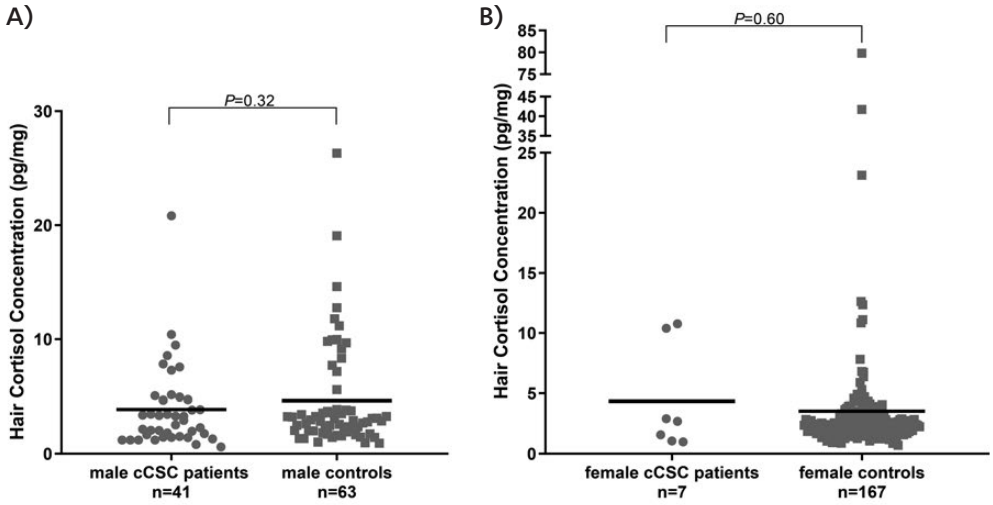

Figure 1. Hair cortisol concentrations (HCC) in cCSC patients and population-based controls stratified by sex. **A**, Males. **B**, Females. Data presented as individual values and mean. cCSC = chronic central serous chorioretinopathy, HCC = hair cortisol concentrations.

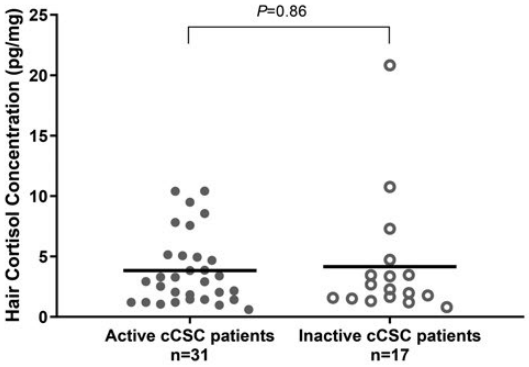

Figure 2. Hair cortisol concentrations (HCC) in cCSC patients with active disease and cCSC patients with inactive disease. Data presented as individual values and mean. cCSC = chronic central serous chorioretinopathy, HCC = hair cortisol concentrations.

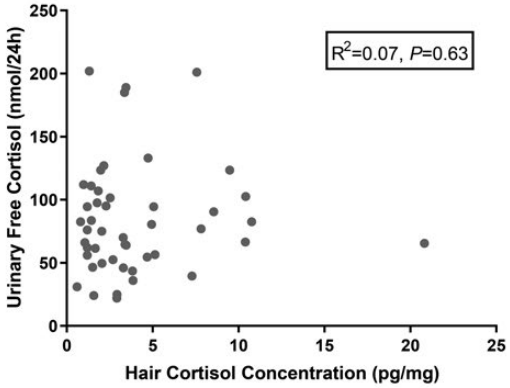

Figure 3. Correlation between HCC and UFC in patients with cCSC. Data presented as individual values. N = 47 cCSC patients. cCSC = chronic central serous chorioretinopathy, HCC = hair cortisol concentrations, UFC = urinary free cortisol.

the mineralocorticoid receptor may be modulated by several genetic receptor variants.[31] With regard to the biological evaluation of patients using the clinically available screening tests for cortisol, we recently reported significantly higher 24 hour UFC levels in cCSC patients, albeit within the normal reference range, with preservation of normal diurnal rhythmicity.[8] In the light of clinical cortisol testing with HCC as a measure for long-term cortisol exposure, the current analysis does not show increased HCC in cCSC patients. We propose that either the HCC technique is not sensitive enough to detect minor and perhaps short-term elevations in cortisol concentrations within a normal range, keeping in mind that a wide individual variation in normal cortisol levels and glucocorticoid sensitivity. Altered glucocorticoid sensitivity due to glucocorticoid receptor gene polymorphisms has been shown to modify manifestations of several diseases.[32-35] Or perhaps these minor increases in cortisol concentrations on a tissue level leading to cCSC specific alterations in choroid and retina

are not reflected by increased cortisol concentrations in hair. On the other hand, based on our findings, one could also postulate that the long-term exposure to cortisol is not increased in cCSC. Perhaps a short peak or a prolonged temporary elevation in cortisol levels is sufficient to induce pathological alterations in the choroid and/or retina, and may have anticipated the current chronic status. We cannot rule out that accidentally non-reported exogenous corticosteroid use may have lowered the HCC in the control group. However, since this was extensively interrogated, we believe the potential effect of non-reported corticosteroids to be limited. An alternative explanation for the absence of increased HCC in cCSC patients, is that the relationship between cortisol and cCSC is not as straightforward as suggested so far.

Our study also has limitations. The cross-sectional character does not allow drawing conclusions on any (absence of a) causal relationship. In controls, the proximal 3 cm of each hair sample was cut into 1 cm segments, and an average of the 3 HCC values was used for the current analysis. Since our patient population consisted mainly of men with most of them having short hair, HCC were only measured in the proximal 1 cm of hair. However, since Noppe et al described that the HCC decline gradually from proximal to more distal hair segments,[10] this would imply higher HCC in controls when only the most proximal cm of hair had been used, resulting in an even smaller difference between female cCSC patients and controls. Last, with our choice to stratify the study groups for analysis, potential residual confounding was introduced. Yet, because stratification on only gender (i.e. two strata) was applied, we consider the effect of this stratification negligible.

In conclusion, HCC as a clinical measure for long-term cortisol exposure in cCSC patients are not elevated when compared to population-based controls. In addition, no difference in HCC were found between different cCSC disease stages. Therefore, the results of this study argue against the use of HCC in monitoring cCSC disease activity. Further research unravelling the role of cortisol and the stress axis in the pathophysiology of cCSC is required.

CONFLICTS OF INTEREST

The authors report no conflicts of interest in this work.

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AUTHORS CONTRIBUTIONS

All authors listed have made substantial, direct, and intellectual contribution to the work, and approved it for publication. FH, ED, and MS collected the data. FH wrote the paper and designed the figures. AP, CB, NB, OD, ER, MS, JB, and GD have made substantial contributions to the concept and design of the work and interpretation of data. All evaluated the paper.

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CHAPTER 5

MALADAPTIVE PERSONALITY TRAITS, PSYCHOLOGICAL MORBIDITY AND COPING STRATEGIES IN CHRONIC CENTRAL SEROUS CHORIORETINOPATHY

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ABSTRACT

Purpose

'Type A' behavioural characteristics and psychosocial stress have traditionally been associated with chronic central serous chorioretinopathy (cCSC). However, a characteristical personality profile could not be identified in these patients and the presumed association with stress is subject to controversy, due to a lack of convincing studies using validated measuring instruments. In this study, we aimed to assess maladaptive personality traits, psychological morbidity and coping strategies in patients with cCSC, in order to identify potentially modifiable psychosocial aspects which could be used in support to current standard treatment.

Methods

A cross-sectional study in a cohort of 86 patients with cCSC using validated questionnaires. Findings were compared to both Dutch population reference data and reference data from patients treated for Cushing's disease.

Results

Maladaptive personality traits were not more prevalent in patients with cCSC than in the general population, and psychological morbidity was not increased. Patients with cCSC were shown to make more use of passive coping, active coping and seeking social support. Interestingly, personality, psychological morbidity and coping characteristics of patients with cCSC were more comparable to features of patients treated for Cushing's disease than to population-based data.

Conclusion

Maladaptive personality traits such as type A behavioural characteristics are not more prevalent in patients with cCSC. Patients with cCSC make more use of certain coping strategies, which could be addressed by psychosocial care to improve self-management. Further research is needed establish whether the course of disease can be improved by altering coping and reducing 'stress'.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a specific and relatively common chorioretinal disease in which choroidal congestion, thickening and hyperpermeability damage the retinal pigment epithelium and subsequently induce serous subretinal fluid accumulation and detachment of the neuroretina.

The pathogenesis of CSC is currently unclear, but many studies indicate a pathophysiological association with stress pathways, due to the relation with both exogenous and endogenous corticosteroid excess, as well as overactivity of the hypothalamus–pituitary–adrenal (HPA) axis [1-9]. Both acute and chronic psychosocial stress have been suggested to predispose to CSC [10, 11]. It has also been suggested that people with a type A behaviour pattern have an increased risk to develop CSC [12-15]. The term 'type A behaviour' was introduced by Friedman & Rosenman [16] and was characterized as follows: an intense, sustained drive to achieve self-selected but usually poorly defined goals, profound inclination and eagerness to compete, persistent desire for recognition and advancement, continuous involvement in multiple and diverse functions constantly subject to deadlines, habitual propensity to accelerate the rate of execution of many physical and mental functions, and extraordinary mental and physical alertness. The concept of personality types has been studied in medical psychology as a predictor of the cause, course and quality of life of somatic diseases such as cancer, rheumatic diseases and coronary artery disease [17-19]. It has been hypothesized that type A behaviour might be linked to CSC by increased levels of circulating catecholamines and corticosteroids, since these hormone levels are found to be higher in people with type A behavioural characteristics compared to those with type B behavioural characteristics (more relaxed and less hurried) [20-23]. A recent meta-analysis indeed concluded that patients with CSC demonstrated significantly more type A behavioural characteristics than healthy controls (odds ratio (OR) = 2.53; confidence interval (CI) 1.08–5.96) [24]. Despite this proposed association between CSC and type A behavioural characteristics [25], a typical CSC personality profile could not be identified in previous studies. Only type A behavioural characteristics have been previously observed [26].

It is well known that personality affects coping behaviour [27]. Coping behaviour encompasses the way people react on a behavioural, cognitive and emotional level to situations that require adjustments in dealing with possible adverse events [28], which has an effect on the amount of stress experienced [29]. Specific coping styles (e.g. emotion-oriented coping) have even been reported to have an effect on disease severity, for example in multiple sclerosis [30]. For CSC, several mostly small-sized studies have reported an association between severe psychosocial stressful events and the onset of disease, with one study describing this association especially in patients with poor coping mechanisms [11,31,32]. However, coping behaviour may be a valuable starting point for psycho-education or self-management training in order to improve quality of life. Also psychological morbidity such as apathy or irritability may be a potential point of engagement for self-management programmes. To date, these psychological factors have not been evaluated in CSC patients.

To the best of our knowledge, no previous systematic studies have been published assessing personality traits in patients with CSC, and there are no studies in a large cohort of patients with CSC that have systematically evaluated coping strategies using a specific coping-oriented validated

questionnaire. Since CSC seems to be related to stress [1-9], a detailed assessment of potential associations with personality traits, psychological morbidity and coping mechanisms is essential to identify potentially psychosocial aspects that could be modifiable with self-management programmes.

The primary aim of this study was to assess maladaptive personality traits (i.e. traits related to type A behavioural pattern), in patients with cCSC. For this purpose, we compared personality traits of patients with cCSC to personality traits of Dutch population reference data, but also to personality traits of patients treated for Cushing's disease (since these patients were exposed to excessive HPA - axis activity). In addition, this study aimed to assess psychological morbidity (i.e. apathy and irritability) and coping strategies in patients with cCSC by comparing these patients with the same reference groups. Finally, we aimed to assess the association between personality and coping in patients with cCSC. Since previous studies have pointed towards a higher prevalence of type A behavioural characteristics in patients with cCSC and considering the above-mentioned definition of type A behaviour [16], we assessed whether patients with cCSC report more stimulus seeking, callousness, rejection, conduct problems and narcissism. Furthermore, considering the recently described hyperactivity of the HPA axis in patients with cCSC [9] and the previously described maladaptive personality traits in patients exposed to hypercortisolism (i.e. Cushing's disease) [33,34], we hypothesized that patients with CSC would report more maladaptive personality traits, more psychological morbidity (i.e. apathy, irritability) and less effective coping strategies compared to reference data from the general population. In accordance with previous literature in patients with other chronic diseases [35-39], we hypothesized that more maladaptive personality traits are associated with less effective coping strategies in patients with cCSC.

SUBJECTS AND METHODS

Study design

We conducted a cross-sectional study in a cohort of patients with cCSC. Patients were asked to complete a set of validated questionnaires on personality traits, psychological morbidity (i.e. apathy and irritability) and coping strategies at home, using an online survey. In addition, a clinical evaluation took place during a single visit to the outpatient clinic of the Division of Endocrinology of the Leiden University Medical Center.

Study population

Eighty-six consecutive adult patients with cCSC, who were followed at the Department of Ophthalmology of Leiden University Medical Center, a tertiary referral center for CSC, were invited to complete the questionnaires. The cCSC diagnosis had been confirmed by fundoscopy, digital colour fundus photography (Topcon Corp., Tokyo, Japan), fundus autofluorescence (Spectralis Heidelberg retinal angiography (HRA) + optical coherence tomography (OCT); Heidelberg Engineering, Heidelberg, Germany), spectral-domain OCT (Spectralis HRA + OCT), fluorescein angiography (Spectralis HRA + OCT) and indocyanine green angiography (Spectralis HRA + OCT), according to current standards [4-7,13,40]. On multimodal imaging, the following characteristics had

to be present within the past 2 years: serous subretinal fluid on OCT, ≥1 area of multifocal diffuse leakage or irregular retinal pigment epithelium window defects on fluorescein angiography, and corresponding hyperfluorescence on indocyanine green angiography. Patients were divided into active or nonactive cCSC at the moment of evaluation, defined by the presence of subretinal fluid. We excluded patients diagnosed with acute CSC, defined by either a focal leakage spot or a smoke stack pattern on fluorescein angiography [4-7,13,40], as well as patients in whom evidence of other retinal diagnoses was detected. The patients also participated in a study on endocrinological phenotyping focussed on the HPA axis (data presented elsewhere) [9], for which other exclusion criteria were the use of corticosteroids or sleep medication prior to the development or during the time-course of cCSC, excessive alcohol intake (>21 units/week), either night shift work or travelling from another time zone in the 6 weeks prior to evaluation. Endocrinological evaluation of the patients included a detailed medical history and complete physical examination and was performed by two endocrinolo,gists. After reassessment of the retinal imaging by two independent ophthalmologists, five patients were considered to have less typical cCSC findings on imaging. Written informed consent was obtained from all participants, and approval of the institutional review board and the ethics committee was obtained (NL50816.058.14). Research was conducted following the tenets of the Declaration of Helsinki.

Questionnaires

Dimensional assessment of personality pathology short form

This questionnaire consists of 136 items assessing personality, which are subdivided into 18 subscales: submissiveness, cognitive distortion, identity problems, affective lability, stimulus seeking, compulsivity, restricted expression, callousness, oppositionality, intimacy problems, rejection, anxiousness, conduct problems, suspiciousness, social avoidance, narcissism, insecure attachment and self-harm [41,42]. The maximal scores for each subscale differ from 30 to 40, and higher scores indicate more pronounced maladaptive personality traits. No formal cut-off scores for these subscales exist [41,42]. We hypothesized that if type A behavioural characteristics would be more prevalent in patients with cCSC, these patients would report more stimulus seeking, callousness, rejection, conduct problems and narcissism.

Apathy Scale

The Apathy Scale (AS) of Starkstein was used to assess apathy [43]. The scale consists of 14 questions on a four-point scale, measuring different features of apathy in the two previous weeks. Total scores in a range from 0 to 42 points are calculated, with higher scores indicating greater apathy. A total score of 14 points or more defines apathy [44].

Irritability Scale

Irritability was assessed by the Irritability Scale (IS) [44]. This scale consists of 14 items on a four-point scale, assessing different features of irritability in the two previous weeks. Total scores range from 0 to 42 points, with higher scores indicating greater irritability. A total score of 14 points or more defines irritability.

Utrecht Coping Scale

The Utrecht Coping Scale (UCS) is an established Dutch coping list with well-documented validity and reliability [45]. It contains 47 statements where one indicates whether he/she finds these applicable to him- or herself. This scale assesses the way a person acts to minimize the impact of stressful events, with seven subscales that represent different coping styles. These subscales include active coping (i.e. immediate action in case of problems, considering problems as a challenge, keeping calm, goal-oriented problem-solving), distraction-seeking, avoidance, seeking social support, passive coping (i.e. isolation, worrying about the past, using soothing resources, fleeing in fantasies), expression of emotions, and positive reframing (i.e. optimism, trying to reconsider things in a positive light). The different items have a four-point scale ranging from 1 (seldom or never) to 4 (very often). Item scores on each subscale are summed to create a total score, with scores of 4 or 5 indicating high use of that specific coping style [46]. Data from an a-select sample of the Dutch railway workers (1493 men, aged between 19 and 65 years) were used as reference data. A cohort of 42 Cushing's disease patients (six men and 36 women) with a mean age of 54 (±12) years was used for comparison [47].

Reference data

Outcomes of the questionnaires were compared to reference of a random sample of the Dutch population and reference data from patients treated for Cushing's disease as reported previously by Tiemensma et al. [33,34,47]. For comparison of dimensional assessment of personality pathology short form (DAPPsf) outcomes, reference data from the publisher of this questionnaire were available (48 van Kampen 2009). The sample used for obtaining these data consisted of 58 men aged 15–34 years, 94 men aged 35–54 years, 146 women aged 15–34 years and 172 women aged 35–54 years. The sample of patients treated for Cushing's disease used for comparison consisted of eight men and 43 women with a mean age of 53 (±13) years [33,34]. Concerning the AS and IS, reference data were derived from the healthy control population described by Tiemensma et al. [33,34] consisting of 35 men and 33 women with a mean age of 59 (±11) years. No male-only reference data were available. The same cohort of patients with Cushing's disease was used for the comparison of the AS and IS scores [33,34]. Data from a random sample of the Dutch railway workers (1493 men, aged between 19 and 65 years) were used as reference data for the comparison of UCS outcomes. Moreover, the cohort of 42 patients treated for Cushing's disease (six men and 36 women) with a mean age of 54 (±12) years was used for comparison [47].

Statistical analysis

Data were presented as mean and standard deviation (SD), unless mentioned otherwise. The primary analyses comprised the comparison of questionnaire outcomes between patients with cCSC and reference data from the general population. Secondary analyses comprised the comparison between patients with cCSC and patients treated for Cushing's disease. Groups were compared using pooled t-tests. The level of significance was set at $p \leq 0.01$ in order to correct for multiple testing. Normality of data was tested using the Shapiro–Wilk test. Correlations between personality and coping were assessed using Pearson's correlation in case of normally distributed data, and data

with a non-normal distribution were correlated using Spearman correlation. Only moderate-to-strong correlations (correlation coefficient of >0.5) were described.

A post hoc sensitivity analysis excluding the five less typical cCSC patients was performed. Data were analysed using spss Statistics (version 23; IBM Corp., Armonk, New York, USA).

RESULTS

Baseline characteristics

A total of 86 patients with cCSC (77 males [90%]) with a mean age of 48.7 years (range, 24–77 years) were included. In all patients, subretinal fluid had been present <2 years ago. In 58 patients with cCSC (67%), subretinal fluid was present at the moment of evaluation, indicating active cCSC. The mean duration from first cCSC diagnosis at an ophthalmologist to inclusion in our study was 3.9 years (range, 0.2–37.1 years). A history of hypertension was reported by 23 patients (27%), dyslipidaemia by 18 patients (21%) and psychiatric disorders by 16 patients (19%) (Table 1). Apart from being slightly overweight (mean body mass index 26.2 kg/m2), patients appeared to be healthy on physical examination, with a mean blood pressure within the normal range. None of the patients fulfilled the criteria for Cushing's syndrome.

Personality traits

Dimensional assessment of personality pathology short form

The DAPPsf was completed by 81 patients with cCSC (94%). Compared to reference data from the general population, patients with cCSC reported only more intimacy problems ($p < 0.01$), but less submissiveness ($p < 0.01$), less cognitive distortion ($p < 0.01$), less affective lability ($p < 0.01$), less stimulus seeking ($p < 0.01$), less compulsivity ($p < 0.01$), less oppositionality ($p < 0.01$), less anxiousness ($p < 0.01$), less suspiciousness ($p < 0.01$), less social avoidance ($p < 0.01$), less narcissism ($p < 0.01$) and less insecure attachment ($p < 0.01$) (Table 2 and Figure 1). Interestingly, there was no increased prevalence of type A behavioural characteristics in patients with cCSC (i.e. no more stimulus seeking, callousness, rejection, conduct problems and narcissism).

Compared to patients treated for Cushing's disease, patients with cCSC reported more conduct problems ($p < 0.01$), but less affective lability ($p < 0.01$), less cognitive distortion ($p < 0.01$) and less oppositionality ($p < 0.01$). For the remaining personality traits, no large difference was observed between patients with cCSC and patients treated for Cushing's disease.

Compared to patients with active cCSC ($n = 54$), patients with inactive disease ($n = 27$) reported more affective lability, submissiveness and social avoidance ($p < 0.01$, $p < 0.01$ and $p < 0.01$, respectively).

Psychological morbidity

The AS was completed by 83 patients with cCSC (97%) (Table 3). The mean score of patients with cCSC was 12.2 (range, 3–26). Clinically relevant apathy (a score of ≥14) was present in 34.9% of the patients with cCSC. No differences in reported apathy were found between patients with cCSC and the reference data from the general population, and the apathy score was lower than

Table 1. Clinical characteristics of chronic central serous chorioretinopathy (cCSC) patients.

	cCSC patients *n = 86*
Mean age, years (SD)	48.7 (10.8)
Sex, male/female	77/9
Duration of cCSC disease, years (range)	3.9 (0.2–37.1)
History of hypertension, *n* (%)	23 (26.7%)
History of diabetes mellitus, *n* (%)	6 (7.0%)
History of dyslipidaemia, *n* (%)	18 (20.9%)
History of psychiatric disorders[a], n (%)	16 (18.6%)
History of thromboembolic events, *n* (%)	0 (0%)
History of cardiac events[b], *n* (%)	5 (5.9%)
History of sexual disorders[c], n (%)	19 (22.1%)

SD = standard deviation.

[a] Consisting of depression, anxiety or panic disorder, posttraumatic stress disorder, burnout, alcohol abuse and schizophrenia.

[b] Consisting of myocardial infarction, endocarditis and atrial fibrillation.

[c] Consisting of impotence, hirsutism, menstrual cycle disorders and loss of libido.

Table 2. Personality traits in chronic central serous chorioretinopathy (cCSC).

DAPPsf	**cCSC patients (*n* = 81)**	**Reference data (*n* = 475)**	**p-Value**	**Cushing's disease patients (*n* = 51)**	**p-Value**
Submissiveness	16.2 (6.6)	19.7 (6.3)	<0.01	19.0 (7.7)	0.03
Cognitive distortion	9.0 (4.3)	12.1 (5.4)	<0.01	11.5 (5.6)	<0.01
Identity problems	10.6 (4.7)	12.2 (5.6)	0.02	13.0 (6.6)	0.01
Affective lability	16.5 (6.9)	21.0 (7.3)	<0.01	21.7 (7.8)	<0.01
Stimulus seeking	15.2 (5.4)	18.0 (5.8)	<0.01	16.4 (4.8)	0.19
Compulsivity	21.9 (7.1)	24.2 (6.5)	<0.01	23.8 (6.6)	0.12
Restricted expression	20.9 (5.9)	21.3 (6.5)	0.65	21.2 (7.3)	0.80
Callousness	17.2 (5.2)	18.8 (5.4)	<0.01	16.1 (4.5)	0.23
Oppositionality	19.0 (7.0)	23.1 (7.2)	<0.01	22.9 (8.8)	<0.01
Intimacy problems	20.9 (5.9)	16.9 (5.7)	<0.01	18.8 (6.4)	0.06
Rejection	19.3 (6.7)	20.1 (5.7)	0.28	17.2 (5.7)	0.06
Anxiousness	12.8 (6.2)	17.8 (5.7)	<0.01	15.3 (6.2)	0.03
Conduct problems	10.8 (4.8)	11.5 (4.4)	0.21	9.0 (1.8)	<0.01
Suspiciousness	12.7 (5.4)	15.0 (5.9)	<0.01	12.6 (5.9)	0.96
Social avoidance	11.0 (5.2)	13.8 (5.5)	<0.01	12.3 (6.3)	0.20
Narcissism	15.7 (5.7)	18.7 (6.2)	<0.01	15.0 (5.5)	0.47
Insecure attachment	10.9 (4.9)	13.7 (5.6)	<0.01	13.3 (6.6)	0.02
Self-harm	7.0 (2.7)	8.0 (4.2)	0.05	7.3 (2.9)	0.58

Data are presented as mean (SD).

cCSC = chronic central serous chorioretinopathy; DAPPsf = Dimensional assessment of personality pathology short form; SD = standard deviation.

scores of patients treated for Cushing's disease (p = 0.03 and p = 0.01, respectively). Although no significant differences were found, the scores of patients with cCSC were in between the scores of the reference data from the general population and the reference data from patients treated for Cushing's disease (Figure 2). No difference was observed in total scores between patients with active cCSC and patients with inactive disease (p = 0.26). The IS was also completed by 83 patients with cCSC (97%) (Table 3). Mean patient score was 9.8 (range, 0–26). Clinically relevant irritability (a score of ≥14) was present in 29.3% of patients with cCSC. No differences in reported irritability were observed between patients with cCSC and the reference data from the general population (p = 0.79), nor to reference data from patients treated for Cushing's disease (p = 0.15). Although no statistically significant differences were found, the scores of patients with cCSC were in between the scores of the reference data from the general population and the reference data from patients treated for Cushing's disease, which was in line with the outcome of the assessment of apathy (Figure 2). Total scores did not differ between patients with active cCSC and patients with inactive cCSC (p = 0.36).

Coping strategies

Eighty-three patients with cCSC (97%) completed the UCS (Table 4). Compared to the reference data, patients with cCSC reported to use more passive coping strategies and to seek more social support (p < 0.01 and p < 0.01, respectively). Because the reference data were male only, data from male patients with cCSC between 19 and 65 years of age (n = 67) were compared separately. This category of patients with cCSC made more use of active coping compared to the reference population (p < 0.01), in addition to the aforementioned seeking social support (p < 0.01) and passive coping (p < 0.01). Patients with inactive disease (n = 27) made more use of avoiding compared to cCSC patients with active disease (n = 56, p < 0.01). No differences in coping strategies were observed between patients with cCSC and patients treated for Cushing's disease.Data are presented as mean and SD. cCSC = chronic central serous chorioretinopathy; DAPPsf = Dimensional assessment of personality pathology short form; SD = standard deviation; *=statistically significant (defined as p-value <0.01).

Correlation between personality and coping strategies in cCSC

Moderate-to-strong correlations were found between several maladaptive personality traits and passive coping. More affective lability (p < 0.01, R2 = 0.759), cognitive distortion (p < 0.01, R2 = 0.656), identity problems (p < 0.01, R2 = 0.675), insecure attachment (p < 0.01, R2 = 0.558), oppositionality (p < 0.01, R2 = 0.522), social avoidance (p < 0.01, R2 = 0.515) and anxiousness (p < 0.01, R2 = 0.711) correlated with using more passive coping.

Post hoc analysis without patients with less typical cCSC

Analyses performed without the five patients with atypical cCSC revealed only a few minor differences. These results are shown in Appendix.

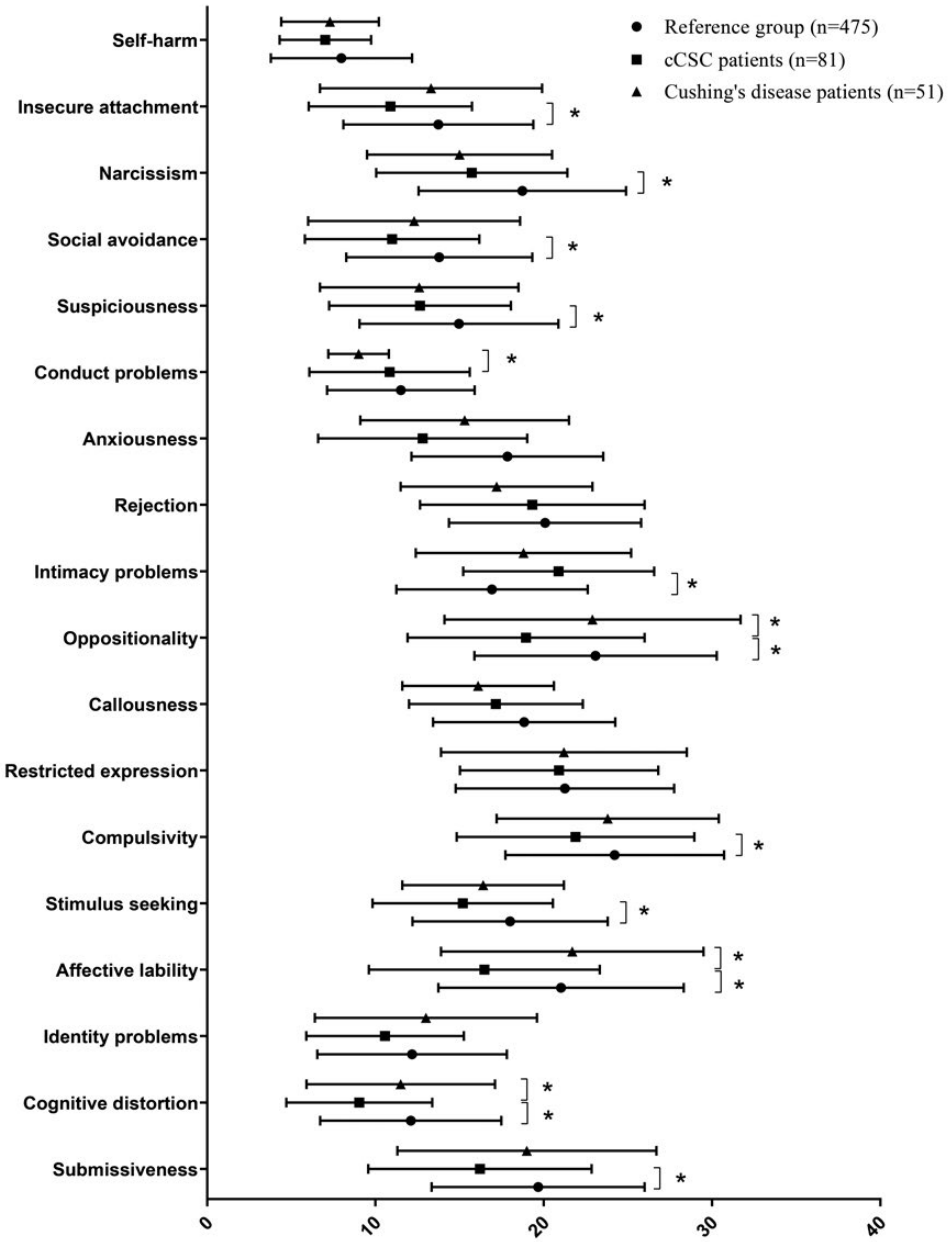

Figure 1. DAPPsf personality traits in patients with cCSC. Data are presented as mean and SD. cCSC = chronic central serous chorioretinopathy; DAPPsf = Dimensional assessment of personality pathology short form; SD = standard deviation; *=statistically significant (defined as p-value <0.01).

Table 3. Apathy and irritability in patients with cCSC.

	cCSC patients (*n* = 83)	Reference data (*n* = 68)	p-Value	Cushing's disease patients (*n* = 51)	p-Value
Apathy	12.2 (5.0)	10.5 (4.8)	0.03	14.8 (6.5)	0.01
Irritability	9.8 (6.2)	9.5 (5.7)	0.79	11.5 (7.7)	0.15

Data are presented as mean (SD).

cCSC = chronic central serous chorioretinopathy; SD = standard deviation.

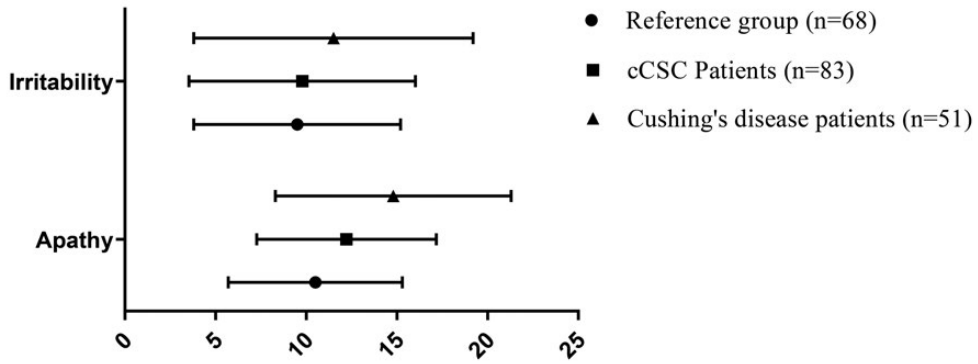

Figure 2. Apathy and irritability in patients with cCSC. Data are presented as mean and SD. cCSC = chronic central serous chorioretinopathy; SD = standard deviation.

Table 4. Coping in patients with cCSC.

	cCSC patients (*n* = 83)	Reference data (*n* = 1493)	p-Value	Cushing's disease patients (*n* = 42)	p-Value
Active coping	19.3 (4.1)	18.3 (3.5)	0.01	17.5 (3.5)	0.02
Seeking distraction	16.1 (3.6)	15.5 (3.6)	0.14	17.7 (3.1)	0.01
Avoiding	15.5 (3.3)	14.8 (3.3)	0.05	16.3 (3.4)	0.23
Seeking social support	12.6 (2.7)	11.3 (3.0)	<0.01	13.3 (4.0)	0.28
Passive coping	11.8 (3.3)	10.7 (2.9)	<0.01	12.0 (3.4)	0.71
Expressing emotions	5.7 (1.6)	6.2 (1.7)	0.01	5.9 (1.6)	0.65
Fostering reassuring thoughts	11.5 (2.5)	11.6 (2.5)	0.64	12.3 (2.7)	0.11

Data are presented as mean (SD).

cCSC = chronic central serous chorioretinopathy; SD = standard deviation.

DISCUSSION

In this study, personality traits, psychological morbidity and coping strategies were systematically assessed in a cohort of patients with cCSC. We did not find a higher prevalence of maladaptive personality traits such as type A behavioural characteristics in cCSC as compared to the general population, which is in contrast to what has been suggested previously [14,15,24,25]. On the level of conduct, patients did not report more psychological morbidity in the form of apathy or irritability. Patients with cCSC made more use of certain coping strategies (e.g. seeking social support, passive coping, and in males also active coping) compared to the general population.

In contrast to earlier studies suggesting more type A behavioural characteristics (i.e. persistent desire for recognition and advancement, and habitual propensity to accelerate the rate of execution of many physical and mental functions) in these patients [14,15,24,25], we did not find any evidence to support this. Critical evaluation of the available literature revealed that type A behavioural characteristics were mainly assessed using behavioural outcome measures (i.e. Jenkins activity survey) in previous studies [14,15,24,25], while this inventory has been shown not to correlate with personality characteristics and psychopathology [49]. In another study, type A behavioural characteristics were not strictly defined, as the term itself may have appeared in the medical charts or patients were included as being type A based on a description of patients by themselves, family members or physicians as being 'tense', 'high strung' or 'highly ambitious' [49]. The conclusion of a recent meta-analysis suggesting more type A behavioural characteristics in patients with cCSC was based on these small studies lacking a type A phenotyping protocol, making the conclusion less reliable [24].

The fact that patients with cCSC seem to report more intimacy problems was interpreted as a chance finding, since all the other traits point in the opposite direction, with generally less maladaptive personality traits in patients with cCSC compared to the general population. Interestingly, the personality profile of patients with cCSC in our cohort tended towards more similarities with the profile of patients treated for Cushing's disease than to the general population, since 14 out of the 18 DAPPsf subscales outcomes of patients with cCSC were comparable with outcomes of patients treated for Cushing's disease, where only eight out of 18 were comparable with the general population (Figure 1, with lines resembling patients with cCSC in between the lines corresponding with reference data of the general population and patients treated for Cushing's disease). However, these findings were not statistically significant.

Cushing's disease is a rare condition which is characterized by exposure to excessive cortisol levels. Therefore, these patients can be regarded as a human model to study the effects of cortisol excess on personality and behaviour. Maladaptive personality traits and psychological morbidity, such as somatic arousal, negative affect, irritability and apathy, have well been documented in patients with Cushing's disease [33,34]. Patients with cCSC showed less affective lability, cognitive distortion and oppositionality compared to patients treated for Cushing's disease, whereas they reported more conduct problems, although the significance of this difference was omitted in the post hoc analysis excluding patients with less typical cCSC. Apathy and irritability scores of patients with cCSC in our cohort were lower, yet not statistically significant compared to scores of patients treated for Cushing's disease. We have recently demonstrated that patients with cCSC

have an activated HPA axis in the presence of high normal serum levels of cortisol [9]. In line with this biochemical resemblance of an activated HPA axis in both patient groups, with patients with Cushing's disease at the far end of the spectrum of HPA - axis activation and patients with cCSC showing a slightly activated HPA axis, the present study showed there may also be similarity between patients with cCSC and patients treated for Cushing's disease regarding the spectrum of personality features. Despite this relative degree of similarity, there was no statistically significant difference in the tested personality traits between the current cCSC cohort and a healthy general population. In literature, a possible association between the occurrence of CSC and a combination of stressful life events and unfavourable coping styles has been reported, with patients with acute CSC reporting more unfavourable stress coping compared to patients with cCSC [32]. Our current data suggest that patients with cCSC seek more social support. This may be explained by the fact that cCSC results in visual impairment affecting quality of life [50], which makes patients more dependent on others. Moreover, patients with cCSC reported to use more active coping, but also more passive coping. Although this may seem to be somewhat counterintuitive, it should be noted that coping behaviour is situation dependent, so that individuals can adapt their coping strategy based on the situation [51].

The comparison of validated questionnaires outcome of a large cohort of patients with cCSC with both healthy controls and a cohort of patients treated for Cushing's disease enabled to describe personality traits, psychological morbidity and coping strategies within a broad spectrum of HPA - axis activity. Nevertheless, to find an ideal control group is challenging and the lack of a gender- and age-matched control group can be considered a potential limitation of this study. However, population-based reference data were available and considered to be a worthy alternative, since these data were derived from large population-based cohorts [48]. The gender and age distribution of our cohort is in accordance with available literature [6,40]. Yet, since the majority of our population is male (90%), our results may not be generalizable to female patients with cCSC. This study aimed to investigate personality traits in patients with cCSC, and with the validated questionnaires used, we did not find an association with type A behavioural characteristics in these patients.

Using validated measures, we found no evidence for a higher prevalence of maladaptive personality traits such as type A behavioural characteristics in patients with cCSC, nor any clear differences in the generic personality traits as compared to the general population. This finding is of interest, as ophthalmologists often assume and report stress-related and type A behavioural characteristics in patients with cCSC, and therefore, the advice on stress reduction to these patients appears common in their management strategies [5,52,53]. However, our paper indicates that psychological interventions targeting these personality features in cCSC, as was suggested in previous literature [10,54], may not be useful. The results of the present study contribute to the psychological phenotyping of patients with cCSC, which may be used to design disease-specific support programmes that address coping mechanisms in patients with cCSC.

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APPENDIX

Post-hoc analysis without patients with less typical cCSC

Analyses performed without the 5 patients with atypical cCSC revealed, in addition to the previously reported result, that patients with inactive cCSC only reported more social avoidance compared to patients with active disease ($P<0.01$). Furthermore, when outcomes of the patients with cCSC were compared to reference data from the general population, no difference in compulsivity was observed anymore ($P=0.01$), and less callousness was observed in patients with cCSC ($P<0.01$). When comparing our patient data to patients treated for Cushing's disease, no difference on conduct problems was observed anymore ($P=0.01$). On the AS, patients with typical cCSC reported less apathy than patients treated for Cushing's disease ($P<0.01$). The exclusion of 5 patients with less typical cCSC also showed some minor differences on the UCS. When the males and females together were compared to the reference group of the general population, patients with cCSC made more use of the coping styles active coping ($P<0.01$), seeking social support ($P<0.01$), and passive coping ($P<0.01$). When male only data were compared to this group, the same differences were found (all $P<0.01$). Excluding the 5 patients with less typical cCSC did not significantly affect the remainder of the aforementioned results.

PART II

ORGANIZATION, OUTCOME EVALUATION AND QUALITY OF CARE FOR CUSHING'S SYNDROME

CHAPTER 6

MORTALITY REMAINS INCREASED IN CUSHING'S DISEASE DESPITE BIOCHEMICAL REMISSION: A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

The aim of this systematic review and meta-analysis was to investigate whether mortality is increased in patients biochemically cured after initial treatment for Cushing's disease. This is a systematic review and meta-analysis of follow-up studies in patients cured from Cushing's disease after initial treatment was performed. Eight electronic databases were searched from 1975 to March 2014 to identify potentially relevant articles. Original articles reporting the standardized mortality ratio (SMR) for patients cured of Cushing's disease were eligible for inclusion. SMRs were pooled in a random effects model. I^2 statistics was used for quantification of heterogeneity. Eight cohort studies with a total of 766 patients were included. Out of eight studies, seven showed an SMR above 1.0 for cured patients. The pooled SMR was 2.5 (95% CI 1.4–4.2). The I^2 statistics showed evidence for statistical heterogeneity (78%, Q-statistics $P<0.001$), which was largely explained by two outliers. This meta-analysis reveals that mortality remains increased in patients with Cushing's disease even after initial biochemical cure remission, suggesting that cure does not directly reverse the metabolic consequences of long-term overexposure to cortisol. Other conditions such as hypopituitarism, including persistent adrenocortical insufficiency after surgery, may also contribute to the increased mortality risk.

INTRODUCTION

Cushing's disease is characterized by endogenous glucocorticoid excess resulting from an adrenocorticotropic hormone (ACTH)-secreting pituitary adenoma. The incidence of Cushing's disease is estimated to be 1.2–2.4/million per year [1], although it is higher in selected patient populations such as poorly controlled diabetics and in young patients with osteoporosis or hypertension [2]. Glucocorticoid excess induces changes in body composition (sarcopenia, osteoporosis, and central obesity), an adverse metabolic profile (dyslipidemia, hypercoagulability, insulin resistance, and diabetes mellitus), and hypertension [3]. Moreover, the association between Cushing's disease and neuropsychiatric disorders is well established [4]. Untreated Cushing's disease has a poor prognosis as the 5-year survival is estimated to be only 50% [5]. The increased mortality in Cushing's disease is mainly caused by macrovascular disease (myocardial infarction and stroke), but poorly controlled diabetes mellitus and infections may also play a role [6]. Selective removal of the corticotrope adenoma by transsphenoidal surgery remains the standard treatment for Cushing's disease. In patients diagnosed with Cushing's disease, mortality is increased compared with the general population [6, 7]. However, whether mortality is also increased in patients cured after initial therapy is yet to be elucidated. In addition, data on the factors predictive of mortality in this population are rare.

The question whether mortality remains increased after initial cure is important for risk stratification in order to devise strategies for follow-up and treatment of co-morbidities. For proper patient management, it is also important to provide adequate information to the patients. The primary aim of this systematic review and meta-analysis was to answer the question whether mortality is increased or not after initial biochemical cure for Cushing's disease.

METHODS

Search strategy

To review currently available studies on mortality rates in Cushing's disease cured by transsphenoidal surgery, we conducted a search for all publications in English, French, German, Spanish, Dutch, and Danish languages on the topic (all languages spoken by the authors). The following databases were searched from 1975 to March 2014: PubMed, Cochrane Library, Web of Science, EMBASE, CINAHL, Central, Academic Search Premier, and Science Direct. We constructed a search string focusing on Cushing's disease, transsphenoidal surgery, mortality, and standardized mortality ratio (SMR), with the cooperation of a trained librarian. These keywords were database-specifically translated. We restricted the search to articles published after 1975, as transsphenoidal surgery for Cushing's disease was introduced since then. Original studies were eligible for inclusion if they met the following criteria:

1. A cohort study including minimally ten patients with Cushing's disease cured after initial therapy.
2. A mean follow-up period of at least 1 year.
3. Mortality risk expressed as SMR.

Studies were excluded when restricted to children. In the event of (partial) duplication of cohorts, the study with the longest follow-up period was included.

Data review and analysis

All identified articles were entered in EndNote version 7 (Thomson Reuters, Philadelphia, PA, USA). The initial selection of studies by title and abstract was performed by one reviewer (F M van Haalen) and the remaining studies were retrieved for closer examination by three reviewers (F M van Haalen, L H A Broersen, and O M Dekkers) and disagreement was solved by consensus. Retrieved articles were screened using a gauge for judgment meeting our inclusion and exclusion criteria. From included studies, we extracted the recruitment period, the duration of follow-up, inclusion and exclusion criteria, the number of patients included, the number of patients cured, SMR, the number of patients lost to follow-up, the methods used for diagnosis, the criteria for cure, surgical, radiological, and histological details, and other therapies used besides transsphenoidal surgery. Finally, we searched whether predictors for mortality in cured patients were reported.

Definition of cure

For definition of cure, we used the definition as provided in the individual articles. In all articles included, patients were considered cured in case of biochemical remission (i.e. eucortisolism or hypocortisolism). Minimal requirements were suppressed post-surgical cortisol with the need for replacement therapy, or, if no replacement therapy was used, normal 24-h urinary free cortisol (UFC) and/or the normal overnight 1mg dexamethasone suppression test (DST).

Risk of bias assessment

For all included studies, the risk of bias was assessed using the following components, as they could potentially bias an association between the exposure (cure of Cushing's disease by transsphenoidal surgery) and outcome (SMR).

1. Loss to follow-up <5% was considered a low risk of bias.
2. No exclusion of patients with late recurrences was considered a low risk of bias, as exclusion of these patients may underestimate the mortality risk.
3. Adequate definition of cure of Cushing's disease represents a low risk of bias.
4. Ascertainment of exposure to Cushing's disease by histological assessment of the adenoma was considered a low risk of bias.

Statistical analysis

The pooled SMR after successful treatment of Cushing's disease was the primary outcome measure of this analysis. For all studies, the SMR was extracted with its accompanying CI. Meta-analysis for SMR was performed using the metan command in Stata 12.1 (Stata Corp., College Station, TX, USA) in a random effects model. For one article [8], the SMR was calculated from the observed and expected mortalities mentioned in the article, and the CI was calculated using the method by Vandenbroucke [9]. I2 statistics and Cochran's Q-test were used to quantify statistical heterogeneity.

Meta-analysis of SMR for patients not cured of Cushing's disease was performed to compare the results with the pooled SMR for patients cured of Cushing's disease. A meta-regression was performed to compare the mortality risk in cured patients vs uncured patients using the metareg command in Stata.

RESULTS

The initial search resulted in a total of 1089 publications, of which 1058 were excluded based on the title and abstract. Of the remaining 31 articles, eight cohort studies were included [1, 7, 8, 10, 11, 12, 13, 14]; see Figure 1 for the flow chart. In one study, results were stratified by the size of the adenoma (microadenoma vs macroadenoma). Included studies were published between 2001 and 2013.

Study characteristics

A summary of characteristics of included studies reporting SMR in cured Cushing's disease is presented in Table 1. Eight studies included a total number of 766 cured patients who were diagnosed with Cushing's disease, the majority (79%) of whom were females.

Reported mean age at diagnosis was similar in all studies, ranging from 36 to 45 years. In all studies, the proportion of patients initially treated by transsphenoidal surgery was >50%, with 100% in three studies. A similar definition of cure was used by six studies (resolution of symptoms and clinical signs, adrenal insufficiency requiring cortisol replacement therapy, or no replacement, and normal UFC and/or a normal overnight 1mg DST). In two studies, only post-operative morning cortisol measurements below 1.8 g/dl (50 nmol/l) were used as a definition for cure [12, 13]. Furthermore, only limited data were provided on surgical, radiological, and histological details stratified by cure status.

Risk of bias assessment

Loss to follow-up was not mentioned in any of the included studies. Only one article [12] excluded eight patients (11% of total) with late recurrence of the disease. Therefore, the risk of underestimation of the SMR due to exclusion of late recurrences is considered to be low. The percentage of adenomas histologically proven to be ACTH secreting was only reported in three of the included studies, varying from 71.1 to 100%. The mean duration of follow-up was 8.9 years. Definition of cure was adequate in all articles.

Meta-analysis of mortality risk in patients cured of Cushing's disease

Reported SMRs ranged between 0.3 and 10.0, with seven out of eight studies reporting an SMR with a point estimate >1.0 (Figure 2). We estimated a pooled SMR of 2.5 (95% CI 1.4–4.2) in a random effects model, with evidence of statistical heterogeneity: I2=78% (Cochran's Q statistic P<0.001). Two reported SMRs were clear outliers (0.3 and 10.0) [1, 13]. A sensitivity analysis without these two datasets showed a similar pooled SMR of 2.1 (95% CI 1.5–3.0), but with lower heterogeneity (I2=40%, Cochran's Q statistic P=0.125). In addition, we searched for possible predictors of mortality in cured Cushing's disease, such as age at diagnosis, sex, tumor size and invasiveness, time to cure, persistent

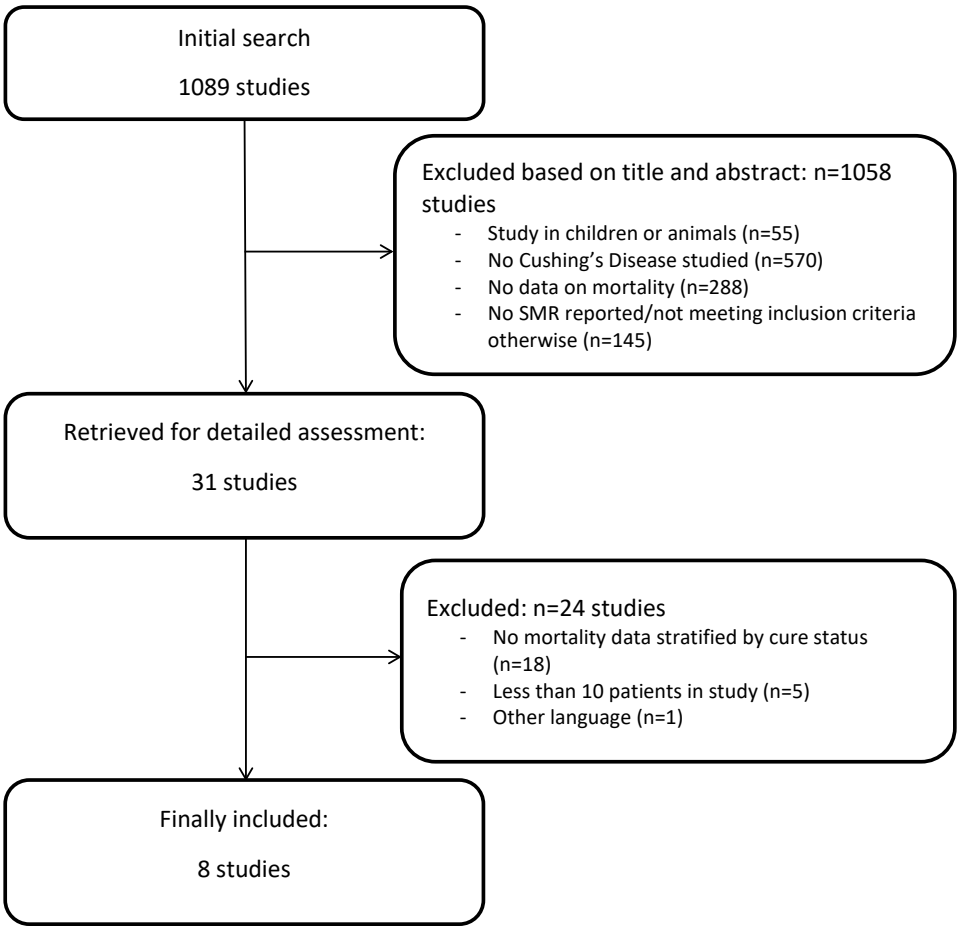

Figure 1. Flow-chart of study inclusion.

adrenal insufficiency, hormonal axis deficiency after surgery, and pre-operative disease severity. Unfortunately, we could not extract sufficient data from the original articles for additional analyses.

Meta-analysis of SMRs for patients not cured of Cushing's disease

For seven articles, the SMR for patients without initial cure by transsphenoidal surgery was also reported (Figure 3). Reported SMRs ranged between 2.4 and 16.0. We found a pooled SMR of 4.6 (95% CI 2.9–7.3). The seven included studies showed some evidence of statistical heterogeneity (I2=40%, Cochran's Q statistic P=0.113).

A meta-regression was performed to address the question whether mortality risk in uncured Cushing's disease (SMR 4.6) was significantly higher compared with cured Cushing's disease (SMR 2.5). This analysis showed an increased risk for uncured patients with Cushing's disease compared with cured patients with Cushing's disease: 1.8 (95% CI 0.9–3.7).

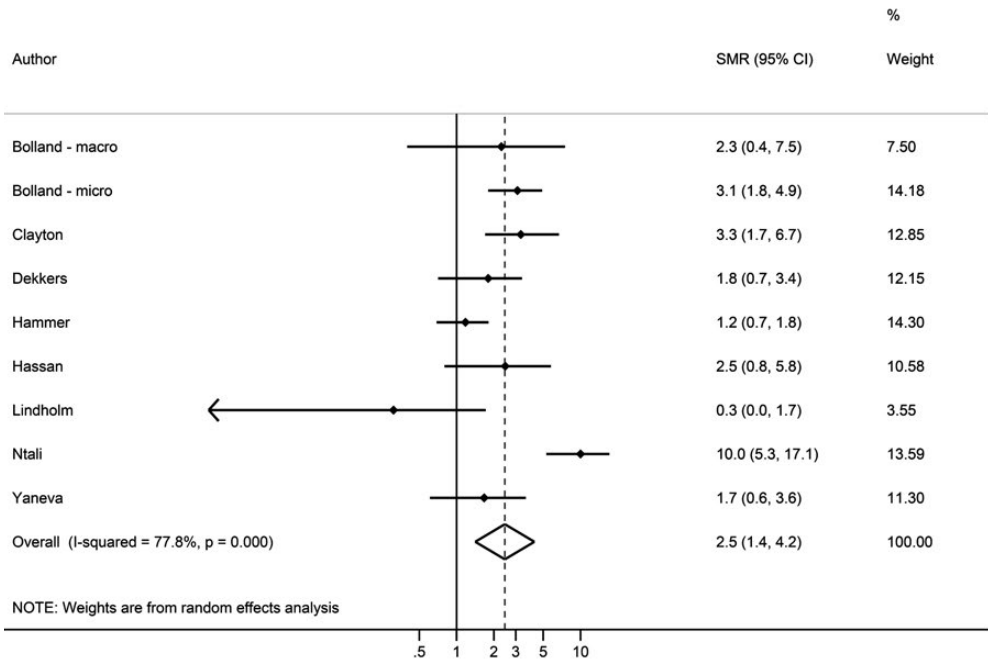

Figure 2. Meta-analysis of mortality risk in cured Cushing's disease.

DISCUSSION

This systematic review and meta-analysis shows that mortality risk is increased despite biochemical cure in Cushing's disease. The pooled SMR was 2.5 (95% CI 1.4–4.2), and remained increased in a sensitivity analysis excluding two outliers. The persistently increased mortality risk despite remission of hypercortisolism suggests irreversible effects of long-term glucocorticoid excess exposure.

To date, results of two previous meta-analyses on mortality in patients successfully treated for Cushing's disease have been published. The first study by Clayton et al. [10] included four studies, all of which were also included in the present meta-analysis. This study found a pooled SMR of 1.2 (95% CI 0.5–3.2) for cured patients, and a pooled SMR of 5.5 (95% CI 2.7–11.3) for patients with persistent disease. The second meta-analysis by Graversen et al. [15] included three studies, all of which were also included in the meta-analysis by Clayton et al. and in the present meta-analysis. They also found a pooled SMR of 1.2 (95% CI 0.5–3.0) in cured patients and 3.7 (95% CI 2.3–6.0) in patients with persistent disease. We additionally included four recently published studies, thereby increasing the power of the meta-analysis, which resulted in the finding that mortality is increased despite cure. Two studies included in our meta-analysis were clear outliers. The study by Lindholm et al. [1] showed an SMR of 0.3 (95% CI 0.0–1.7), and the study by Ntali et al. [13] with an SMR of 10 (95% CI 5.3–17.1). Both SMRs had large CIs, pointing toward the uncertainty that accompany the effect estimates. However, exclusion of these two studies in a sensitivity analysis did not significantly change the results and interpretation of our conclusion.

Table 1. Summary of included studies reporting mortality in Cushing's disease.

First author (year)	Lindholm (2001)	Hammer (2004)	Dekkers (2007)	Bolland - macroadenoma (2011)
Period covered	1985-1995	1975-1998	1977-2005	1960-2005
Gender (M/F)	23/50	50/239	18/56	8/22
Mean age at diagnosis (yrs)	41[a]	36[b]	39[b]	45
Number of cured patients	45	236[c]	59	19
Mean follow-up (yrs)	8.1[a]	11.1[a]	12.8	6.9
Definition of cure	Subnormal plasma cortisol after ACTH test and/or normal UFC / or panhypopituitarism	Normal basal cortisol or DST and/or UFC, symptoms resolution, and no additional therapy	Normal DST and UFC in 2 consecutive samples	Adrenal insufficiency requiring replacement therapy; or no glucocorticoid therapy and normal UFC; or normal DST at last FU
Initial transsphenoidal surgery (%)[f]	53.6	100	100	NR
Recurrent disease during follow-up (%)	4 (2/45)	9 (13/150)	14 (8/59)	26 (5/19)
Post-surgical hormone deficiencies	27% (12/45) panhypopituitarism	27 (=11%) total postoperative hormonal replacement	18% pituitary insufficiency (44% at least one axis)	NR

NR, not reported; ACTH, adrenocorticotropic hormone; UFC, 24-h urinary free cortisol; DST, dexamethasone suppression test; FU, follow-up. [a]Median. [b]Age at operation instead of diagnosis. [c]Of which 150 had a FU >6 months. [d]Out of 159 with initial transsphenoidal surgery. [e]Out of 154 with initial transsphenoidal surgery. [f]Characteristics based on total cohort and not on cured patients only. [g]100% in patients diagnosed after 1985.

A limitation of the present systematic review is that included studies mainly provided patient characteristics for the whole cohort rather than stratified by cure status. This limited the possibility to assess potential causes of increased mortality in more detail by meta-regression techniques. Similarly, the percentage of post-treatment hypopituitarism could not be abstracted from all included studies and the percentages reported varied widely from 8.7 to 71% [8, 12]. It is also important to consider that patients included were not necessarily cured by transsphenoidal surgery, but were also treated by transcranial surgery, radiotherapy, or adrenalectomy. However, as shown in Table 1, the vast majority of patients were treated by initial transsphenoidal surgery. The three studies in which 100% of patients were initially treated by transsphenoidal surgery [8, 11, 12] also point toward an increased mortality risk after biochemical cure (SMR 1.2, 1.8, and 2.5 respectively).

From a pathophysiological as well as epidemiological point of view, it is sensible to assume that mortality in cured Cushing's disease is increased when compared with the general population. There is increasing evidence that glucocorticoid excess-related morbidity decreases after successful treatment of Cushing's disease, but does not normalize. It has been shown that patients cured from Cushing's disease still have a high prevalence of atherosclerosis and maintain an increased cardiovascular risk, probably due to residual abdominal obesity and/or insulin resistance

Bolland - microadenoma (2011)	Clayton (2011)	Hassan (2012)	Ntali (2013)	Yaneva (2013)
1960-2005	1958-2010	1988-2009	1967-2009	1965-2010
36/122	9/51	15/57	45/137	43/197
36	NR	40	40[a]	38
117	54	52	99[d]	85[e]
7.5	1.3[a]	4.6[a]	12[a]	7.1[a]
Adrenal insufficiency requiring replacement therapy; or no glucocorticoid therapy and normal UFC; or normal DST at last FU	Symptoms resolution; normal UFC and DST (and normal plasma cortisol day curve for those on metyrapone), < 3 yrs after treatment	Normal morning postsurgical cortisol and continued biochemical cure during FU	Undetectable postsurgical cortisol and continued biochemical cure during FU	Absence of clinical hypercortisolism; normal/low UFC or 17-OH and ketosteroids (earlier cases) and normal DST at last clinical visit
NR	58.3[g]	100	87.4	66
15 (18/117)	NR	13 (8/60)	9 (9/99)	NR
NR	NR	71% (51/72) deficiency of at least one hormone	22-54% for various hormones	NR

syndrome [16]. In addition, MRI studies showed favorable changes in body fat distribution and a decrease in some cardiovascular risk factors (for example, insulin resistance, leptin, and total cholesterol), but other markers such as adiponectin and C-reactive protein did not change after remission [17]. In line with this, it was shown in a large cohort study that the risk for myocardial infarction and stroke remained increased during a long-term follow-up [6]. Subtle cognitive impairments and an increased prevalence of psychopathology after long-term cure of Cushing's disease are also documented [18, 19]. To address the question whether mortality is increased after biochemical cure, it is important not to adjust for baseline imbalances in cardiovascular risk, as the higher prevalence of risk factor in patients with Cushing's disease is a direct consequence of the disease. The SMR is a ratio measure that provides such unadjusted estimates.

Besides direct cortisol-excess related effects, hypopituitarism including secondary adrenocortical failure after surgical cure of Cushing's disease may contribute to the observed increased mortality, because hypopituitarism per se is associated with an increased mortality [20, 21]. Current glucocorticoid replacement therapy does not mimic physiological cortisol secretion, resulting in over- or under-replacement, and improved replacement modalities improve adverse cardiovascular risk profile and the quality of life [22]. Unfortunately, included articles did not provide detailed clinical data to address

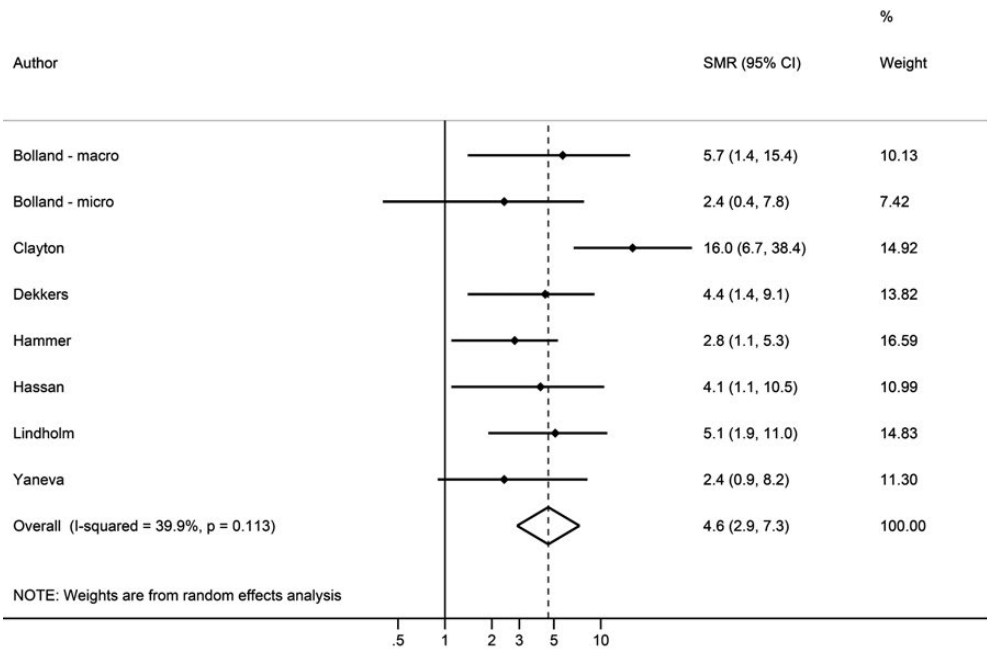

Figure 3. Meta-analysis of mortality risk in uncured Cushing's disease.

the effect on mortality of the factors mentioned above. Accordingly, we were not able to assess whether the increased mortality risk despite cure was mainly due to patients with a late recurrence, as late recurrences appear to be increasingly recognized [23] and might contribute to mortality. By not excluding late recurrences, the analyses were performed based on characteristics known at the time of prediction without the risk of selection bias.

In conclusion, this meta-analysis shows that mortality in Cushing's disease remains elevated after biochemical cure. This finding suggests that biochemical cure does not fully reverse the metabolic consequences of long-term overexposure to cortisol, which is supported by evidence showing persistent multisystem morbidity after biochemical cure. Other conditions, such as hypopituitarism (including adrenocortical insufficiency) after surgery, may also contribute to the increased mortality risk. Future research should aim to disentangle risk factors contributing to mortality in cured patients.

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CHAPTER 7

LONG-TERM EFFECTS OF CUSHING'S DISEASE ON VISUOSPATIAL PLANNING AND EXECUTIVE FUNCTIONING

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ABSTRACT

Background

Patients with remitted Cushing's Disease (CD) often present persisting impairments in executive and cognitive functioning domains. Little research has been conducted regarding the functional neural correlates of an important executive functioning skill, namely the ability to plan, in these patients. We used functional magnetic resonance imaging (fMRI) to examine visuospatial planning related brain activity in patients with remitted CD and matched controls.

Methods

fMRI scans were made using a 3-Telsa scanner while remitted CD patients (n=21) and age-, gender-, and education matched healthy controls (HCs; n=21) completed a parametric Tower of London (ToL) task. Psychological and cognitive functioning were assessed using validated questionnaires. Clinical severity was assessed retrospectively using the Cushing's syndrome Severity Index (CSI).

Results

CD Patients were on average 45.1 (SD=7.1) years old, 81% female, and in remission for mean 10.68 (SD=7.69) years. No differences were found in number of correct trials, response times per ToL trial, or in the region of interest analyses. Exploratory whole-brain analyses found that CD patients showed more activation in several brain regions associated with higher cognitive processes on 2-, 3-, and 5-step trials compared to HCs. Over-recruitment of the right parietal operculum cortex in the patients was significantly negatively associated with the prior active disease state on the CSI ($r=-0.519$, $p=0.02$).

Conclusions

The increased brain activation during the ToL in remitted CD patients versus controls signals over-recruitment of certain brain areas involved in higher cognitive processes. CD may thus result in long-lasting, subtle scarring effects during demanding executive functioning tasks, despite remission.

INTRODUCTION

Cushing's disease (CD) is characterized by hypercortisolism caused by a pituitary adenoma secreting excessive amounts of adrenocorticotropic hormone (ACTH); [1]. A variety of psychiatric symptoms can be induced by hypercortisolism, whereby the most common is major depressive disorder. However, mania, anxiety, and cognitive dysfunction also often co-occur [2]. Although CD can be effectively treated, usually by means of transsphenoidal surgery, increased mortality [3], residual psychopathological and physical morbidity [4-6], and reduction in quality of life [7] often remain. Furthermore, several important skills within the cognitive functioning domain have also often been found to remain impaired [6,8,9].

It is likely that these residual symptoms are associated with the detrimental effects of long-term exposure to hypercortisolism on brain function and structures [10]. Several neuroimaging studies have observed changes in both brain structure and function in patients with active CD [10-12]. As for the structural changes of the brain, certain abnormalities appear to persist after successful treatment of CD. The decreased hippocampal volume often found in patients with active CD, seems to normalize in patients with remitted CD [5,13,14]. In contrast to this, altered gray matter volumes of, for example, the anterior cingulate cortex (ACC) tend to persist after remission [10,15].

Regarding the functional brain alterations in CD, functional magnetic resonance imaging (fMRI) studies with remitted CD patients have also demonstrated various abnormalities in brain activity in this patient population in comparison to healthy controls (HCs). Resting-state functional MRI (rs-fMRI) studies with remitted CD patients have found increased resting-state functional connectivity (RSFC) between the limbic network and the ACC, the default mode network in the left lateral occipital cortex [16], and elevated RSFC in the medial temporal lobe, the hippocampus, and the prefrontal cortex networks [17]. In these studies, functioning of the executive control network was similar in both remitted CD patients and healthy controls, however, this resting state study did not include specific goal-oriented tasks that require high cognitive effort. Perhaps differences in functional activity within this network may only manifest when the cognitive demands are higher, as has been found to be the case in patients with other stressrelated psychopathologies, such as depression and post-traumatic stress disorder (PTSD) [18-20].

Cognitive functioning has been examined by means of standard neuropsychological testing in active CD patients [5,6], as well as in remitted patients after a follow-up period of up to 18 months [8]. These studies found that cognitive and executive functioning (i.e., psychomotor functioning, visuoconceptual tracking, processing speed, auditory attention, auditory working memory, verbal fluency, reading speed, and brief attention) are (and perhaps remain) impaired in active and remitted CD patients. An important cognitive function necessary to lead a functional life is the cognitive skill of planning. Cognitive planning encompasses the neurological processes that are involved with the strategy formulation, coordination, evaluation, and selection of a thought sequence, and the necessary actions that are needed in order to achieve that goal [21]. Reductions of these cognitive abilities in patients with remitted CD may lead to lasting effects on planning abilities, affecting one's daily functionality, psychological state, and quality of life. To date, alterations in brain activity patterns with regard to cognitive planning and executive functioning within the remitted

CD patient population have not been studied. A task that is often used to detect alterations in brain activation with regard to cognitive planning and executive function is the Tower of London (ToL) task [22].

In this study, we examined whether patients with remitted CD display altered performance and brain activity patterns in comparison to healthy controls (HCs) with regard to cognitive planning and executive functioning using the ToL paradigm. Based on previous research on cognitive functioning in CD patients, we hypothesized that remitted CD patients will complete less trials correctly, complete less trials in total, and take more time to complete a ToL trial in comparison to healthy controls. Furthermore, taking the differences in brain activation found in earlier studies with CD patients into account [10,16], we hypothesized increased activation in the ACC, an area involved in several complex cognitive functions and critically active when engaging in a cognitively demanding task [23], and often implicated in earlier findings within this patient population, in comparison to matched healthy controls. In addition, we performed an exploratory whole-brain analysis to examine whether other task-related differences in activation can be identified. Furthermore, potential associations between brain activity, psychological, cognitive, and clinical measures were explored.

METHODS AND MATERIALS

Subjects

Participants were all remitted CD patients (aged 18-60 years) who were being monitored at the Leiden University Medical Center (LUMC). Of the 49 invited participants, 96% responded to the invitation, and based on primary in- and exclusion criteria, 31 patients were ultimately screened for further study eligibility (details with regard to this study protocol have previously been published elsewhere; [10]). Healthy controls (HCs) were recruited via advertisements in grocery stores and internet. HCs were matched to each patient based on gender, age, and level of education. A HC specific exclusion criterion was a history of or current psychiatric disorder. Further exclusion criteria for both the remitted CD and HC groups were neurological problems, MRI contraindications, a (history of) drug or alcohol abuse, and/or left-handedness. Six remitted CD patients were excluded due to one of these exclusion criteria. Finally, one remitted CD and their matched HC were excluded because behavioral data was not recorded, leaving the final sample to consist of 24 remitted CD patients and 24 matched HCs.

All remitted CD patients had received transsphenoidal surgery following a diagnosis of active CD based on clinical supervision as well as biochemical and radiological conformation conform current international guidelines and multiple positive test outcomes. Detailed information with regard to these criteria have previously been published elsewhere [5]. Following surgery, CD remission was confirmed by means clinical evaluation and multiple biochemical test outcomes (for example, normal 24-hr urinary cortisol excretion rates (<220 nmol/24-hr), normal midnight saliva cortisol (below 5.7 nmol/L), and normal overnight suppression of plasma cortisol levels (<50 nmol/l) by dexamethasone (1 mg)). Patients with remaining glucocorticoid dependency were substituted with hydrocortisone (on average 20 mg/day, divided over three doses), and evaluated twice yearly.

Prior to study participation, persistent biochemical cure of CD was confirmed in concurrence with the abovementioned diagnostic tests. Disease duration was identified as the moment earliest somatic signs were presented in a patient's history. Duration of remission was calculated from either the date of curative transsphenoidal surgery or from the date of normalization of biochemical tests in the case of initial persistent disease persistence following surgery. Written informed consent was obtained from all participants and the study protocol was approved by the medical ethical committee of the Leiden University Medical Center (LUMC). The protocol was written in accordance with the principles of the Helsinki declaration. Patient and treatment characteristics were obtained from patient medical records.

Behavioral and clinical severity assessment

Psychopathology and cognitive functioning were assessed using the following scales: the 10 item Montgomery-Åsberg Depression Rating Scale (MADRS)[24], and the 28 item Inventory of Depression Symptomatology (IDS) [25] to assess the severity of depressive symptoms. An interviewer assessed the MADRS, all other scales used were self-report. Anxiety was evaluated using the blood injury phobia, social phobia, blood injury subscales, and total score of the 15 item Fear Questionnaire (FQ) [26], and the 21 item Beck Anxiety Inventory (BAI) [27]. The 14 item Irritability Scale (IS) and the 14 item Apathy Scale (AS) were used to assess the severity of irritability and apathy, respectively [28,29]. Participants with total scores of more than 14 points were considered to be irritable or apathetic. Failures in memory, motor function, and perception were assessed using the 25 item Cognitive Failure Questionnaire (CFQ)[30]. Higher sum scores indicate greater symptom severity.

CD symptom severity during the active and the remitted disease state were established using the 8 item Cushing's syndrome Severity Index (CSI)[31]. The CSI score during active disease was estimated retrospectively. The remission score was based on the last annual evaluation. Total CSI scores were used for both active and remitted disease states and scores on this index can range between 0 and 16 (higher total score indicates greater symptom severity). The necessary information in order to score the CSI was obtained from the patient's clinical history and medical records. The index was scored by two independent raters that reached consensus in case of discrepancy. Finally, prior to and after the fMRI ToL task, anxiety levels were monitored by means of a Visual Analogue Scale (VAS) [32] ranging from 0 to 100, where a higher score indicates a higher level of anxiety.

Task paradigm

An event-related parametric version of the ToL was used. A detailed description of this task has been previously published [33]. In brief, participants were presented with either a baseline or test trial. In the baseline trials, participants were requested to count the number of yellow and blue beads presented on the screen. In the test trials, participants were requested to count the minimum number of steps from the 'start' condition to the 'goal' condition. The test trials ranged from 1 to 5 steps (see Figure 1 for examples). The task was pseudorandomized and self-paced, with a maximum response duration of 60 seconds for each trial. The trial was presented by means of E-Prime software (Psychological Software Tools, Pittsburg, PA, USA). Responses and response times were logged by means of button boxes. No feedback was given with regard to the answers.

Image Acquisition

The ToL paradigm was part of a larger fMRI protocol, which included a resting-state scan and an emotional faces paradigm. In each session, the ToL was administered as the first fMRI paradigm in each session. The task duration was 17 minutes and 36 seconds. Imaging data were acquired in the LUMC using a Philips 3T system (Philips Healthcare, Best, The Netherlands; software version 3.2.1). A SENSE-32 channel headcoil was used for transmission and reception of radio frequencies. For each subject, anatomical imaging was acquired by means of a transvers 3D gradient-echo T1-weighted sequence (repetition time (TR) = 9.8, echo time (TE) = 4.6 ms, flip angle = 8º, Field of view (FOV) matrix size = 256 x 256, voxel size = 1.17x1.1.7x1.2 mm, 140 slides), which were examined by a neuroradiologist blinded for patient details. Other than age-related white matter intensities and effects of post-transsphenoidal surgery, no further macroscopic abnormalities were detected. ToL fMRI echoplanar images (EPI) were acquired using a T2*-weighted gradient-echoplanar imaging sequene (EPI) (TR = 2200 ms, TE = 30 ms, flip angle = 80º, 38 transverse slices, no slice gap, FOV = 220 × 220 mm, voxel size = 2.75 × 2.75, 3 mm slice thickness), which was then registered to the MNI T1-template brain.

Data analysis

Task performance and clinical characteristics

Psychometric and task performance data were analyzed using IBM SPSS Statistics for Windows version 24 (IBM Corp. Armonk, N.Y., USA). If the data did not meet the assumptions required for parametric analyses, the appropriate nonparametric tests were performed (i.e. Mann-Whitney U test). VAS scores and performance were analyzed using paired samples t-tests. Proportion correct scores and mean response times per trial were entered as dependent factors in the analyses.

Image processing

Preprocessing and analyzing of the ToL data was conducted using FSL v.5.0.8. Preprocessing included artefact removal with FSL FIX [34], motion correction (realignment), grand mean scaling, and spatial smoothing with 6mm Gaussian kernel. ICA-AROMA [35] was used for motion artefact removal, and high-pass filtering was used. FSL FEAT was used to create first-level statistical parametric maps.

The fMRI ToL paradigm was modelled in an event-related manner with regressors (i.e. explanatory variables) made by convolving each event-related stimulus function (baseline, 1-5 step trials), with a canonical hemodynamic response function, and then modulated using reaction times. Low-frequency noise was stripped by applying a high-pass filter (set at a cut-off of 128 seconds) to the time series at every voxel.

Main effects of task and between-group comparisons

Analysis were conducted in line with the ToL analysis approach reported in van Tol et al. [36]. Contrast images for task load, which ranged from trial type 1-5 with weighting [-1.5, -1.0, -0.5, 1, 2] respectively, were calculated per subject on a voxel-by-voxel basis and then entered into a secondlevel analyses

for between group comparisons (remitted CD and HC). Thus, in all cases, activation of the regions specified were modulated by the complexity of the task.

Thresholds for the main effects of the task and between group comparisons were corrected using a cluster z-threshold of 2.3 with $p < 0.05$. Between group comparisons were conducted using the ACC as a region of interest (ROI), followed by an exploratory whole-brain analysis per trial step. Significant clusters per trial step were tested for correlations measures of psychiatric symptom severity, cognitive functioning and clinical severity. The questionnaires used for

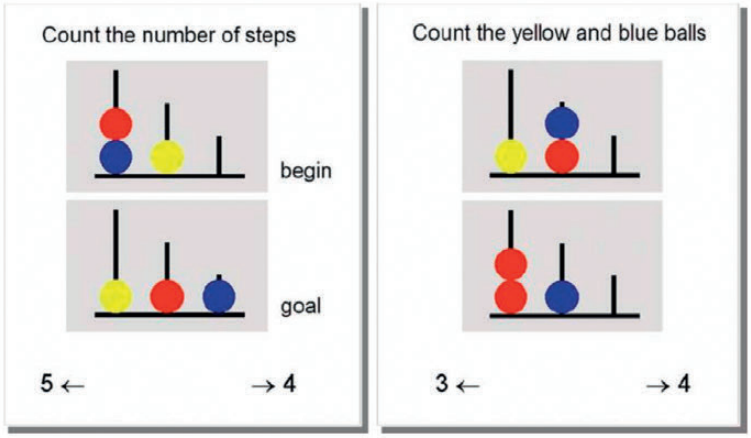


Figure 1. Example of a 5-step planning trial in the Tower of London (left figure), and a baseline trial with no planning involved (i.e. participants were asked to count the number of yellow and blue balls presented (right figure)).

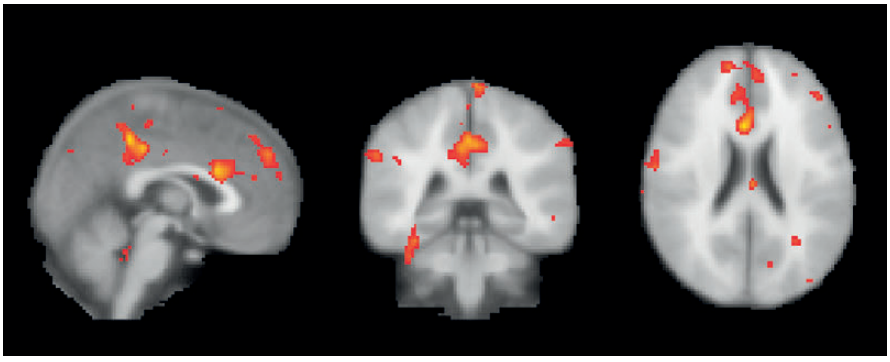


Figure 2. Mean activity during task performance across the subjects displayed at cluster z-threshold of 2.3 with $p < 0.05$ [-39, 36, 30].

the behavioral assessment show considerable overlap, therefore correction for multiple testing using the BenjaminiHochberg [37] method with an FDR set at 5% was considered too stringent. Therefore, we corrected for multiple testing using an FDR set at 20%. We report the uncorrected Pearson's correlations for normally distributed data, and the Spearman's rho for data that is not normally distributed.

RESULTS

Sample characteristics

Three subjects (1 remitted CD patient and 2 HCs) and their respective matched pairs (thus n = 6), were excluded from the analyses because they did not meet the prespecified overall performance percentage of more than 75% correct responses. This was done in order to increase the likelihood of capturing task-based planning activity and to reduce possible non-task related bias, resulting in a total of 21 pairs of participants. Remitted CD patients and the HCs were well-matched as they did not differ significantly in gender, age, education, and intercranial volume (ICV). Mean MADRS, IDS, BAI, and AS scores differed significantly between remitted CD and HC groups (all $p < 0.02$), whereas mean scores on the total FQ score and its subscales, and the IS did not (see Table 1 for further details). Mean disease duration in the remitted CD was 7.6 years and duration of remission, 10.7 years. Mean scores on the CSI were 7.95 (SE = 0.428) during the active phase and 2.33 (SE = 0.340) upon remission of CD.

Behavioral results

Mean VAS scores, mean accuracy scores, and mean response types per trial type are reported in Table 2. Remitted CD patients reported significantly higher levels of anxiety in comparison to healthy controls ($p = 0.02$) both before and after the task ($p = 0.006$). Overall, mean accuracy decreased with increasing task load. This did not differ significantly between the groups on any of the step trials. Also, performance speed increased as task load increased in both groups, although no differences in response times on any of the trial steps were found. An overview of the mean number of trials per trial type and group can be found in Appendix 1. Although the remitted CD group completed less trials per step in comparison to the HC group, this did not differ significantly between groups on any of the trial steps.

fMRI results

Main task effects

No participants were excluded from the analyses due to movement or scanning artifacts. The task effects across all participants identified two significant activity clusters: (i) in the superior frontal gyrus and the frontal pole, and (ii) in the cingulate gyrus (posterior division) and the precuneus cortex ($p < 0.05$ for both clusters after cluster correction; see Table 4 and Figure 2). No main effects of increasing task load were found in both the remitted CD group and the HC group. This is likely due to a lack of power due to less trials in the more difficult steps (Appendix 1). No significant activity clusters were found in the ROI. Significant activity clusters were found in the parietal operculum

Table 1. Demographic and clinical characteristics remitted CD patients and matched HCs.

	CD patients (n=21)	Matched controls (n=21)	*p*-value
Gender (female, (%))	17(81%)	17(81%)	1.00[a]
Age (years)	45.9 ± 7.1	44.6 ± 7.7	0.57[b]
Education (years)			1.00[a]
Low	5 (23.8%)	5 (23.8%)	
Medium	10 (47.6%)	10 (47.6%)	
High	6 (28.6%)	6 (28.6%)	
ICV mm^3 (mean)	$1.5110^6 \pm 1.4110^5$	$1.5210^6 \pm 1.6910^5$	0.76[b]
MADRS (mean)	5.43 ± 3.91	1.38 ± 1.80	<0.001[c]
Inventory of Depressive Symptomatology (mean)	45.55 ± 12.60	36.10 ± 6.07	0.02[c]
Beck Anxiety Inventory (mean)	28.15 ± 6.10	24.05 ± 3.34	0.02[c]
Fear Questionnaire (mean)	22.85 ± 17.10	14.52 ± 9.94	0.07[b]
Agoraphobia subscale	5.30 ± 6.69	2.67 ± 3.26	0.52[c]
Blood injury phobia subscale	6.45 ± 9.04	3.76 ± 4.28	0.73[c]
Social phobia subscale	11.10 7.33	8.10 ± 4.89	0.13[b]
Irritability Scale	11.90 ± 8.99	8.52	0.23[c]
Apathy Scale	13.6 ± 6.6	7.8 ± 3.8	0.002[c]
Cognitive Failures Questionnaire	35.60 ± 14.17	29.0 ± 9.46	0.09[b]
Disease duration (years)	7.55 ± 8.39		
Duration of remission (years)	10.68 ± 7.69		
Cushing's Syndrome Severity Index (CSI)			
Active phase (total)	7.95 ± 1.96		
Remission phase (total)	2.33 ± 1.56		

Data are presented as mean ± standard deviation or number (%), with a significance level set at P<0.05.

ICV = Intercranial volume; MADRS = Montgomery-Åsberg Depression Rating Scale

[a] p-values were tested with X^2 test

[b] p-values were tested with independent samples t-test

[c] p-values tested with Mann-Whitney U test

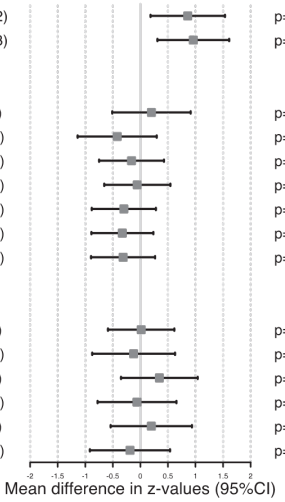
cortex on 2 step trials (z = 3.75, p = 0.005 after cluster correction), and in the supramarginal gyrus on 3 step trials in the remitted CD patient group (z = 3.27, p = 0.02 after cluster correction) in comparison to HCs. Group comparisons on the other trial steps did not reveal further significant differences (see Table 3). However, at a lower threshold (1.9), significant activity clusters were found in the remitted CD group on 4 and 5 step trials (see Appendix 2).

Correlation analyses

After adjusting for multiple comparisons using the Benjamini-Hochberg procedure [37], activation in of the right parietal operculum cortex in remitted CD patients was significantly negatively associated with the prior active disease state on the CSI (r = -0.519, p = 0.02). No other significant associations between significantly activated clusters and scores on behavioral scales, measures of disease duration, duration of remission, and clinical disease severity were found.

Table 2. Overview of proportion correct answers and response times (in seconds) per group (remitted Cushing's Disease and Healthy Controls).

Variable	RCD (n = 21)	HC (n = 21)	Mean difference	p-value
VAS prior to ToL (total score)	37.86 (29.63; 46.09)	19.81 (11.68; 27.94)	18.05 (3.87; 32.22)	p=0.01
VAS after ToL (total score)	34.05 (27.03; 41.07)	16.14 (9.04; 23.24)	17.90 (5.78; 30.03)	p=0.006
Proportion correct:				
- Baseline	0.98 (0.97; 0.99)	0.97 (0.96; 0.98)	0.00 (-0.01; 0.02)	p=0.56
- 1 step	0.93 (0.91; 0.95)	0.96 (0.94; 0.98)	-0.04 (-0.10; 0.03)	p=0.23
- 2 steps	0.91 (0.89; 0.93)	0.92 (0.90; 0.94)	-0.02 (-0.08; 0.05)	p=0.58
- 3 steps	0.91 (0.87; 0.95)	0.91 (0.87; 0.95)	-0.01 (-0.08; 0.06)	p=0.85
- 4 steps	0.79 (0.75; 0.83)	0.83 (0.79; 0.87)	-0.05 (-0.14; 0.05)	p=0.30
- 5 steps	0.73 (0.67; 0.79)	0.80 (0.74; 0.86)	-0.07 (-0.19; 0.05)	p=0.24
- Total	0.92 (0.91; 0.93)	0.93 (0.92; 0.94)	-0.17 (-0.49; 0.15)	p=0.27
Response time(s):				
- Baseline	3.26 (2.95; 3.57)	3.24 (2.81; 3.67)	0.12 (-0.52; 0.54)	p=0.96
- 1 step	4.86 (2.84; 6.88)	5.00 (2.47; 7.53)	-0.14 (-1.01; 0.73)	p=0.74
- 2 steps	6.73 (5.67; 7.79)	6.01 (5.30; 6.72)	0.72 (-0.74; 2.19)	p=0.31
- 3 steps	8.44 (7.24; 9.64)	8.62 (7.33; 9.91)	-0.18 (-2.24; 1.88)	p=0.86
- 4 steps	13.15 (10.72; 15.58)	12.15 (10.29; 14.01)	1.00 (-2.72; 4.72)	p=0.58
- 5 steps	15.32 (13.73; 16.91)	16.23 (13.76; 18.70)	-0.91 (-4.41; 2.59)	p=0.59



* *p*-values were tested using paired samples t-tests

**VAS: Visual Analogue Scale measuring anxiety prior to the ToL task and after the ToL task

DISCUSSION

In this study, we investigated whether patients with remitted CD displayed altered performance and brain activity patterns in comparison to HCs using the ToL, a parametric visuospatial planning task. No differences in performance were found between the groups, neither in the number of trials completed correctly, nor in the number of trials completed in total, nor in the amount of time needed to complete the trials. Mean task activation was found in two brain clusters. Our ROI analysis of the ACC did not yield any significant difference in activation between the groups. However, an exploratory whole-brain analysis found increased brain activity in certain areas in the patient group on 2-, 3-, and 5-step trials. Finally, we found a negative association between activation of the right parietal operculum cortex in remitted CD group with the prior active disease state as measured on the CSI. These findings indicate that CD can result in subtle scarring effects as seen in the altered activity in certain brain areas during demanding executive functioning tasks, despite long-term remission.

Previous research conducted with remitted CD patients found impairments in multiple domains of neurocognitive functioning, such as (auditory) attention, alerting, spatial orienting, processing speed, working memory, verbal fluency, reading speed, and brief attention in remitted CD patients [5,6,8,38]. We therefore hypothesized that remitted CD patients would complete less trials correctly, complete less trials in total, and take more time to complete a ToL trial in comparison

Table 3. Mean task activation and planned paired comparisons of activity related to increasing task load at threshold 2.3.

Mean task activation/ paired comparison	Area	Side	Cluster size	Peak Voxel (MNI) x (mm)	y (mm)	z (mm)	*p*
Mean task activation	Superior Frontal Gyrus/ Frontal Pole	L	801	-6	56	26	0.004**
	Cingulate gyrus, posterior division/ Precuneus Cortex	R	554	2	-40	44	0.03**
ROI							
ACC RCD*>HC	None						
ACC RCD<CD	None						
1 Step							
RCD>HC	None						
RCD<HC	None						
2 steps							
RCD>HC	Parietal Operculum Cortex	R	664	60	-36	26	0.005**
RCD<HC	None						
3 steps							
RCD>HC	Supramarginal Gyrus	R	527	58	-42	20	0.02**
RCD<HC	None						
4 steps							
RCD>HC	None						
RCD<HC	None						
5 steps							
RCD>HC	Occipital Fusiform Gyrus/ Lingual Gyrus	R	599	20	-74	-18	0.01**
RCD>HC	Supramarginal Gyrus	R	478	58	-38	28	0.04**
RCD<HC	None						

*RCD = remitted Cushing's Disease patients

*Thresholded using Cluster correction $z = 2.3$; $p < 0.05$.

to HCs. Surprisingly, remitted CD patients showed no cognitive and executive functioning deficits in comparison to HCs as measured on the ToL. As several studies have identified visuospatial impairments in active CD patients [39], albeit using other measurement instruments, our findings suggest that certain visuospatial impairments may improve upon remission of CD. However, further insight into whether remission of CD also remits all or most visuospatial impairments should be confirmed in longitudinal studies comparing performance on the ToL in the active disease state with the long-term remission state.

Findings from an earlier study with HCs aimed at validating the ToL paradigm for fMRI, found mean task activation in a number of brain areas (i.e., in the dorsolateral prefrontal cortex, the cingulate cortex, the cuneus, the supramarginal and angular gyrus in the parietal lobe, and the frontal opercular area of the insula) [40]. Although we did not expect to find precisely the same activation in all of the brain areas found in the aforementioned study due to the differences in our study populations and MRI scanners, we did expect to find a certain amount of overlap. We identified mean group activation in two separate brain clusters: (i) the superior frontal gyrus and

the frontal pole,and (ii) the posterior division of the cingulate gyrus and the precuneus cortex. Our first cluster (i.e., the superior frontal gyrus (part of the dorsolateral prefrontal cortex), and the frontal pole), partially overlapped with one of the activated areas found in the Lazeron et al. [40] paper (i.e. the dorsolateral prefrontal cortex). This area has been found to be involved in the management of uncertainty, where increasing uncertainty leads to increased activation [41], and the frontal pole has been implicated in cognition, perception, and working memory [42]. With regard to the second cluster identified (i.e. the cingulate gyrus (an area in the cingulate cortex) and the precuneus cortex), this largely overlaps with an area found in the Lazeron et al. [40] study (i.e. the cingulate cortex and the precuneus). These overlapping findings increase the validity of our current findings, and provide further evidence regarding the specific brain areas that are recruited during visuospatial planning tasks.

Considering the differences in brain structure and activation found in earlier studies with this same population of remitted CD patients [10,16], we hypothesized to find increased activation in the ACC, an area involved in several complex cognitive functions and critically active when engaging in a cognitively demanding task [23], in comparison to matched HCs. However, we did not find any differences in activation between the groups. This indicates that although the ACC has previously been implicated in displaying altered resting-state brain activity, altered gray matter volumes, and altered white matter integrity in this same patient group [10,14,16], they do not present increased recruitment of this area during the ToL task. It could, however, be the case that both patients and HCs overrecruit the ACC in this type of visuospatial planning and executive functioning task, as it is an area involved in several complex cognitive functions [43].

As mentioned earlier, certain regions were not identified in our mean group activation that were identified in the Lazeron et al. [40] paper. Interestingly, several of these areas were found to be more activated in the remitted CD group on a number of the trial steps. Increased right parietal operculum cortex recruitment was found as a function of increased planning load on 2-step trials in the remitted CD group. This is an area involved in mathematical thought, visuospatial cognition, and imagery of movement, among other functions [44]. Also, increased right supramarginal gyrus recruitment was found as a function of increased planning load on 3- and 5-step trials. This brain area has been found to be involved in complex cognitive functions, such as calculation and visuospatial awareness [45]. These findings indicate that remitted CD patients need to overrecruit these brain regions to attain a similar performance level as the HC's. Moreover, increased recruitment in the occipital fusiform implicated in higher processing for visual information such as the processing of color information, word recognition, and working memory capacity, amongst others [46-48], and lingual gyri was found on the 5-step trials. Both regions (i.e. the occipital fusiform and lingual gyri) have demonstrated to play an important role in color perception [49-50]. In sum, this indicates that remitted CD patients primarily characterize themselves in increased recruitment of the abovementioned brain regions on certain trial steps, and not in executive and cognitive functioning as measured in the number of trials answered correctly or the time needed to answer each trial.

Although no differences in altered brain activity were found on the 4-step trials, we believe this was likely due to lack of power (i.e. too few trials to be able to identify a possible effect). We therefore ran further exploratory whole-brain analyses at a lower threshold (i.e. 1.9) for all trial steps (see

Appendix 2). Although we cannot interpret these results as we interpret the results set at the more stringent and accepted threshold, we did find increased activation in the precuneus of the remitted CD group, an area that was also found to be overrecruited in the mean task activation. Moreover, activation in this area was also observed in 5-step trials at this lower threshold. Previous studies have shown activation in the precuneus during action generation tasks [51], as well as in visuospatial and -motor imagery [52-53]. It has also been suggested to be involved in the direct visual route from vision to action, functioning in extracting visual-motor and spatial relationship features [54].

A negative association was found between the right parietal operculum cortex in remitted CD patients and the CSI score of the prior active disease state. This indicates that the more severe the active disease state (as was measured using the CSI), the less activation in the right parietal operculum cortex, a region that has been found to be involved in mathematical thought, specifically in the knowledge of numbers and their relations [55]. These findings seem to imply that this brain region may be less proficient in increasing activation in remitted CD patients who have experienced a more severe active phase of CD. This highlights a possibly interesting region to study further in this patient population, as well as perhaps in other stress-related disorders. There were no further significant associations found between activated brain clusters and scores on behavioral scales, measures of disease duration, and duration of remission.

The hypothesis has been posited that studying patients with remitted CD could offer further insight into the effects of prolonged cortisol exposure on, amongst others, the brain, as these findings may in turn be (partially) generalizable to other remitted stress-related disorders (such as depression and/or anxiety), as well as to conditions treated with synthetic glucocorticoids. A previous study investigating the neural correlates of the ToL task in out-patients with (remitted) depression and anxiety found that only patients with a current moderate or severe depression had increased dorsolateral prefrontal cortex activation as a function of increasing task load, whereas patients with current mild or remitted depression, with a current diagnosis of anxiety disorder(s) (such as generalized anxiety disorder and/or panic disorder and/or social anxiety disorder) did not, in comparison to HCs [36]. Thus, it seems that the prolonged excess exposure of endogenous cortisol on the brain in the magnitude as is the case with CD, leads to seemingly permanent alterations in brain activation of certain brain regions after long-term disease remission in contrast to, for example, patients with remitted depression.

Due to the cross-sectional nature of this study, causal conclusions cannot be drawn as we cannot be certain whether the found differences in brain activity were present prior to the onset of CD. A further possible limitation of this study is the use of the CSI to evaluate disease severity during the active phase. Although this instrument has been validated repeatedly, it does make use of retrospective assessments, which may lead to less accurate estimations. Study strengths were the homogeneity of the patient population (i.e. all of the patients included in the study were treated by means of transphenoidal surgery), and the selection of the age-, gender-, and education matched HCs. Nevertheless, heterogeneity was present in the remitted CD patient group regarding duration of the disease and duration of remission, and this therefore may have decreased the precision of the effect estimates of the study.

In conclusion, we found no evidence for pervasive cognitive impairments for the domain of visuospatial planning and executive functioning as measured on the ToL task in remitted CD patients. Yet, differences in brain activation were found in the remitted CD patient group on 2-, 3- and 5-step trials, suggesting that the over-recruitment of a number of brain regions reflects persistent alterations in these specific brain regions after recovery from CD. Overall, remitted CD patients displayed increased activation on certain ToL trial steps in a number of brain areas involved with higher cognitive functions. The increased activation implies that remitted CD patients require more effort as measured in increased brain activity in certain brain regions to successfully complete a visuospatial planning task, although they do not need more time to do so accurately, indicating a subtle scarring due to CD. In the future, longitudinal studies are necessary to provide further insight with regard to the onset and course of alterations in cognition and brain activity patterns in the CD patient population during the active disease state and the transition into remission.

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APPENDIX

Appendix 1. Overview of number of trials per step per group (remitted Cushing's Disease and Healthy Controls) on the Tower of London task.

	remitted CD (n=21; no. of trials, (%))	HC (n = 21; no. of trials, (%))	Rounded mean no. of trials per participant remitted CD group (%)	Rounded mean no. of trials per participant HC group (%)
Baseline	1376 (40.6)	1410 (40.5)	66 (40.7%)	67 (40.4%)
1 step	647 (19.1)	669 (19.2)	31 (19.1%)	32 (19.3%)
2 steps	535 (15.8)	539 (15.5)	25 (15.5%)	26 (15.7%)
3 steps	347 (10.2)	358 (10.3)	17 (10.5%)	17 (10.2%)
4 steps	292 (8.6)	303 (8.7)	14 (8.6%)	14 (8.4%)
5 steps	195 (5.7)	200 (5.7)	9 (5.6%)	10 (6.0%)
Total	3392 (100.0)	3479 (100.0)	162 (100%)	166 (100%)

Appendix 2. Effects paired testing of steps at threshold 1.9.

Paired comparison	Area	Side	Cluster size	Peak Voxel (MNI) x (mm)	y (mm)	z (mm)	p*
1 step							
RCD>HC	Cingulate Gyrus, posterior division	R/L	1361	6	-48	20	0.005
RCD<HC	None						
2 steps							
RCD>HC	Supramarginal gyrus, posterior division/ parietal operculum cortex	R	2242	60	-36	26	6.53e-05
RCD<HC	None						
3 steps							
RCD>HC	Supramarginal Gyrus (posterior division)/	R	1105	58	-42	20	0.02
RCD>HC	Intracalcarine Cortex	L	1008	-16	-70	4	0.03
RCD<HC	None						
4 steps							
RCD>HC	Precuneous Cortex	R	965	0	-44	56	0.03
RCD<HC	None						
5 steps							
RCD>HC	Lingual Gyrus/Occipital Fusiform Gyrus	R	5398	-2	-36	-10	1.01e-09
RCD>HC	Supramarginal Gyrus, posterior division	R	1692	58	-38	28	<0.001
RCD>HC	Precentral Gyrus	L	1289	-58	4	36	0.007
RCD>HC	Precuneous Cortex	R	1265	2	-44	66	0.008
RCD<HC	None						

CHAPTER 8

CURRENT CLINICAL PRACTICE FOR THROMBOPROPHYLAXIS MANAGEMENT IN PATIENTS WITH CUSHING'S SYNDROME ACROSS REFERENCE CENTERS OF THE EUROPEAN REFERENCE NETWORK ON RARE ENDOCRINE CONDITIONS (ENDO-ERN)

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ABSTRACT

Background

Cushing's syndrome (CS) is associated with an hypercoagulable state and an increased risk of venous thromboembolism (VTE). Evidence-based guidelines on thromboprophylaxis strategies in patients with CS are currently lacking. We aimed to map the current clinical practice for thromboprophylaxis management in patients with CS across reference centers (RCs) of the European Reference Network on Rare Endocrine Conditions (Endo-ERN), which are endorsed specifically for the diagnosis and treatment of CS. Using the EU survey tool, a primary screening survey, and subsequently a secondary, more in-depth survey were developed.

Results

The majority of the RCs provided thromboprophylaxis to patients with CS (n = 23/25), although only one center had a standardized thromboprophylaxis protocol (n = 1/23). RCs most frequently started thromboprophylaxis from CS diagnosis onwards (n = 11/23), and the majority stopped thromboprophylaxis based on individual patient characteristics, rather than standardized treatment duration (n = 15/23). Factors influencing the initiation of thromboprophylaxis were 'medical history of VTE' (n = 15/23) and 'severity of hypercortisolism' (n = 15/23). Low-Molecular-Weight-Heparin was selected as the first-choice anticoagulant drug for thromboprophylaxis by all RCs (n = 23/23). Postoperatively, the majority of RCs reported 'severe immobilization' as an indication to start thromboprophylaxis in patients with CS (n = 15/25). Most RCs (n = 19/25) did not provide standardized testing for variables of hemostasis in the postoperative care of CS. Furthermore, the majority of the RCs provided preoperative medical treatment to patients with CS (n = 23/25). About half of these RCs (n = 12/23) took a previous VTE into account when starting preoperative medical treatment, and about two-thirds (n = 15/23) included 'reduction of VTE risk' as a goal of treatment.

Conclusions

There is a large practice variation regarding thromboprophylaxis management and perioperative medical treatment in patients with CS, even in Endo-ERN RCs. Randomized controlled trials are needed to establish the optimal prophylactic anticoagulant regimen, carefully
balancing the increased risk of (perioperative) bleeding, and the presence of additional risk factors for thrombosis.

BACKGROUND

Cushing's syndrome (CS) is characterized by excessive tissue exposure to glucocorticoids, caused by either exogenous administration of synthetic glucocorticoids, or excessive endogenous secretion of cortisol. Endogenous CS is rare, with an estimated incidence of 0.2–5.0 cases per million inhabitants per year in various populations, whereas its prevalence is close to 39–79 cases per million inhabitants [1]. Endogenous CS is most commonly caused by a pituitary corticotroph adenoma (Cushing's Disease, CD), accounting for 70% of all CS cases, and least frequently by adrenocorticotropic hormone (ACTH)-secreting non-pituitary tumors (ectopic ACTH and corticotropin-releasing hormone syndrome, CRH). ACTH-independent CS, is most commonly caused by an unilateral adrenal adenoma, or in fewer cases by bilateral micronodular, or macronodular adrenal hyperplasia, or adrenal carcinoma [1].

In recent years, the association between CS and hypercoagulability has gained growing interest. Multiple cohort studies reported an increased risk for venous thromboembolism (VTE), which encompasses pulmonary embolism (PE) and deep vein thrombosis (DVT), in patients with CS, both during the active phase of disease, and in the postoperative period after transsphenoidal surgery or adrenalectomy, and even after biochemical remission [2]. In their systematic meta- analysis, Wagner et al. found an almost 18-fold higher incidence of VTE in patients with CS compared with the general population [3]. A national multicenter cohort study by Stuijver et al. [4] showed an incidence rate of VTE in CS of 14.6 per 1000 person-years, whereas the risk for postoperative VTE in patients with ACTH-dependent CS was 3.4%.

The underlying mechanisms of, and contributing factors for the hypercoagulable state in patients with CS are still under investigation, with observed/reported coagulation profiles in patients with CS being heterogeneously affected. The hemostatic abnormalities most consistently reported in the various studies include increased levels of procoagulant factors, e.g. von Willebrand Factor (vWF), and factor VIII, and increased levels of fibrinolytic inhibitors, e.g. plasminogen activator inhibitor-1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI), and alpha 2-antiplasmin. The currently available reports did not find a correlation between the severity of hypercortisolism and hemostatic abnormalities [2, 3].

However, to date, there have been no prospective studies that have evaluated the effects of prophylactic anticoagulation on the occurrence of VTE in patients with CS, and consequently, evidence-based guidelines on thromboprophylaxis strategies in patients with CS are lacking [5]. Only retrospective series showing a decrease in VTE associated mortality and morbidity after the introduction of postoperative antithrombotic prophylaxis with unfractionated heparin followed by warfarin [6], low-molecular weight heparin with or without mechanical interventions [7], or aspirin [8] have been reported. We, therefore, anticipated and hypothesized that European Reference Centers (RCs) applied various thromboprophylaxis strategies for patients with CS. Using the EU survey tool, a primary screening survey, and subsequently a secondary, more in-depth survey were developed and sent to RCs of the European Reference Network on Rare Endocrine Conditions (Endo-ERN), which are endorsed specifically for the diagnosis and treatment of CS, thus allowing mapping of the current clinical practice for thromboprophylaxis management in patients with CS.

RESULTS

Response rates

Forty-three out of 54 RCs completed the primary survey, of which one RC was excluded because the RC did not treat patients with CS resulting in a final response rate of 78% (n = 42). The secondary survey was sent to the 42 responding RCs of the primary survey, and was completed by 27 RCs of which one RC was excluded due to the lack of both new and chronic patients in their center in the past 2 years. This resulted in a response rate of 62% (n = 26). One response was partial (up to and including the section 'Treatment of CS', see Supplemental file 5). Figure 1 shows an overview of the geographical distribution of RCs per country. Notably, no information on the Cushing population and available treatment modalities due to non-response or exclusion from analysis of both surveys was available for The Czech Republic and Latvia. Slovakia was included for analysis of only the primary survey, and thus, information was partly available.

Figure 1. European Landscape of RCs participating in MTG Pituitary and/or MTG Adrenal of Endo-ERN and responder status. Completion of both primary and secondary survey (green icons). Completion of only the primary survey or was included for analysis of only the primary survey (blue icons). Non-responder to the surveys or exclusion from analysis of both surveys (red icons). Endo-ERN The European Reference Network on Rare Endocrine Conditions, MTG main thematic group, RC reference center.

Primary survey

The results of the primary survey are summarized in Table 1. The majority of the RCs reported to treat patients with CD (n = 40/42), and benign adrenal CS (n = 39/42). More than half of the RCs (n = 27/42) reported treating the entire spectrum of CS at their center including benign adrenal CS, malignant adrenal CS, CD, and ectopic CS. These RCs were heterogeneously spread across Europe. Additionally, the majority of the RCs (n = 36/42) provided all treatment modalities regarding CS, including surgery, medical treatment, and radiotherapy and administered combination therapy (i.e. combination of surgery and ≥1 of the other treatment modalities). The geographical distribution of the RCs, that provided all treatment modalities for patients with CS, showed almost complete coverage of the countries with the exception of Slovakia and Cyprus that have no RC providing all treatment modalities. An overview of the RC's countries that treated the whole spectrum of CS and provided all treatment modalities is shown in Figures 4A and 4B included in an supplemental file (see Supplemental file 1).

Sixteen of 42 RCs routinely provided preoperative medical treatment, and nearly three-quarters of RCs (n = 31) routinely provided thromboprophylaxis to patients with CS, of which the majority (n = 25) gave thromboprophylaxis only in the inpatient setting, while six RCs also prescribed thromboprophylaxis in the ambulatory setting. Eleven of 42 RCs reported to have a dedicated thromboprophylaxis protocol/policy available at their center. Twenty-four of 42 RCs systematically registered TE events, of which the majority (n = 17) specifically registered PE, DVT, and arterial thrombosis (AT), while seven RCs only registered PE, and DVT specifically. Eighteen RCs systematically registered bleeding complications, and twenty-two RCs documented the severity and outcome of the bleeding.

Secondary survey

Definitions

The section on definitions was completed by 26 RCs. First, the definitions of new and chronic patients being used by RCs varied greatly. The majority of the RCs used the following definitions: (a) new patients were defined as patients not previously seen by their center (n = 8), or as treatment naive patients, in addition to any patient not previously seen by their center (n = 8), and (b) chronic patients were defined as patients under active treatment (n = 7). An overview of all used definitions of new and chronic patients by the different RCs is presented in Table 4 enclosed in an supplemental file (see Supplemental file 2).

Epidemiology

Twenty-six RCs were included in the analysis for the section on epidemiology. Complete estimated numbers of new and chronic patients under local care, and numbers of performed transsphenoidal surgeries and adrenalectomies in 2019 and 2020 were provided (Table 5; see Supplemental file 3). Among the participating RCs, the number of new patients with CS ranged from 0 to 45 in 2019, and from 0 to 56 in 2020. The number of patients with CS under chronic care ranged from 1 to 196 in 2019, and from 0 to 215 in 2020. The highest number of both new and chronic patients with CS was

Table 1. Results of the primary survey.

Characteristics	Total number of RCs (N = 42)
Etiology of CS treated at RC[a]	
Benign adrenal CS	39 (93%)
Malignant adrenal CS	31 (74%)
Cushing's disease	40 (95%)
Ectopic CS	33 (79%)
Whole spectrum of CS (i.e. benign adrenal CS, malignant adrenal CS, CD and ectopic CS) treated at RC	27 (64%)
Treatment modalities for CS available at RC	
Surgery + medical treatment	3 (7%)
Surgery + medical treatment + combination therapy[b]	2 (5%)
Surgery + medical treatment + combination therapy[b] + radiotherapy	36 (86%)
Combination therapy[b]	1 (2%)
Preoperative medical treatment routinely provided at RC, yes (%)	16 (38%)
Thromboprophylaxis routinely provided at RC, yes (%)	31 (74%)
If yes, setting[a]	
In the inpatient setting	25/31 (81%)
In the ambulatory setting	6/31 (19%)
Presence of a thromboprophylaxis protocol for patients with CS, yes (%)	11 (26%)
Registration of bleeding complication, yes (%)	18 (43%)
Documentation of severity and outcome of bleeding, yes (%)	22 (52%)
Registration of TE events, yes (%)	24 (57%)
If yes, specific registration of	
PE + DVT	7/24 (29%)
PE + DVT + AT	17/24 (71%)

AT arterial thrombosis, CS Cushing's syndrome, CD Cushing's disease, DVT deep vein thrombosis, PE pulmonary embolism, RC reference center, TE thromboembolic

[a] Not mutually exclusive

[b] Combination therapy was defined as combination of surgery and ≥ 1 of the other treatment modalities

reported by France and the Netherlands, respectively. The number of transsphenoidal surgeries that were performed in 2019 and 2020 ranged from 0 to 16, and 0 to 20, respectively. The number of adrenalectomies in 2019 and 2020 ranged from 0 to 21, and 0 to 20, respectively. The highest numbers of performed transsphenoidal surgeries and adrenalectomies were reported by French RCs. Since only the number of CS patients per RC and the number of patients operated on within 1 year were requested in the survey, the number of newly diagnosed patients and patients operated on may not be the same in a single RC due to the fact that patients diagnosed in 1 year, may have had their surgery in another year.

Thromboprophylaxis in Cushing's syndrome

The section on thromboprophylaxis in CS was completed by 25 RCs. Ten RCs answered that thromboprophylaxis was routinely provided to all patients with CS. Thirteen centers provided

thromboprophylaxis only in selected and/or severe cases with or without risk factors for venous thromboembolism. Two centers never provided thromboprophylaxis to patients with CS.

Treatment duration of thromboprophylaxis

From the twenty-three RCs that provided thromboprophylaxis routinely, or only in selected/severe cases, the majority (n = 11) started thromboprophylaxis from diagnosis onwards. Six centers started thromboprophylaxis on the day of the surgery, or 1 day prior. Four centers started thromboprophylaxis preoperatively, of which three centers provided specifics regarding the moment of thromboprophylaxis initiation; namely at an average of 7, 14 and 18 days preoperatively. Furthermore, three RCs started thromboprophylaxis postoperatively, of which two RCs started at an average of 1 day, and one RC at an average of 3 days postoperatively. Two RCs reported that the start of thromboprophylaxis for patients with CS varied, and depended on presentation. Having started thromboprophylaxis in patients with CS, the time at which thromboprophylaxis was abrogated was standardized in approximately one-third of the RCs (n = 8/23), and individualized in two-thirds (n = 15/23), as shown in Table 2. The standardized discontinuation of thromboprophylaxis varied greatly between the RCs. One out of eight RCs stopped somewhere between 1 week before to 2 weeks after surgery, one RC stopped between 5 and 6 days postoperatively and two RCs between two to 4 weeks postoperatively. Furthermore, three RCs stopped at 1 month postoperatively and one RC at 3 months postoperatively. The individualized discontinuation of thromboprophylaxis, on the other hand, depended most frequently on the mobility (n = 9/15), and to a lesser extent on remission according to normalization of cortisol production (n = 6/15). One RC used crosslinked fibrin (XDP), prothrombin time (PT), aPTT and fibrinogen to make an individualized decision on the duration of thromboprophylaxis. Four out of 15 RCs reported that treatment duration varied according to the status of the patient, improvement of clinical parameters (e.g. hypertension, hyperglycemia and hypercortisolism) and/or current risk factors.

Factors influencing the initiation of thromboprophylaxis

The three most frequently selected factors influencing the start of thromboprophylaxis were 'previous VTE' (n = 15/23), 'severity of hypercortisolism' (n = 15/23), and 'limitation of mobility' (n = 13/23), as depicted in Figure 2. Risk factors for VTE—other than positive history—including older age, cancer and current smoking influenced the start of thromboprophylaxis at ten out of 23 centers. Eight centers started thromboprophylaxis in all patients with CS regardless of the presence of risk factors. Known hereditary thrombophilia (e.g. factor V Leiden/Prothrombin 2021a), and vWF promoter polymorphism haplotype 1 were reported to be used in the decision to start thromboprophylaxis by seven, and three centers, respectively, while non-0 blood group (BG) was not considered by any center. Four centers considered the subtype of CS in the decision of starting thromboprophylaxis (Figure 2). The prothrombotic considered subtypes of CS most frequently named by these centers were ectopic ACTH/CRH syndrome (n = 3/4) and malignant adrenal CS (n = 3/4), and, to a lesser extent, CD (n = 1/4).

Table 2. Time for initiation and time for abrogation of thromboprophylaxis in patients with Cushing's syndrome.

Characteristic	Total number of RCs (N = 23)
Time for initiation of thrombo-prophylaxis[a]	
From diagnosis onwards	11 (48%)
X days preoperatively (mean):	4 (17%)
X = 7	1/4 (25%)
X = 14	1/4 (25%)
X = 18	1/4 (25%)
Not specified	1/4 (25%)
Start on the day before/of the surgery	6 (26%)
X days postoperatively (mean):	3 (13%)
X = 1	2/3 (67%)
X = 3	1/3 (33%)
Other: varies, depends on presentation	2 (9%)
Time for abrogation of thrombo-prophylaxis	
Standardized	8 (35%)
Stop 1 week before until 2 weeks after surgery	1/8 (13%)
Stop between 4 and 6 days postoperatively	1/8 (13%)
Stop between 2 and 4 weeks postoperatively	2/8 (25%)
Stop at 1 month postoperatively	3/8 (38%)
Stop at 3 months postoperatively	1/8 (13%)
Individualized[a]	15 (65%)
Stop upon achieving remission according to normalization of cortisol production	6/15 (40%)
As soon as the patient is no longer immobile	9/15 (60%)
Based upon hemostatic parameters	1/15 (7%)
Other: Varies, depends on patient status, improvement of clinical parameters and/or risk factors	4/15 (27%)

RC reference center

[a] Not mutually exclusive

Anticoagulant treatment and hereditary screening for thrombophilia in Cushing's syndrome

All twenty-three RCs that routinely provided thromboprophylaxis, or only in selected/severe cases reported low-molecular-weight-heparin (LMWH) as the first-choice anticoagulant drug for thromboprophylaxis in patients with CS. Direct oral anticoagulants including apixaban, rivaroxaban, dabigatran and edoxaban were not reported. A thromboprophylaxis protocol for patients with CS was provided by only one of 23 centers. All 25 RCs including the centers that never provided thromboprophylaxis answered the question whether they routinely screened for hereditary thrombophilia during diagnostic work up. One RC reported to perform this screening test routinely.

Role of venous thromboembolism in preoperative medical treatment of CS

Twenty-five RCs completed the section on preoperative medical treatment in CS. Twenty-three RCs answered that preoperative medical treatment was provided to patients with CS (routinely to all

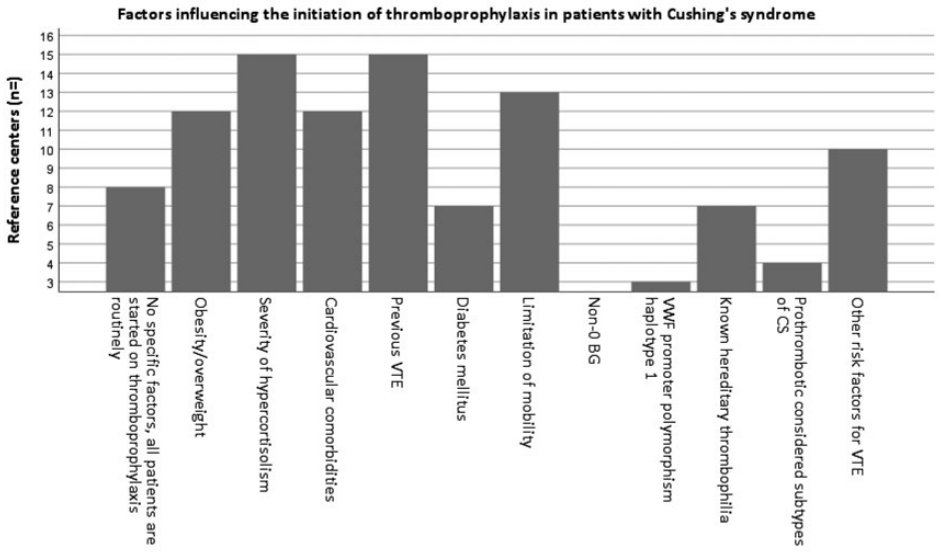

Figure 2. Proportion of responses including each factor influencing initiation of thromboprophylaxis in patients with Cushing's syndrome (not mutually exclusive).BG blood group, CD Cushing's disease, CS Cushing's syndrome, VTE venous thromboembolism, vWF von Willebrand Factor.

patients or only in selected and/or severe cases). About half of these RCs (n = 12/23) took a previous VTE into account when starting preoperative medical treatment, and about two-thirds (n = 15/23) included 'reduction of VTE' as a goal of treatment.

Indications for the initiation of postoperative thromboprophylaxis

Twenty-five RCs completed the section on postoperative thromboprophylaxis (if not (routinely) provided preoperatively) and follow-up care in CS. Five RCs reported not to routinely prescribe thromboprophylaxis in the postoperative setting (Figure 3). The most frequently selected indication for postoperative thromboprophylaxis was 'severe immobilization' (n = 15/25); 'known thromboembolic risk' was reported by one center as shown in Figure 3.

Follow-up care

Six out of 25 centers included hemostatic parameters in routine postoperative laboratory testing. These hemostatic parameters are shown in Table 3. Nine out of 25 centers routinely provided graduated compression stockings to patients with CS after surgery. From this group of RCs the treatment duration was until hospital discharge at five centers and until complete mobilization at one center. The remaining three centers did not specify the treatment duration.

DISCUSSION

This study examined the current clinical practice for thromboprophylaxis management in patients with CS across Endo-ERN RCs. This study provides valuable insight into the large variety

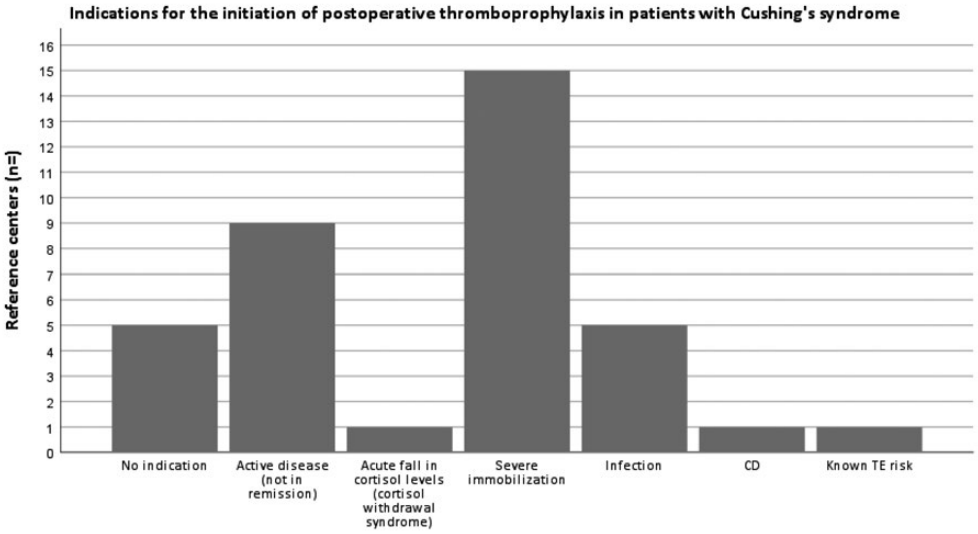

Figure 3. Proportion of responses from each indication for the initiation of postoperative thromboprophylaxis in patients with Cushing's syndrome (not mutually exclusive). CD Cushing's disease, TE thromboembolic.

of thromboprophylaxis strategies for patients with CS, and the limited availability of protocols on thromboprophylaxis even in the reference centers of Endo-ERN that have been endorsed as expert centers for the diagnosis and treatment of CS.

CS is associated with hypercoagulability and an increased risk of VTE (i.e. PE or DVT) both during the active phase of the disease, in postoperative setting, and even after biochemical remission [2]. There are currently no treatment studies on thromboprophylaxis of CS and no guidelines on the use of thromboprophylaxis for patients with CS, and therefore thromboprophylaxis management is committed to each center's clinical practice [5].

The in-depth assessment of thromboprophylaxis management showed that the majority of the RCs provided thromboprophylaxis routinely to all patients with CS or only in selected/severe cases (n = 23/25), however, a thromboprophylaxis protocol for patients with CS was unavailable in the vast majority of them (n = 22/23). Thromboprophylaxis was mostly started from diagnosis onwards, whereas the moment of stopping thromboprophylaxis was merely based on individual characteristics rather than standardized treatment duration. Because active CS is associated with a moderate to high risk on VTE [2,3,4] there is a rationale to start with thromboprophylaxis at diagnosis. On the other hand, treatment with anticoagulation is accompanied by an increased risk of major bleeding, which has been reported to be between 2.8 and 6 per 100 person years [3]. However, the bleeding tendency in CS may be only theoretical, as no increased bleeding complications were found in patients with CS undergoing laparoscopic adrenalectomy [9]. Although CS is associated with bruising and poor wound healing, these manifestations are thought to be the result of alterations in synthesis of skin components rather than specific coagulation disorders [10]. Future studies should assess additional risk factors to determine which patients are particularly at risk for VTE and would benefit from thromboprophylaxis. The individualized

Table 3. Characteristics of postoperative care.

Characteristic	Total number of RCs (N = 25)
Hemostatic blood testing as standard postoperative care	
Yes, namely:	6 (24%)
Thrombocytes + INR	1/6 (17%)
Platelet count + aPTT + PT + vWF + AT III + PS + PC	1/6 (17%)
aPTT + PT	1/6 (17%)
aPTT + INR + D-dimer + fibrinogen	1/6 (17%)
aPTT + PT + INR + D-dimer	1/6 (17%)
aPTT + PT + fibrinogen + XDP	1/6 (17%)
Graduated compression stockings as standard postoperative care	
Yes	9 (36%)

aPTT activated partial thromboplastin time, AT-III antithrombin III, PC protein C, PS protein S, PT prothrombin time, RC reference center, vWF von Willebrand Factor, XDP serum crosslinked fibrin.

decision to abrogate depended mostly on the mobility status of the patient. Risk factors that influenced the initiation of thromboprophylaxis in patients with CS were most frequently reported to be 'previous VTE' and 'severity of hypercortisolism', and LMWH was selected as the first-choice anticoagulant drug by all RCs. Furthermore, the majority of RCs reported 'severe immobilization' as an indication to start postoperative thromboprophylaxis in patients with CS if not (routinely) provided preoperatively, and lastly, did not provide standardized testing for hemostatic parameters in the postoperative care of CS.

A thromboprophylaxis protocol for patients with CS was provided by only one center. This center referred to a recently published article by Barbot et al [11]. In this article, perioperative multidisciplinary management of patients with sellar lesions submitted for transsphenoidal surgery was described and suggested. Specifically for patients with CD, the clinical practice included elastic compression stockings for every patient from the day of admission until full mobilization, treatment with enoxaparin 4000 U once daily, doubling the dose for patients with a body weight above 80 kg for 30 days, starting 24 h after the surgical procedure. However, this protocol did not compromise the whole spectrum of CS [11].

As no studies have been conducted on thromboprophylaxis management in patients with CS, we compared our findings with currently available reports on closely related topics. First, in our study, multiple factors were reported that were taken into account in the decision of thromboprophylaxis initiation in patients with CS. Currently available studies reported multiple risk factors that may be associated with the hypercoagulable state of CS and to our knowledge, no evidence- based VTE risk assessment model for patients with CS has been published thus far [3, 12,13,14]. In our study, the severity of hypercortisolism was one of the most frequently reported factors that influenced the initiation of thromboprophylaxis. One study found that patients with CS developing VTE had significantly higher plasma cortisol concentrations, compared with CS patients without VTE [12]. However, this was a retrospective study with a very small sample size. Multiple studies found no correlations between

the severity of hypercortisolism, and coagulation and fibrinolysis indexes, which was confirmed by Wagner et al. in their recently published systematic meta-analysis [3, 15, 16].

Furthermore, in our study we found a limited role for the measurement of coagulation parameters in the thromboprophylaxis management of CS applied by the Endo-ERN expertise centers. Only one RC reported that the ending of thromboprophylaxis in patients with CS depended on the results of hemostatic variables, including XDP, PT, aPTT and fibrinogen. Additionally, only six RCs reported that hemostatic parameters were screened routinely during follow-up care after transsphenoidal surgery or adrenalectomy. Results of studies examining the hemostatic profiles in patients with CS and the effect of (successful) treatment on these profiles were diverse. A prospective study by Manetti et al. [16] showed an improvement of coagulations indices after successful surgery including vWF, thrombin-antithrombin, antithrombin III, PAI-1, alpha 2-antiplasmin and aPTT. Kastelan et al. [17] found extensive significant improvements of coagulation factors in patients with CS after remission and concluded that the risk of TE 6 months after successful treatment was not greater than the risk faced by healthy individuals. In contrast, a cohort study by Dekkers et al. [13] reported high risks of VTE during the first 3 months following surgery in patients with CS. Furthermore, a study by van der Pas et al. [15] showed no significant changes in aPTT and vWF:Ag in patients with CD after successful pharmaceutical treatment, and additionally showed persistent elevated levels of PAI-1 and alpha 2-antiplasmin. A reason for these contradicting findings may well be the differences in follow-up duration. A systematic meta-analysis by Wagner et al. [3] confirmed the association between CS and VTE, and changes in coagulation parameters including vWF, protein C, protein S, aPTT, fibrinogen and factor VIII, but found no relationship between coagulation parameters and number of thrombotic events. However, more evidence is needed to show whether screening for hemostatic parameters and (changes in) laboratory coagulation metrics can define timing, duration and intensity of (extended) thromboprophylaxis before implementation in daily clinical practice.

In our study we found that four out of 23 centers reported to consider the subtype of CS in the decision of initiation of thromboprophylaxis. The subtypes of CS that were deemed to be associated with an increased risk of TE by these RCs were CD, ectopic ACTH/CRH syndrome and/or malignant adrenal CS. Previous studies showed a higher VTE rate in patients with CD compared to adrenal CS [4, 6]. The reason for the differences in VTE incidence in patients with different etiologies of CS is not clear. Tirosh et al. [18] observed higher AT- III activity and vWF:Ag antigen in patients with CD compared to patients with primary adrenal CS, along with higher baseline mean cortisol levels, and proposed that higher cortisol levels could explain the differences in coagulation profile and increased risk for VTE. However, another study reported no significant differences in coagulation profile between ACTH- dependent and ACTH- independent CS [19]. As to patients with adrenal carcinoma and ectopic ACTH source, the presence of malignancy per se is considered a VTE risk factor, and therefore, these subtypes of CS can be considered prothrombotic in clinical practice, as seen in our study.

The association between preoperative medical treatment and reduction of VTE risk in patients with CS remains controversial. In our detailed assessment of the use of preoperative medical treatment at the different centers, we found that only about half of the responding RCs (n = 12/23) reported to take risk factors for VTE (e.g. older age, cancer and previous VTE) into account in

the decision of starting treatment in patients with CS. In addition, about two-thirds (n = 15/23) reported that reduction of the risk of VTE postoperatively was one of the goals of preoperative medical treatment. Preoperative medical treatment might have a role in reducing the likelihood of VTE by reducing the cortisol withdrawal syndrome (i.e. a rapid and large decrease in cortisol exposure after surgery) that can trigger a rebound inflammatory response by withdrawal of the anti-inflammatory effect of cortisol [3]. Stuijver et al. [4] reported a reduced risk ratio of VTE 3 months postoperatively in patients with CS who were medically pretreated before surgery, in comparison to patients who were not. In contrast, a study by Valassi et al., in which data on preoperative medical treatment from The European Registry on Cushing's syndrome (ERCUSYN) was analyzed, reported no differences in postsurgical morbidities including thromboembolism within 180 days of surgery between patients who received preoperative medical treatment compared to patients who underwent surgery directly. Furthermore, there was little evidence that preoperative medical treatment affected postsurgical outcome [20].

Important limitation of our study is that our findings may be biased due to non-responders and missing data. However, a minimum response rate of 60% was achieved, and the survey questions were mainly independent from each other. We tried to prevent ambiguity in our survey questions by making a clear distinction between start of thromboprophylaxis in an inpatient and/or ambulatory/out-patient setting, and by enquiring about the exact time of initiation of thromboprophylaxis. However, thromboprophylaxis management in general of patients who are not diagnosed with CS or of patients admitted to the RCs for surgery related to a condition other than CS was not surveyed.

CONCLUSIONS

Current clinical thromboprophylaxis management in patients with CS varies considerably across Endo-ERN reference centers. In the absence of prospective studies evaluating thromboprophylaxis on the occurrence of VTE in patients with CS, no evidence-based guidelines on thromboprophylaxis management for patients with CS exist. As the clinical practices have shown to be highly variable, randomized, controlled trials are needed to establish the optimal prophylactic anticoagulant regimen for patients with CS taking into account the increased risk of perioperative bleeding and the presence of additional risk factors for thrombosis.

METHODS

Aim of the study

The aim of this study was to map the current thromboprophylaxis regimens, (perioperative) treatment practices, and follow-up care after treatment for CS across the (inter)nationally endorsed RCs of the Endo-ERN.

Study setting

In March 2017, European Reference Networks for rare and complex diseases (ERNs) were installed. ERNs are virtual networks involving RCs across the EU and their primary aim is to enhance cross-border expert consultation and guide conformity for rare and/or complex diseases [21]. The Endo-ERN

includes 71 RCs in 19 EU member states. Each of the RCs has been endorsed both nationally and subsequently at the European level for specific expertise for CS, RCs participate in the main thematic disease groups of 'Adrenal' and 'Pituitary' [22].

Study design

This was a survey based study, with a primary and secondary survey which are included in Supplemental files 4 and 5, respectively.

The questionnaires included compulsory questions presented in open-ended and multiple choices and in yes/no-format. The surveys were developed using the EU Survey tool and RCs were approached by email which included a link to the survey. A reminder email was sent approximately 4 weeks after the initial mail-out. RCs that did not respond to the reminder email within 2 weeks after the reminder mail-out were considered non-respondents. Partial completions of the questionnaires were included in the study analysis due to the independent character of the survey questions. The exclusion criteria of the primary survey was the absence of patients with CS, and of the secondary survey was the lack of new and chronic patients with CS in their center in 2019 and 2020. A response rate of 60% was considered sufficient for analysis.

Study parameters

Primary survey

First, a primary survey was developed and send to 54 participating RCs of the Endo-ERN endorsed for the diagnosis and treatment of CS. The primary survey included eighteen questions which served as a screening tool to capture the first essential data for the development of the secondary survey. The questionnaire addressed current practices related to key performance indicators, treatment of CS, and cortisol-lowering treatment prior to surgery, i.e. preoperative medical treatment, prophylactic anticoagulation treatment, and monitoring for thromboembolic events (TE) and bleeding complications in patients with CS.

Secondary survey

Next, we developed a secondary survey based on the outcome of the primary survey questionnaire. The secondary survey included 35 questions and was sent to all responders of the primary survey. The section on thromboprophylaxis in CS in the secondary survey was fully completed by RCs that provided thromboprophylaxis to patients with CS. RCs that never provided thromboprophylaxis to patients with CS were requested to answer the questions on 'hereditary screening for thrombophilia in CS', 'indications for the initiation of postoperative thromboprophylaxis' and 'follow-up care'. Information on treatment duration of thromboprophylaxis in patients with CS was assessed with questions on the time for initiation of thromboprophylaxis, and the time at which thromboprophylaxis was abrogated. Furthermore, remission of CS was defined as normalization of cortisol production in the survey.

The main goal of the secondary survey was a more in-depth assessment of thromboprophylaxis management in daily clinical practice in patients with CS, protocols for thromboprophylaxis, if

any, and (perioperative) treatment practices and follow-up care after transsphenoidal surgery or adrenalectomy in patients with CS. Furthermore, the epidemiological distribution of new and chronic CS patients and performed surgeries were assessed. This was done for both 2019 and 2020 to avoid distortion of information as a result of the COVID-19 pandemic. Lastly, to prevent information bias definitions of new and chronic patients were surveyed too.

Statistical analyses

Descriptive statistics were used to present data, with categorical variables being presented as number (n), and continuous variables being described as means with ranges. Statistical analysis was performed using SPSS version 25.0.

ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
aPTT	Activated partial thromboplastin time
AT	Arterial thrombosis
CLT	Clot lysis time
CRH	Corticotropin-releasing hormone
CD	Cushing's disease
CS	Cushing's syndrome
DVT	Deep vein thrombosis
Endo-ERN	The European Reference Network on Rare Endocrine Conditions
ERN	European Reference Network
LMWH	Low-molecular-weight-heparin
PAI-1	Plasminogen activator inhibitor-1
PE	Pulmonary embolism
PT	Prothrombin time
RC	Reference center
TAFI	Thrombin activatable fibrinolysis inhibitor
TE	Thromboembolic
TSS	Transsphenoidal surgery
VTE	Venous thromboembolism
vWF	Von Willebrand Factor
XDP	Serum crosslinked fibrin

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APPENDIX

Supplemental file 1

Title: Characteristics of care for Cushing's syndrome patients at the reference centers

Description: Overviews of participating reference centers (RC's) per country that treated the complete spectrum of Cushing's syndrome (CS) and that provided the complete spectrum of treatment modalities for CS.

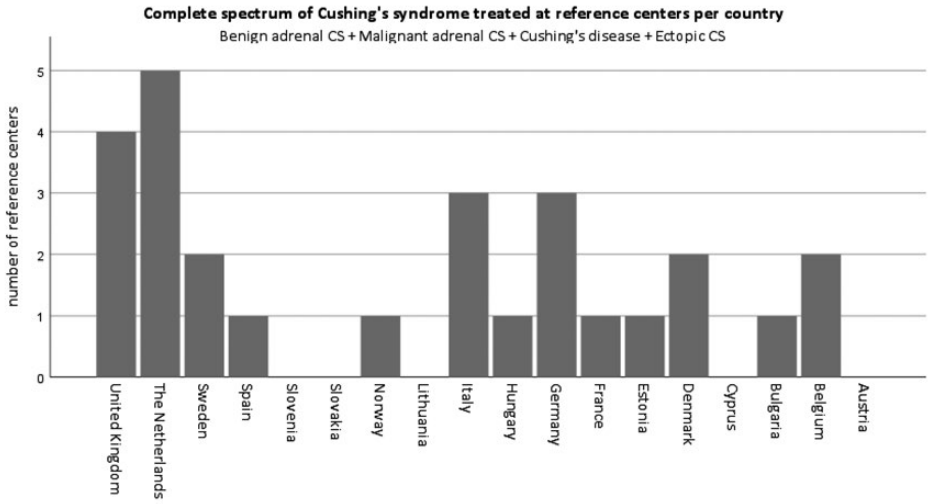

Figure 4A. Overview of the number of reference centers per country that treated the complete spectrum of Cushing's syndrome (CS).

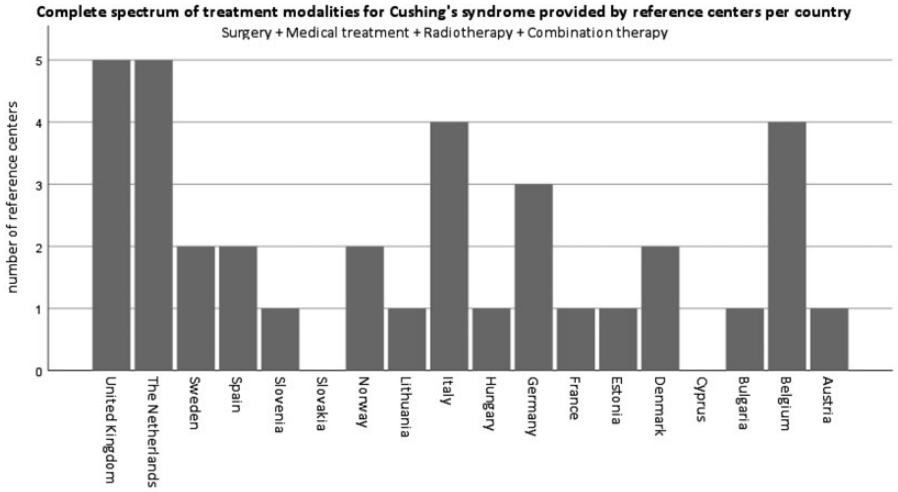

Figure 4B. Overview of the number of reference centers per country that provided the complete spectrum of treatment modalities for CS (n=36). CS, Cushing's syndrome.

Supplemental file 2

Title: Definitions used by the reference centers

Description: Overview of used definitions of new patients and patients under chronic care as reported by participating reference centers (RCs).

Table 4. Definition(s) of new patients and of patients under chronic care.

Definition(s) of new patients at RC	Total number of RCs (n=26)
Treatment naive patients	6 (23%)
Patients not previously seen by RC	8 (31%)
Treatment naive patients + Patients not previously seen by RC	8 (31%)
Patients not previously seen by RC + Patients with recurrent disease after initial remission	1 (4%)
Treatment naive patients + Patients not previously seen by RC + Patients with recurrent disease after initial remission	1 (4%)
Treatment naive patients + Patients not previously seen by RC + Patients with recurrent disease after initial remission + Any patient with an exceeding interval between the last and present consultation depending on the Health Record of the RC	2 (8%)
Definition(s) of patients under chronic care at RC	**Total number of RCs (n=26)**
Patients under active treatment at RC	7 (27%)
Patients with previous treatment at RC	4 (15%)
Patients with previous treatment currently under affiliated centers referred to RC for a single consultation only, diagnostic tests, or for specific procedure	2 (8%)
Patients under active treatment at RC + Patients with previous treatment at RC	6 (23%)
Patients under active treatment at RC + Patients with previous treatment currently under affiliated centers referred to RC for a single consultation only, diagnostic tests, or for specific procedure	2 (8%)
Patients under active treatment at RC + Patients with previous treatment at RC + Patients with previous treatment currently under affiliated centers referred to RC for a single consultation only, diagnostic tests, or for specific procedure	5 (19%)

Supplemental file 3

Title: Epidemiological data of Cushing's syndrome patient population across the Endo-ERN

Description: Numbers of patients newly diagnosed with CS, patients with CS under chronic care, performed transsphenoidal surgeries (TSSs) and adrenalectomies in 2019 and 2020 at participating reference centers (RCs).

Table 5. Numbers of patients newly diagnosed with CS, patients with CS under chronic care, performed TSS and adrenalectomies in 2019 and 2020 at RCs. Missing values are shown as X. CD, Cushing's disease; CS, Cushing's syndrome; RC, reference center; TSS, transsphenoidal surgery.

	Total new CS patients (n=)		Total chronic CS patients (n=)		TSSs (n=)		Adrenalectomies (n=)	
Name RC	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
Aarhus University Hospital	14	13	81	86	7	7	X	X
Assistance Publique - Hôpitaux de Marseille	45	56	148	171	15	20	20	20
Assistance Publique-Hopitaux de Paris -Consortium Hôpitaux Cochin, Robert Debré, Necker, St Antoine, La Pitié Salpétrière	44	37	92	101	16	10	21	18
Hospital-University of Padova	15	17	168	184	7	5	5	11
Azienda Ospedaliera Universitaria "Federico II", Napoli	7	1	67	74	3	1	6	2
Azienda Ospedaliero Universitaria Città della Salute e della Scienza di Torino	5	4	2	1	2	1	3	2
UCL Cliniques Universitaires Saint-Luc	9	8	77	78	3	4	6	5
Erasmus MC: University Medical Center Rotterdam	22	17	196	215	7	5	7	6
Fundacio de Gestio Sanitaria Hospital de la Santa Creu i Sant Pau	11	12	42	43	5	6	3	3
Great Ormond Street Hospital - NHS Foundation Trust	2	5	4	4	0	3	2	2
Lithuanian University of Health Sciences	5	7	22	22	3	3	4	3
IRCCS Ospedale Policlinico San Martino – Genova – Italy	6	3	24	26	2	2	1	1
IRCCS Istituto Auxologico Italiano and BIOMETRA	21	18	29	19	2	2	10	10
Karolinska University Hospital	9	7	99	105	4	4	2	2
Leiden University Medical Center	15	14	52	51	15	15	3	2
Oslo University Hospital HF	11	12	33	36	5	5	5	5
Scientific Institute San Raffaele	2	0	10	10	0	0	1	0

Table 5. continued.

	Total new CS patients (n=)		Total chronic CS patients (n=)		TSSs (n=)		Adrenalectomies (n=)	
Name RC	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
Radboud University Nijmegen Medical Centre - including Amalia's children Hospital	29	20	113	113	11	10	8	4
Sahlgrenska University Hospital	6	6	84	87	3	5	2	1
Tartu University Hospital	4	3	13	12	2	2	1	1
The Cyprus Institute of Neurology and Genetics	1	1	1	0	0	0	0	1
University Hospital Southampton - NHS Foundation Trust	9	8	26	35	4	5	2	1
University Hospital Würzburg	X	X	X	X	X	X	X	X
University Hospitals Birmingham - NHS Foundation Trust	X	X	X	X	5	5	X	X
University Medical Centre Groningen	8	5	73	81	4	2	3	2
University Medical Centre Ljubljana	0	0	1	1	0	0	0	0

Supplemental file 4

Title: Primary survey

Description: The primary survey includes 18 questions serving as a screening tool to capture the first essential data for the development of the secondary survey. The questionnaire addressed current practices related to key performance indicators, treatment of Cushing's syndrome (CS) and pre-treatment prior to surgery, i.e. preoperative medical treatment (PMT), prophylactic anticoagulation treatment, and monitoring for thrombo-embolic (TE) events and bleeding complications in patients with CS.

1. Please select for which Main Thematic Group(s) your HCP participate in? (multiple options possible)
   a. MTG- Pituitary
   b. MTG- Adrenal

2. Would you be interested in participating in studies on the topic of Thromboprophylaxis in patients with Cushing's syndrome? (multiple options possible)
   a. Yes I would like to participate in retrospective chart study
   b. Yes I would like to participate in a RCT
   c. No

3. Does your center have a specific clinical trial unit? (yes/no)

4. Do you see patients with Cushing's syndrome? (yes/no)

5. Number of new patients per year:
   a. 0-5
   b. 5- 10
   c. 10- 20
   d. >20

6. Total number of patients under chronic care:
   a. 0-10
   b. 10-50
   c. 50-100
   d. >100

7. Please select the appropriate boxes for underlying cause of Cushing Syndrome: (multiple options possible)
   a. Adrenal CS, benign
   b. Adrenal CS, malignant
   c. Cushing's disease
   d. Ectopic- CS

8. Do you collect clinical data of your patient cohort in a specific database? (yes/no)
   If yes: Have (any part of) these data been published? (yes/no)
   If yes, provide reference

9. Are the following treatment modalities provided at your center? (multiple options possible)
   a. Surgery
   b. Medical treatment
   c. Radiotherapy
   d. Combination therapy (e.g. surgery and 1 of the treatment modalities)

10. Please select which medical treatment to you provide treat cortisol excess? (multiple options possible)
    a. Ketoconazole
    b. Metyrapone
    c. Pasireotide
    d. Other
       If other, please specify

11. Do you routinely pre-treat prior surgery? (yes/no)
    If yes, which medical agent? Please briefly specify protocol regarding duration and dose:

12. Do patients at your center routinely receive thromboprophylaxis? (multiple options possible)
    a. Yes, in the inpatient setting
    b. Yes, in the ambulatory setting
    c. No, only selected and/or severe cases with or without risk factors
    d. No

    If yes, please specify:
    a. All patients
    b. Only severe cases with or without other risk factors

13. Which kind of thromboprophylaxis? (multiple options possible)
    a. Low molecular weight heparin
    b. NOAC
    c. Other

    If other, please specify
14. Do you have a specific protocol for thromboprophylaxis? (yes/no)
    If yes, please select the specific duration of treatment (multiple options possible)
    a. From diagnosis onwards
    b. Peri- operatively

c. 6 weeks after surgery
d. 12 weeks after surgery
e. During hospitalization for other reason than elective pituitary or adrenal surgery

15. Do you specifically register bleeding complications? (yes/no)

16. Do you document the severity and outcome of the bleeding complications? (yes/no)

17. Do you specifically register thrombo-embolic events? (yes/no)
    If yes, do you register separately (multiple options possible)
    a. Pulmonary embolism
    b. Deep vein thrombosis
    c. Arterial thrombosis

18. Remarks/comments

Supplemental file 5

Title: Secondary survey

Description: The secondary survey includes 35 questions. The main goal of the secondary survey was a more in- depth assessment of thromboprophylaxis (TP) management in daily clinical practice in patients with Cushing's syndrome (CS), protocols for TP, if any, and (perioperative) treatment practices and follow-up care after transsphenoidal surgery (TSS) or adrenalectomy in patients with CS. Furthermore, the epidemiological distribution of new and chronic CS patients and performed surgeries were assessed for both 2019 and 2020, and definitions of new and chronic patients were surveyed too

Section 1: Definitions & Epidemiology

1. How do you define a new patient? (multiple options possible)
   a. Treatment naive patients
   b. Patients not previously seen by the reference center
   c. Any patient with an exceeding interval between the last and present consultation depending on the Health Record of the reference center (for instance more than 12 months)
   d. Patients with recurrent disease after initial remission
   e. Other, namely:

2. How do you define a patient under chronic care? (multiple options possible)
   a. Patients under active treatment at the reference center
   b. Patients with previous treatment at the reference center (e.g. patients in complete remission after treatment)
   c. Patients with previous treatment currently under affiliated centers referred to the reference center for a single consultation only, diagnostic tests, or for specific procedure
   d. Other, namely:

3. Do you have a specific database containing clinical characteristics of patients with Cushing's syndrome? (yes/no)
   » If yes, what kind of database? (for example Excel sheets or files of Electronic Health Record Software)
   » If no, are you interested in clinical data collection? (yes/no)

4. What is the number of patients newly diagnosed with the following subtypes of Cushing's syndrome (CS) in your center in 2019 and 2020?

CS subtype	Number of new diagnoses (2019)	Number of new diagnoses (2020)
Cushing's disease		
Ectopic ACTH		
Benign adrenal CS		
Malignant adrenal CS		

5. What is the number of patients under chronic care with the following subtypes of Cushing's syndrome (CS) in your center in 2019 and 2020?

CS subtype	Number of patients under chronic care (2019)	Number of patients under chronic care (2020)
Cushing's disease		
Ectopic ACTH/CRH syndrome		
Benign adrenal CS		
Malignant adrenal CS		

6.1 Does discharge of follow- up depend on etiology of Cushing's syndrome? (yes/no)
» If yes; please specify:

6.2 Is discharge of follow- up related to remission status in patients with Cushing's syndrome? Please specify (multiple options possible)
a. Discharge upon remission
b. Discharge X months/years in remission
» Please specify:
c. Not related to remission status; lifelong follow- up
d. Not related to remission status; patients are discharged of follow- up when...
» Please specify:

7. What is the frequency of transsphenoidal surgeries for Cushing's disease (CD) performed in 2019 and 2020?

Year	Number of transsphenoidal surgeries for CD
2019	
2020	

8. What is the frequency of adrenalectomies for Cushing's syndrome (CS) performed in 2019 and 2020?

Year	Number of adrenalectomies for CS
2019	
2020	

Section 2: Treatment of CS

The first- line treatment of all forms of Cushing's syndrome (CS) is surgery. If surgical resection of the primary tumour is not successful or not an option, second- line treatment includes medical treatment. Potential indications for medical therapy of CS include: 1) persistent or recurrent Cushing's syndrome after transsphenoidal or adrenal surgery; 2) non- feasibility for surgery; 3) acute complications of severe hypercortisolism; 4) pretreatment before surgery.

The following questions are about the first-choice medical therapy in the treatment of the different subtypes of CS, and about pretreatment before surgery (i.e. Preoperative Medical Treatment; PMT).

9. Which medical drug is first-choice in the treatment of Cushing's disease? (multiple options possible)
   - » Ketoconazole (x), metyrapone (x), mitotane (x), etomidate (x), osilodrostat (x), levoketoconazole (x), cabergoline (x), pasireotide (x), lanreotide (x), octreotide (x)
   - » Other, namely:
   - » Please specify in case of multiple options:

10. Which medical drug is first-choice in the treatment of ectopic ACTH/CRH syndrome? (multiple options possible)
    - » Ketoconazole (x), metyrapone (x), mitotane (x), etomidate (x), osilodrostat (x), levoketoconazole (x), cabergoline (x), pasireotide (x), lanreotide (x), octreotide (x)
    - » Other, namely:
    - » Please specify in case of multiple options:

11. Which medical drug is first-choice in the treatment of benign adrenal CS? (multiple options possible)
    - » Ketoconazole (x), metyrapone (x), mitotane (x), etomidate (x), osilodrostat (x), levoketoconazole (x), cabergoline (x), pasireotide (x), lanreotide (x), octreotide (x)
    - » Other, namely:
    - » Please specify in case of multiple options:
12. Which medical drug is first-choice in the treatment of malignant adrenal CS? (multiple options possible)

» Ketoconazole (x), metyrapone (x), mitotane (x), etomidate (x), osilodrostat (x), levoketoconazole (x), cabergoline (x), pasireotide (x), lanreotide (x), octreotide (x)
» Other, namely:
» Please specify in case of multiple options:

13. Do you provide combination medical therapy (e.g. combination of 2 or more cortisol lowering agents) in patients with severe hypercortisolism?
    a. No
    b. Yes, routinely
    c. Yes, sometimes depending on the case
    » If yes, which medical drugs does the first-choice combination therapy consist of?

Section 3: Preoperative medical treatment (PMT)

14. In the primary survey you indicated that your reference center provides PMT routinely to patients with Cushing's syndrome. Is this still applicable? Please specify.
    a. Yes, PMT is provided to all patients *Continue with question 16*
    b. Only in selected and/or severe cases with or without risk factors *Continue with question 16*
    c. No, PMT is never provided *Continue to part 4: Thromboprophylaxis*

15. In the primary survey you indicated that your reference center does not provide PMT routinely to patients with Cushing's syndrome. Is this still applicable? Please specify.
    a. Yes, PMT is never provided *Continue to part 4: Thromboprophylaxis*
    b. Only in selected and/or severe cases with or without risk factors *Continue with question 16*
    c. No, PMT is provided to all patients *Continue with question 16*

16. Providing PMT (routinely) to patients with Cushing's syndrome, do you take into account the following factors? (multiple options possible)
    a. No specific factors, all patients are medically pretreated before operation
    b. Severity of clinical syndrome as reflected by:
    1. Difficult-to-treat hypertension
    2. Uncontrolled diabetes mellitus or progressive glucose intolerance
    3. Biochemical severe cortisol excess
    4. Clinical severe syndrome/symptoms
    5. Severe psychotic decompensation
    6. Other, namely
    c. Risk factors for VTE (e.g. older age, cancer, current smoking, previous VTE)
    d. Active malignancy with/without treatment

17. What is/are your goals of PMT in patients with Cushing's syndrome? (multiple options possible)

a. Decrease of cortisol excess
b. Complete normalization of cortisol production
c. Improved regulation of hypertension and/or diabetes mellitus
d. Reduction of VTE risk
e. Prevention of cortisol withdrawal syndrome
f. Reduction of infectious complications
g. Reduction of other surgery- related complications (e.g. bleeding)
h. Reduction of psychopathology
i. Other, namely:

18. Which medical drug is first-choice in the preoperative medical treatment of the following subtypes of Cushing's syndrome (CS)? (multiple options possible)
    1. Pituitary CS: Ketoconazole (x), metyrapone (x), mitotane (x), etomidate (x), osilodrostat (x), levoketoconazole (x), cabergoline (x), pasireotide (x), lanreotide (x), octreotide (x)
    » Other, namely:
    » Please specify in case of multiple options:

    2. Benign adrenal CS: Ketoconazole (x), metyrapone (x), mitotane (x), etomidate (x), osilodrostat (x), levoketoconazole (x), cabergoline (x), pasireotide (x), lanreotide (x), octreotide (x)
    » Other, namely:
    » Please specify in case of multiple options:

    3. Malignant adrenal CS: Ketoconazole (x), metyrapone (x), mitotane (x), etomidate (x), osilodrostat (x), levoketoconazole (x), cabergoline (x), pasireotide (x), lanreotide (x), octreotide (x)
    » Other, namely:
    » Please specify in case of multiple options:

19. Do you provide combination preoperative medical treatment therapy (e.g. combination of two or more cortisol lowering agents) in Cushing's syndrome?
    a. No
    b. Yes, routinely
    c. Yes, sometimes depending on the case
    » If yes, which medical drugs does the combination therapy usually consist of?

20. When do you start PMT in patients with Cushing's syndrome?
    a. From diagnosis onwards
    b. ***X*** days preoperatively
    c. X days postoperatively
    d. Other, namely:

21. When do you stop PMT in patients with Cushing's syndrome?
    a. **X** days preoperatively
    b. **X** days postoperatively
    c. Other, namely:

Section 4: Thromboprophylaxis in CS

22. In the primary survey you indicated that your reference center provides thromboprophylaxis routinely to patients with Cushing's syndrome. Is this still applicable? Please specify.
    a. Yes, thromboprophylaxis is provided to all patients *Continue with question 25*
    b. Only in selected and/or severe cases with or without risk factors *Continue with question 25*
    c. No, thromboprophylaxis is never provided *Continue with question 30*

23. In the primary survey you indicated that your reference center provides thromboprophylaxis only in selected and/or severe cases of Cushing's syndrome with or without risk factors. Is this still applicable?
    a. Yes, only in selected and/or severe cases with or without risk factors. *Continue with question 25*
    b. No, thromboprophylaxis is provided to all patients *Continue with question 25*
    c. No, thromboprophylaxis is never provided *Continue with question 30*

24. In the primary survey you indicated that your reference center does not provide thromboprophylaxis to patients with Cushing's syndrome. Is this still applicable?
    a. Yes, thromboprophylaxis is never provided *Continue with question 30*
    b. Only in selected and/or severe cases with or without risk factors *Continue with question 25*
    c. No, thromboprophylaxis is provided to all patients *Continue with question 25*

25. Providing thromboprophylaxis routinely or only in selected and/or severe cases, when do you start thromboprophylaxis in patients with Cushing's syndrome?
    a. From diagnosis onwards
    b. X days preoperatively
    c. Start on the day before/of the surgery
    d. X days postoperatively
    e. Other, namely:

26. In starting (perioperative) thromboprophylaxis do you take into account the following factors? (multiple options possible)
    a. No specific factors, all patients are started on thromboprophylaxis routinely
    b. Obesity/overweight
    c. Severity of hypercortisolism
    d. Cardiovascular comorbidities

e. Previous VTE
f. Diabetes mellitus
g. Limitation of mobility
h. Non- 0 bloodgroup
i. von Willebrand Factor (VWF) promoter polymorphism haplotype 1
j. Known hereditary thrombophilia (e.g. factor V Leiden/Prothrombin 2021a)
k. Subtype of CS
» If yes: Which subtype(s) of CS is/are considered as a prothrombotic factor? (multiple options possible)
  • Cushing's disease
  • Ectopic ACTH/CRH syndrome
  • Adrenal CS, benign
  • Adrenal CS, malignant
l. Other risk factors for VTE (e.g. older age, cancer, current smoking)

27. Which anticoagulant drug is first-choice for (perioperative) thromboprophylaxis in patients with Cushing's syndrome? (multiple options possible)
  a. Low molecular weight heparin
  b. Unfractionated heparin via continuous iv infusion
  c. Apixaban
  d. Edoxaban
  e. Rivaroxaban
  f. Dabigatran
  g. Other, namely:
  h. Please specify in case of multiple options:

28. Having started (perioperative) thromboprophylaxis in patients with Cushing's syndrome, is the treatment duration standardized or individualized?
  a. Standardized
    » Continuation X days/weeks postoperatively.
  b. Individualized (multiple options possible)
  1. Stop upon achieving remission according to normalization of cortisol production.
  2. As soon as the patient is no longer immobile
  3. Based upon hemostatic parameters
    » If yes, which hemostatic parameters?
  4. Other, namely:

29. Can you please share the thromboprophylaxis protocol for patients with Cushing's syndrome (English version)? (yes/no)

30. If thromboprophylaxis is not (routinely) given (perioperatively), is there an indication for starting in the postoperative setting?
    1. No indication
    2. Active disease (not in remission)
    3. Acute fall in cortisol levels (cortisol withdrawal syndrome)
    4. Severe immobilization
    5. Infection
    6. Other, namely:

31. Do you routinely check for hereditary thrombophilia in patients diagnosed with Cushing's syndrome (for example Factor 5 Leiden, PT2021a)? (yes/no)

32. What is the frequency of clinical follow- up visits after surgery in case of uncomplicated surgery and post-operative course ?

33. What is the testing frequency of cortisol levels after surgery?

34. Does the postoperative laboratory testing include hemostatic parameters? (yes/no)
    - » If yes; which hemostatic parameters?

35. Do you routinely provide graduated compression stockings to patients with Cushing's syndrome after surgery? (yes/no)
    - » If yes, what is the duration of treatment?
      - » Not specified
      - » Until hospital discharge
      - » Continuously for *X* weeks postoperatively
      - » Other, namely:

CHAPTER 9

LONG-TERM POSTOPERATIVE CUSHING'S DISEASE FOLLOW-UP USING INTEGRATED OUTCOME SQUARES: UNIFIED OUTCOME AND EVALUATION

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ABSTRACT

Purpose

Both remission and complications determine the success of transsphenoidal surgery in Cushing's disease (CD). Outcome Squared provides a unified outcome classification in time, integrating intended and adverse effects. Particular challenges in evaluating management of CD are the position of postoperative hypocortisolism and need for multiple interventions in outcome evaluation. A retrospective cohort study was used to report on long-term integrated postoperative outcome in patients with CD.

Methods

Seventy-two consecutive CD patients treated by transsphenoidal resection between 2000 to 2016 in our tertiary referral center were included. Results are presented in Outcome Squares.

Results

One year after surgery, good outcome (remission without pituitary deficiencies excluding adrenal insufficiency) was observed in 55.4%, whereas 4.6% of the patients reported poor outcome (no remission, pituitary deficiencies present). In 29.2% remission with pituitary deficiencies was observed, and 10.8% was not in remission without pituitary deficiencies. When ongoing adrenal insufficiency was included as adverse outcome at one year postoperative, only 17% had remission without pituitary deficiency (good outcome), and 68% had remission with pituitary deficiencies. With follow-up, a gradual shift to the good outcome category occurred, mainly due to recovery of the hypothalamus-pituitary-adrenal axis.

Conclusion

The majority of patients are in remission five years after transsphenoidal surgery, though in a considerable number at the expense of persistent pituitary deficiencies. The four different integrated outcome quadrants used provide an uniform, patient-centred integrated overall view of the important balance between efficacy and safety of transsphenoidal surgery in CD, and can be used in individualised patient counselling.

INTRODUCTION

Cushing's disease (CD) is a rare endocrine disease caused by an adrenocorticotropic hormone (ACTH)-secreting pituitary adenoma, resulting in endogenous glucocorticoid excess. Prominent features of glucocorticoid excess include adverse changes in body composition, adverse metabolic profiles, hypertension, and neuropsychiatric disorders.[1-3] If left untreated, the prognosis of CD is poor.[4] Selective removal of the corticotropic adenoma by transsphenoidal surgery remains the treatment modality of first choice, aiming at biochemical remission to eliminate the associated signs, symptoms, and comorbidities, and to improve quality of life.[5] Surgical outcomes traditionally focus on biochemical remission rates. However, the success of surgery is determined by the delicate balance between achievement of remission and the occurrence of long-term complications. This is particular true for CD, where failure to normalize cortisol secretion is potentially life threatening.[4] Main complications such as pituitary insufficiencies, which is described in a range of 3 to as high as 88% of surgically treated patients,[6, 7] potentially due to (over)aggressive surgical approaches, are also associated with comorbidities and reduced quality of life. [8-12]

Pituitary surgery in CD is challenging for several reasons. First, adenoma localization is demanding with previously described detection rates of ACTH-secreting pituitary microadenomas on Magnetic Resonance Imaging (MRI) techniques of approximately 60 to 88% (range 36 to 100%, the latter in a very small case series)[13-16], which means that some patients have no visible or a very small, unclear microadenoma on MRI. In addition, false positive findings on MRI do occur.[17-19] Furthermore, there might be several adenoma localizations, localizations in both sides of the gland or medially, near the stalk,[20, 21] and even extrapituitary and parasellar adenomas have been described.[22, 23] Tumors in CD are often not round or well circumscribed, binodular with small connections, not always enclosed and frequently show a diffuse growing pattern.[24] Most adenomas are deliquescent, but some may have a firm consistency, and therefore may be mistaken for normal pituitary tissue. [24] Consequently, some experienced pituitary surgeons advocate inspection of the total gland by incising the gland carefully, aiming at maximizing total resection. In CD this is generally regarded as safe for preserving pituitary function.[23, 24]

Because CD is associated with high mortality if left untreated and selective adenectomy is challenging for aforementioned reasons, more radical approaches as "hemi- or total hypophysectomy", "sella clean-out" or "bilateral adrenalectomy" resulting in life long hypopituitarism and specifically hypocortisolism are accepted for this condition only, in contrast to all other pituitary tumors where partial adenomectomy or debulking will be proposed if total resection is not feasible.[5, 24] In CD, there is a high tendency for recurrence (15-66% within five to ten years of successful surgery[24-26]), and therefore re-operations may be needed, which may be successful in experienced hands.[5, 27, 28] An unresolved question is whether the risk of recurrence is determined by the quality or approach of the surgery or rather by tumor biology.[29, 30] The management strategy of (repeated) conservative surgery with the goal of remission without pituitary failure or other complications, should be weighed against time exposed to hypercortisolism. Therefore, careful outcome measurements incorporating surgical strategies, preoperative and per-operative evaluation of chances and risks are required to reliably evaluate outcomes in the treatment of CD within and between centers. From

a patient perspective, ultimate outcome after re-intervention is of interest, while surgical series usually analyse only single interventions.

A complicating factor in thorough outcome evaluations is the multitude of endocrine evaluations and tests of CD state postoperatively prohibiting straightforward conclusions. Following surgery early on, there may be a period of profound ACTH and cortisol deficient state, because of downregulation of the activity of the hypothalamic-pituitary-adrenal (HPA)-axis during active disease. This means that in the early postoperative phase remission is reflected by preferred very low early morning cortisol concentrations which should be discriminated from (surgical) damage to the pituitary gland.[5] After recovery of HPA-axis, Cushing's remission is defined differently by normal biochemical tests results, i.e. low midnight salivary cortisol, adequate suppression after low dose dexamethasone, and normal 24 hour free urinary cortisol excretion.[5] In many cases, clinicians will need to deal with discrepant tests, and ultimately decide on the state of disease. Remission or recurrence will be based on the results of subsequent testing, integrated with the re-occurrence of clinical signs and symptoms. Although these considerations are well-adapted in clinical decision making and clinical management, in registries and outcome studies the definitions of disease state are quite heterogenous and not easy to interpret or compare.

For quality evaluations of our pituitary care path, we have recently developed an outcome evaluation method called Outcome Squared (Outcome2). Outcome2 provides a simple, patient-centered, clinically relevant representation of integrated outcomes.[31] Advantages of this method are the unified outcome classification in four categories, based on flexibly chosen definitions of intended and adverse effects, with full integration of efficacy and safety, which is needed to understand complex outcomes as is the case in pituitary surgery for CD. Outcome2 enables integrating intended and adverse effects, ranging from good to poor reflected by four integrated outcome quadrants (IOQs), merged into a cross table called outcome squares (see figure 1). When a strategy of re-interventions is adopted, the intermediate category "no remission and no adverse effects" (IOQ-3) is important, as sequential interventions may ultimately lead to remission without long-term adverse effects (good outcome, IOQ-1). However, since hypercortisolism needs to be controlled, remission with adverse effects (remission with hypopituitarism, IOQ-2) may sometimes be the only option. Furthermore, comparability of (heterogeneous) subgroups is facilitated using Outcome2, and it may therefore be a clinically helpful tool in informing patients about possible outcomes of surgery.

This study is the first to report on long-term outcome measures in patients with CD after transsphenoidal surgery, taking the delicate and clinically important balance between treatment efficacy and safety into account using the Outcome2 approach.

MATERIAL AND METHODS

Study population

All consecutive patients with CD primarily treated with transsphenoidal resection between January 1st 2000 (start of our multidisciplinary pituitary care team) and December 31st 2016 at our tertiary referral and European reference center for pituitary diseases were included in this cohort study.

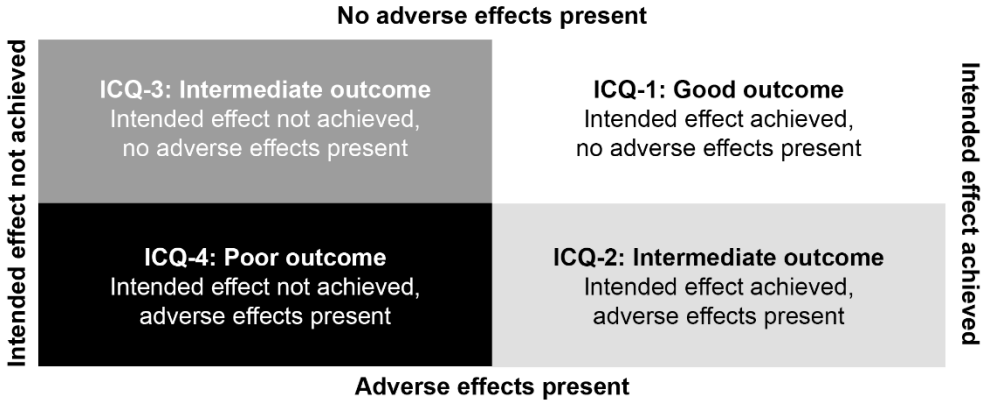

Figure 1. Outcome squares. Figure adapted from de Vries et al.[29].
Abbreviations: IOQ, Integrated Outcome Quadrant.

A retrospective chart review was performed of all patients; no exclusions were made based on tumor size or invasiveness or re-operation. There were no restrictions in adjuvant therapy in case of persistent or recurrent disease, or presurgical medical treatment with cortisol lowering agents.

Preoperative assessment

The diagnosis of CD was made based on both clinical signs and symptoms and biochemical testing, in accordance with the current clinical guidelines at time of diagnosis: increased 24 hour urinary free cortisol (UFC) excretion (> 220 nmol until 2010, > 150 nmol afterwards), insufficient suppression of morning serum cortisol after low-dose dexamethasone (1 mg) in the evening (> 50nmol/L), as well as a non-suppressed ACTH, and increased midnight salivary cortisol (> 5.7 nmol/L, available since 2004). An MRI scan (1.5-3 Tesla) with dynamic sequences was performed in all patients but one (computed tomography was used in this case due to a contraindication for MRI), and in case of inconclusive results, patients underwent bilateral inferior petrosal sinus sampling (IPSS) and usually repeated scanning. When the IPSS results were consistent with a pituitary source of ACTH overproduction, subsequent pituitary surgery with exploration of the sella was performed. Otherwise, imaging studies (CT thorax/abdomen, octreotide or gallium dotatate pet scan) were used to identify a possible ectopic ACTH-producing tumor. When no ectopic ACTH source was found, surgical treatment was only performed after a period of watchful waiting, repeated tests and after imaging or IPSS indicated a pituitary adenoma. All patients were discussed in our multidisciplinary pituitary care team, including endocrinologists, neurosurgeons, neuroradiologists, radiotherapists, ophthalmologists, and specialized pituitary nurses.

Treatment

The primary treatment was either microscopic or endoscopic transsphenoidal adenomectomy (TSA) for all included patients. The microscopic approach was used in all performed surgeries until 2002, and from 2003 onwards, the endoscopic procedure was increasingly used until it became

the standard treatment modality in our center from 2006 on.[32] Three experienced pituitary neurosurgeons performed all operations. Surgical strategy and technique are described in detail elsewhere.[33] When the adenoma was poorly or not visible on the MRI scan, multiple shallow incisions in the pituitary gland were made to localize the adenoma. In case of persistent or recurrent disease, (multiple) re-operations were performed in order to achieve remission, preferably without long-term adverse effects. However, ultimately remission with adverse effects was considered preferable over persistent CD.

Postoperative assessment and follow-up

The first postoperative biochemical evaluation was completed within two weeks after operation, usually with early morning cortisol level only. Three to six months postoperatively, remission state was assessed using both clinical criteria (hydrocortisone independency without any signs of hypercortisolism, and regression of clinical signs, or persisting dependency of hydrocortisone replacement) as well as biochemical criteria (normal suppression of morning cortisol after 1 mg dexamethasone [<50nmol/], normal 24 hour urinary free cortisol excretion, normal midnight salivary cortisol on two separate days, if not on hydrocortisone replacement therapy). Persistent disease was defined as the absence of remission upon evaluation after surgery. Disease recurrence was defined as clinical and biochemical recurrence after a period of remission of at least two to three months, according to the aforementioned criteria. Also in long-term follow-up, remission state was evaluated regularly (at least yearly) using the above mentioned criteria. For this study, follow-up data at three to six months after surgery (hereafter referred to as three months after surgery), one year, two years, and five years were used.

Efficacy parameters, adverse outcome and Outcome squares

For this study, the efficacy parameter intended effect of the intervention in our outcome integration model Outcome2 was defined as achievement of biochemical remission, either by hydrocortisone dependency or by normalization of hypercortisolism according to the current guideline (see above) and interpreted by the treating physician in case of discrepant values. [5] As described by de Vries et al.[31], Outcome2 is also suitable to evaluate alternative intended effects of surgery depending on the surgical goal, for example tumor debulking. In CD, however, the intrinsic features of the condition with its known morbidity and mortality, justifies that the aim of treatment will virtually most always be achieving complete remission of cortisol hypersecretion and not tumor debulking, as was the case in our cohort. However, the postoperative course of patients with CD requires that there are different definitions of biochemical remission in time, because early after surgery there will be adrenal deficiency and many tests to exclude recurrent hypercortisolism cannot be performed during steroid replacement therapy.

Interestingly, early postoperative adrenal insufficiency can first be seen as a preferred intended effect, while persisting in a later stage this turns into an undesired outcome. For this study, HPA-axis deficiency was considered an adverse effect when there was no tendency of recovery one year after surgery (as recovery of adrenal function usually occurs within this time period). To highlight

the consequences of this interpretation and because HPA-axis deficiency and time to recovery is relevant for outcome in patients' perspective, results are presented with and without HPA-axis deficiency included as an adverse effect from one year after surgery onwards to show outcome during follow-up.

In addition to evaluation of HPA-axis, the complication parameter, the "adverse effects" of the intervention, was (new-onset, permanent) pituitary deficiencies, because of the influence of these deficits on long-term comorbidity and quality of life. Pituitary deficiencies were defined as below normal serum values or abnormal values on currently used pituitary function tests, requiring medical management or with associated, irreversible symptoms. (Temporary) HPA-axis deficiency was taken into account separately, given the expected and intended effect of successful surgery on the activity of the HPA-axis. Diabetes insipidus (DI) was defined as polyuria (urine production >300cc/hour for 3 consecutive hours) with urine gravity < 1.005, in addition to at least one related criterium: excessive thirst, serum osmolality > 300 mosmol/kg, or serum sodium > 145 mmol/L,[34] and was also considered a pituitary function deficit if present. In case of hydrocortisone replacement in the context of postoperative steroid withdrawal syndrome with normal cortisol response during dynamic testing, the patients were not classified as HPA-axis deficient. In line with the publication of de Vries et al.[31] we focused on long-term, permanent adverse effects and did not include transient complications in these outcome squares.

For the presentation of the results based on intended effect of the surgery (remission) and adverse outcomes as described above, Outcome2 was used. This resulted in four integrated outcome quadrants (figure 1): good outcome (IOQ-1, intended effect achieved, no adverse effects), poor outcome (IOQ-4, intended effect not achieved, adverse effects present), intended effect achieved, adverse effects present (IOQ-2), and intended effect not achieved, no adverse effects (IOQ-3). Different Outcome squares were constructed for different follow-up periods in order to evaluate surgical outcome over time and also for different clinically relevant subgroups according to tumor size, surgical technique, preoperative medical pretreatment, and whether disease recurrence had occurred or not. The reported outcome includes the effect of possible re-interventions, if applicable. Recurrences and re-interventions shift the classification of patients over the four IOQs at different time points (figure 2). IOQs can be used to define outcome of a single intervention, but also of a multimodality strategy. This may result in an IOQ-1 classification when a patient with recurrent or persistent disease was reoperated and was in remission without adverse effects afterwards.

Statistical analysis

IBM SPSS statistics 25 (IBM Corp. Armonk, NY, USA) was used to perform statistical analysis and to construct Outcome2 two by two tables and piecharts. Descriptive statistics were used for describing the study population (baseline characteristics).

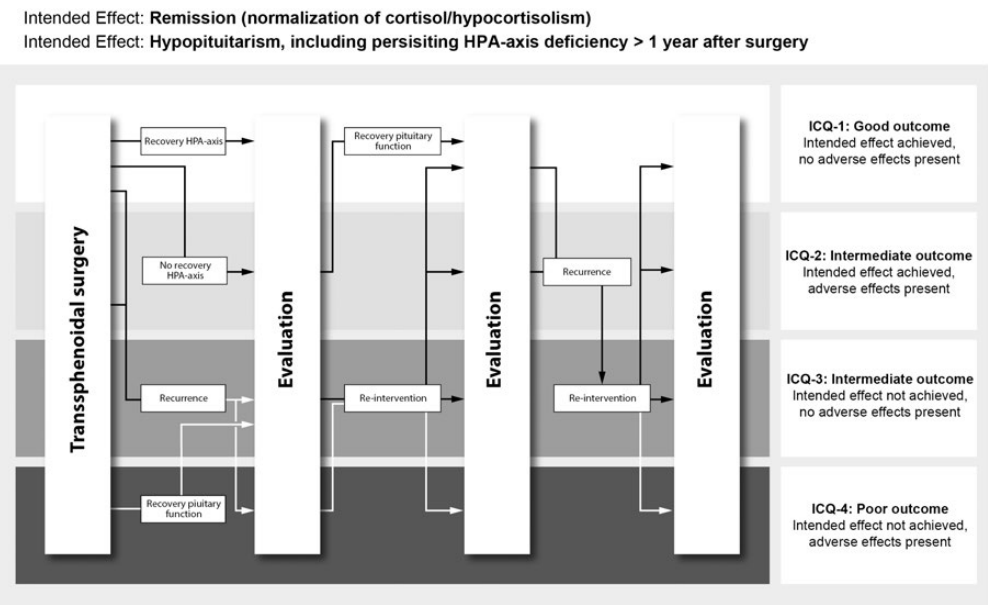

Figure 2. Conceptual framework of Cushing's disease patients shifts in Outcome[2] integrated outcome. Abbreviations: HPA-axis, Hypothalamus-Pituitary-Adrenal axis, IOQ, Integrated Outcome Quadrant.

RESULTS

Study population

In the specified time period, 74 consecutive CD patients were evaluated at our center. Two of them died/or were lost to follow-up before treatment could start. Therefore, 72 patients were surgically treated and included in this study, of whom 53 females (74%, in line with the available literature [35, 36]). The mean age at diagnosis was 45 years (range 10-80), and the mean Cushing Severity Index score[37] was 6.78 (range 0-14) during active disease. In 9 patients (12.5%) no adenoma was visible on preoperative MRI, in 8 patients (11%) a possible/uncertain adenoma was present, and in 53 patients (74%) a clear adenoma could be identified on preoperative imaging (of whom 25 showed a macroadenoma). IPSS prior to surgery was performed in 20 patients (28%). The majority of patients (n=67, 93%) was medically pretreated with cortisol lowering agents prior to surgery (metyrapone, ketoconazole, a combination of both, or pasireotide was used), as is common practice in our center. Fifteen patients (21%) underwent microscopic transsphenoidal surgery, whereas in 50 patients (69%) the endoscopic technique was used. Seven patients (10%) underwent surgery using a combined microscopic and endoscopic approach. In 57 patients (79%) a first adenoma resection was performed, 15 patients (21%) underwent a re-operation (10 because of recurrent disease, five because of persisting disease after first surgery). Sixteen patients (22%) underwent radiotherapy during follow-up, whether or not in combination with repeated transsphenoidal surgery (n= 7), multiple repeated transsphenoidal resections (n=1), or repeated transsphenoidal surgery and adrenalectomy (n=3). No sella clean outs were performed. The mean follow-up period of all patients

was 77 months (range 1-120). In ten patients (14%), recurrence occurred at any time during follow-up. (table 1)

Outcome Squares – Remission status and adverse outcome

Good outcome (IOQ-1, remission (e.g. hydrocortisone dependency or no biochemical signs of hypercortisolism) without adverse effects (e.g. ongoing hypopituitarism other than corticotroph deficiency)) was achieved in 56.5% (n=39) of patients three months after surgery, and in 55.4% (n=36) one year after surgery. Poor outcome (IOQ-4, e.g. no remission (e.g. ongoing hypercortisolism) and adverse outcome present (e.g. hypopituitarism)) was observed in 5.8% (n=4) of the patients after three months, and in 4.6% (n=3) one year after surgery. IOQ-2 (remission and adverse outcome present) listed 21.7% (n=15) of the patients three months after surgery, and 29.2% (n=19) one year after surgery, whereas 15.9% (n=11) of the patients were classified as IOQ-3 (no remission, no adverse outcome present) after three months, and 10.8% (n=7) one year post-operatively.

During prolonged follow-up, the good outcome group decreased only slightly to 53.3% (n=24) five years after surgery, mostly because patients were diagnosed with relative pituitary deficiencies other than corticotroph deficiency or disease recurrence occurred, and therefore patients shifted from IOQ-1 (good outcome) to IOQ-2 (remission and adverse outcome present) or IOQ-3 (no remission and no adverse outcome). The poor outcome group (IOQ-4) became smaller over time, with only one patient (2.2%) in this category five years after surgery, due to successful re-operations leading to remission in the other patients. The single patient in IOQ-4 was a case of mild

Table 1. Clinical characteristics of included Cushing's disease patients.

	CD patients n=72
Age, yrs (mean, range)	45 (10 - 80)
Sex, male / female (no)	19 / 53
CSI at diagnosis (mean, range)	6.78 (0 - 14)
Preoperative MRI (no, %)	
No adenoma visible	11 (15%)
Possible adenoma	8 (11%)
Clear adenoma	53 (74%)
Adenoma type, macroadenoma / microadenoma (no)	25 / 47
IPSS performed (no, %)	20 (28%)
Medical pretreatment (no, %)	67 (93%)
Surgical procedure TSA, microscopic / endoscopic* (no)	15 / 50
Follow-up time in months (mean, range)	77 (1 - 120)
Recurrence of disease (no, %)	10 (14%)
Re-intervention: TSA (one or more)	3
Re-intervention: TSA combined with RT	4
Re-intervention: RT	2

Abbreviations: CD, Cushing's disease, yrs, years, no, number, CSI, Cushing Severity Index score(37), MRI, Magnetic Resonance Imaging, TSA, transsphenoidal adenomectomy, RT, radiotherapy

*7 patients combined microscopic and endoscopic approach

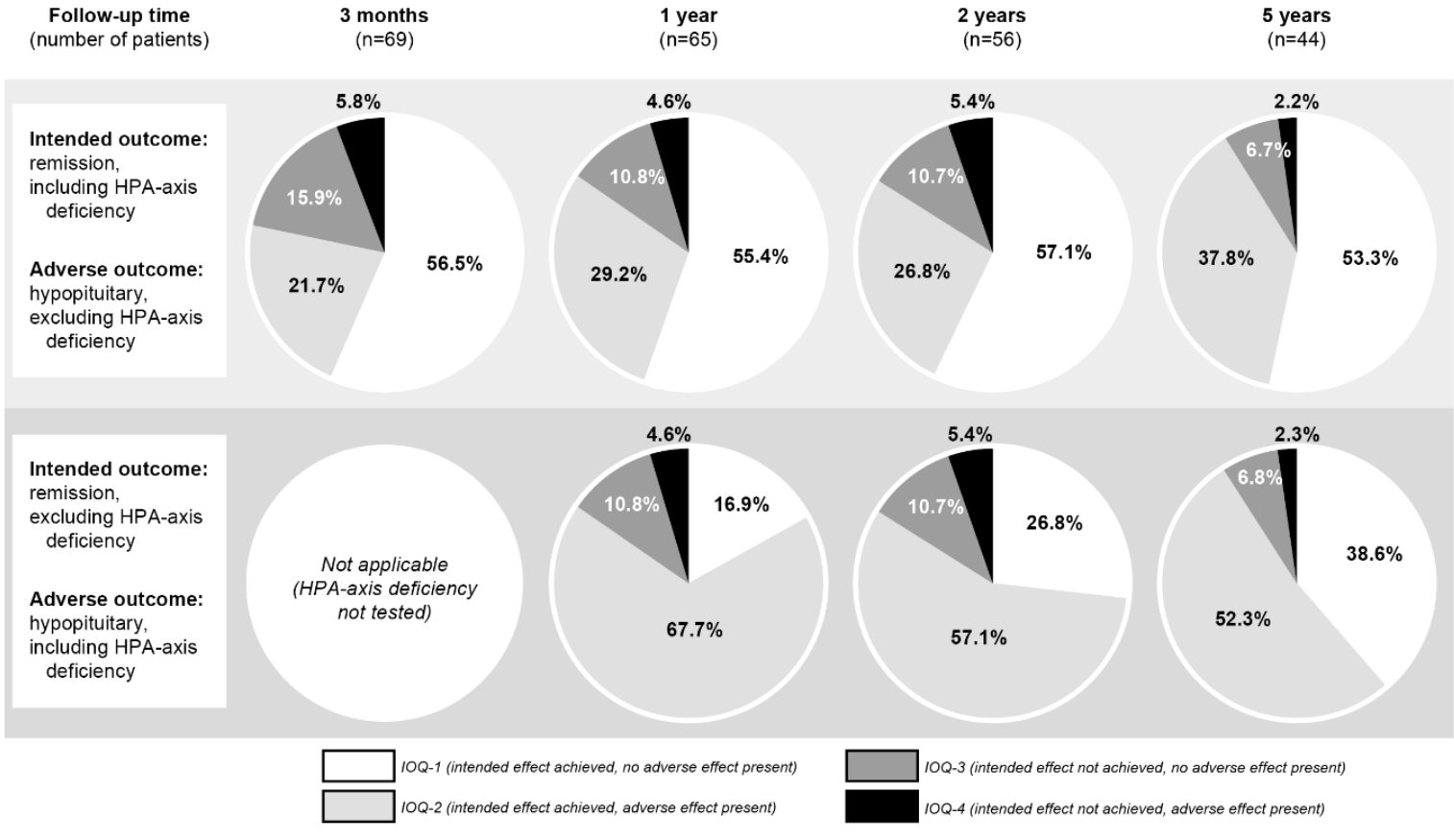

Figure 3. Outcome[2] integrated outcome quadrants after transspheniodal surgery in Cushing's disease over time.

biochemical hypercortisolism due to incomplete resection of the invasive macroadenoma, however without clinical signs and symptoms, and therefore without a wish for further treatment.

Five years after surgery, 37.8% of patients was classified as IOQ-2 (remission and adverse outcome present) and 6.7% as IOQ-3 (no remission, no adverse outcome), the latter group also decreasing over time due to successful re-operations. The patients in IOQ-3 five years after surgery were diagnosed with recurrence of disease just before this time point, and no re-intervention was performed yet at the moment of the five year evaluation. Figure 3 shows pie charts of the Outcome2 integrated outcome quadrants over the follow-up time of five years as described above.

In the third column of figure 3, the same follow-up time is displayed, only in these Outcome2 pie chart series the HPA-axis deficiencies are included in the adverse outcome category. Of the 46 patients with HPA-axis deficiencies three months after surgery, 33 patients (78.6%) had persisting adrenal insufficiency one year after surgery (four patients lost to follow-up), 21 patients (53.8%) two years after surgery (seven patients lost to follow-up), and 12 patients (42.9%) five years after surgery (18 patients lost to follow-up). One year after surgery, 16.9% of the patients (n=11) are in remission without adverse effects (IOQ-1), and 67.7% of the patients in remission (n=44) did have adverse effects mainly due to corticotroph deficiency (IOQ-2). The proportion of patients in IOQ-1 (good outcome) improved gradually over time to 38.6% (n=17) due to restoration of pituitary functioning (mainly recovery of HPA-axis functioning), and 52.3% (n=23) were classified in IOQ-2 after five years of follow-up (remission, but ongoing hypopituitarism). The number of patients in IOQ-3 and IOQ-4 also decreased over time, due to successful re-operations and restoration of the HPA-axis functioning.

Of special interest is the group of CD patients without remission after surgery (IOQ-3 and IOQ-4). One year after initial surgery, three patients were in the poor outcome category (IOQ-4). In two of these patients, poor outcome was also observed two years after surgery (one patient died between one and two years post-surgery), and one patient was still in IOQ-4 five years after surgery despite a second transsphenoidal operation (no five year follow-up data available in the other patient, no re-intervention performed during follow-up). Two of the three IOQ-4 patients had a macroadenoma (one with cavernous sinus invasion), and in one patient there was an uncertain microadenoma visible on preoperative MRI scan. Seven patients were in the intermediate outcome group without remission one year after surgery (IOQ-3). After re-intervention, all patients were in remission five years after initial surgery (four patients in IOQ-1, two patients in IOQ-2, one patient was lost to follow-up). Five of the seven IOQ-3 patients had a macroadenoma (of which four with cavernous sinus invasion), and in one patient preoperative MRI scan showed an uncertain microadenoma.

Analysis of the distribution of patients lost to follow-up at 5 years after surgery (n=27) over the IOQs at three months after surgery, showed that these patients were similarly distributed over the four categories, as was the total group of patients at the timepoint of three months follow-up.

Outcome Squares – subgroup analysis

Macroadenoma versus microadenoma

One year after transsphenoidal surgery, 92.7% (n=38) of all patients with a microadenoma (including invisible adenoma) were in remission, compared to 70.8% (n=17) of patients with a macroadenoma.

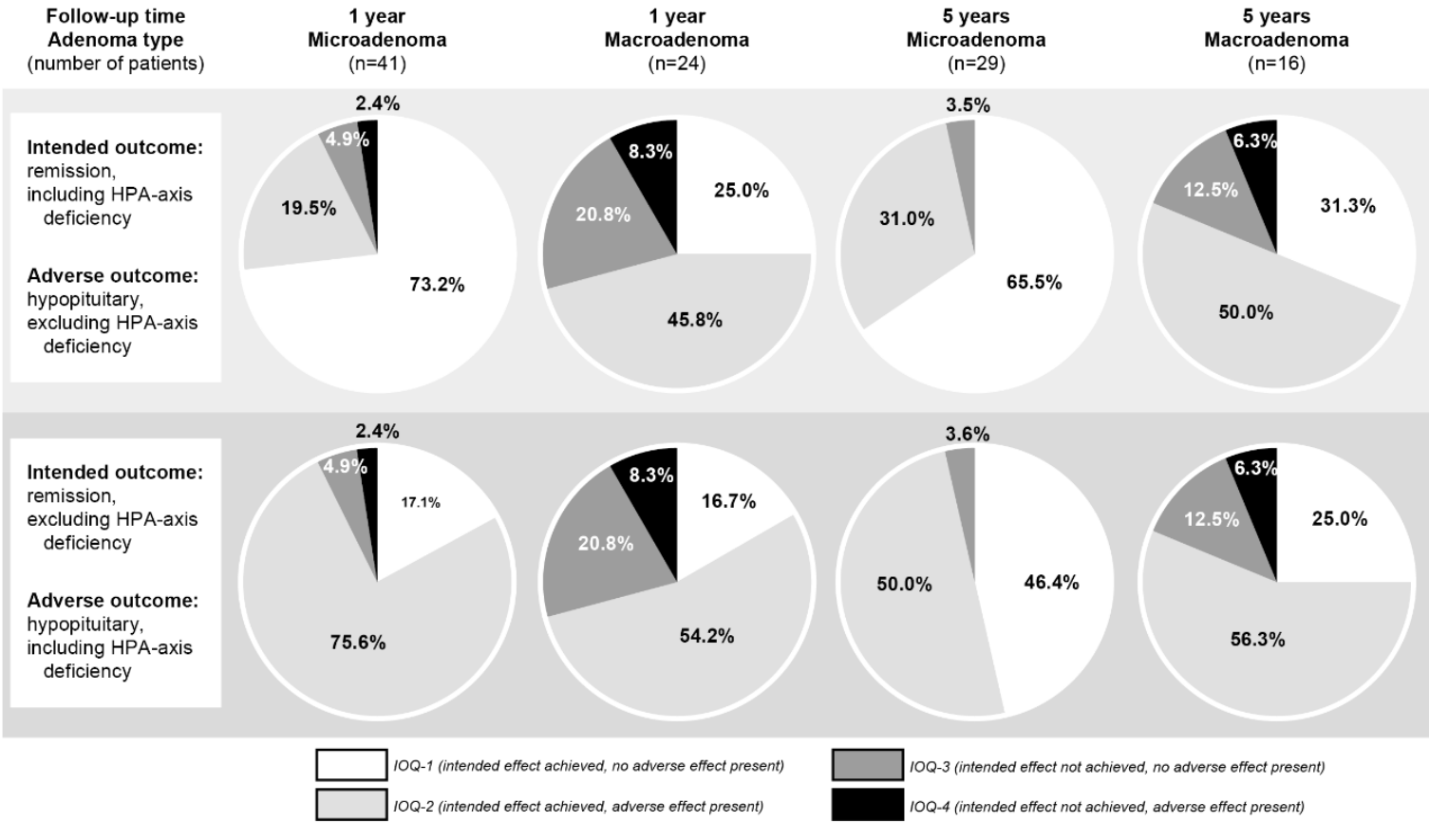

Figure 4. Outcome[2] integrated outcome quadrants in macro- versus microadenoma in Cushing's disease after transphenoidal surgery.

When we defined intended effect as biochemical remission (no hypercortisolism or adrenal insufficiency) and adverse effects as hypopituitarism excluding adrenal insufficiency at one year postoperative, for microadenoma the distribution in IOQs was as follows: IOQ-1 73.2% (n=30), IOQ-2 19.5% (n=8), IOQ-3 4.9% (n=2), and IOQ-4 2.4% (n=1). For macroadenoma patients, the distribution was as follows: IOQ-1 25.0% (n=6), IOQ-2 45.8% (n=11), IOQ-3 20.8% (n=5), and IOQ-4 8.3% (n=2). In an additional Outcome square, deficiency of the HPA-axis was included as an adverse outcome (see figure 4).

It is of note that six of the macroadenoma patients in IOQ-2 already had pre-operative pituitary deficiencies due to the macroadenoma itself, so it is debatable whether this needs to be registered as an adverse effect, with persisting deficiencies after surgery. In nine of the 47 microadenoma patients and in 16 of the 25 macroadenoma patients a re-intervention was performed during follow-up.

The long-term follow-up results (five years after surgery) using the Outcome2 method for macro- and microadenoma CD patients are also shown in figure 4. The decrease in IOQ-4 (poor outcome) patients over time was due to loss of follow-up.

A separate category concerns patients with an invisible adenoma, due to the different surgical approach as described above. In our cohort, 12.5% of the patients (n=9) did not have a visible adenoma on preoperative imaging. When looking at the outcome measurements of these patients, one year after surgery, the distribution of patients per IOQ was as follows: IOQ-1: five patients, IOQ-2 two patients, IOQ-3 one patient, IOQ-4 no patients (in one patients, outcome data was lacking). missing 1. Five years after surgery, four patients had good outcome, and three patients were in IOQ-2 (in two patients no five year follow-up data was available). The one patients in IOQ-3 one year after surgery, shifted to IOQ-2 due to repeated transsphenoidal resection in combination with radiotherapy and adrenalectomy. When including ongoing HPA-axis deficiency as an adverse effect, six patients were in IOQ-2 five years after surgery, and only one patient was in IOQ-1.

Microscopic versus endoscopic transsphenoidal resection

The results of transsphenoidal surgery by surgical technique according to the Outcome2 method are depicted in figure 5. Follow-up data were available on 15 patients who underwent microscopic surgery and 43 patients who were operated on using the endoscopic technique. Figure 5 shows the outcome one year and five years after surgery (HPA-axis deficiency excluded as adverse effect), showing that the results regarding the percentages of patients in the different IOQs did not differ significantly between the two operation technique groups. There was a tendency for more HPA-axis deficiency in the endoscopic group and more remission without deficiencies including HPA-axis deficiency after five years (IOQ-1, endoscopic 45.5% versus microscopic 33.3%). At five years follow-up, the microscopic group had more remission with deficiencies (IOQ-2, endoscopic 40.9% versus microscopic 60.0%). Since only seven patients were operated through combined endoscopic and microscopic procedure, no separate analysis was performed on this specific category.

Recurrence of disease

Since a treatment strategy consisting of multiple interventions if necessary in order to achieve remission is frequently needed in Cushing's disease and adopted by our multidisciplinary pituitary

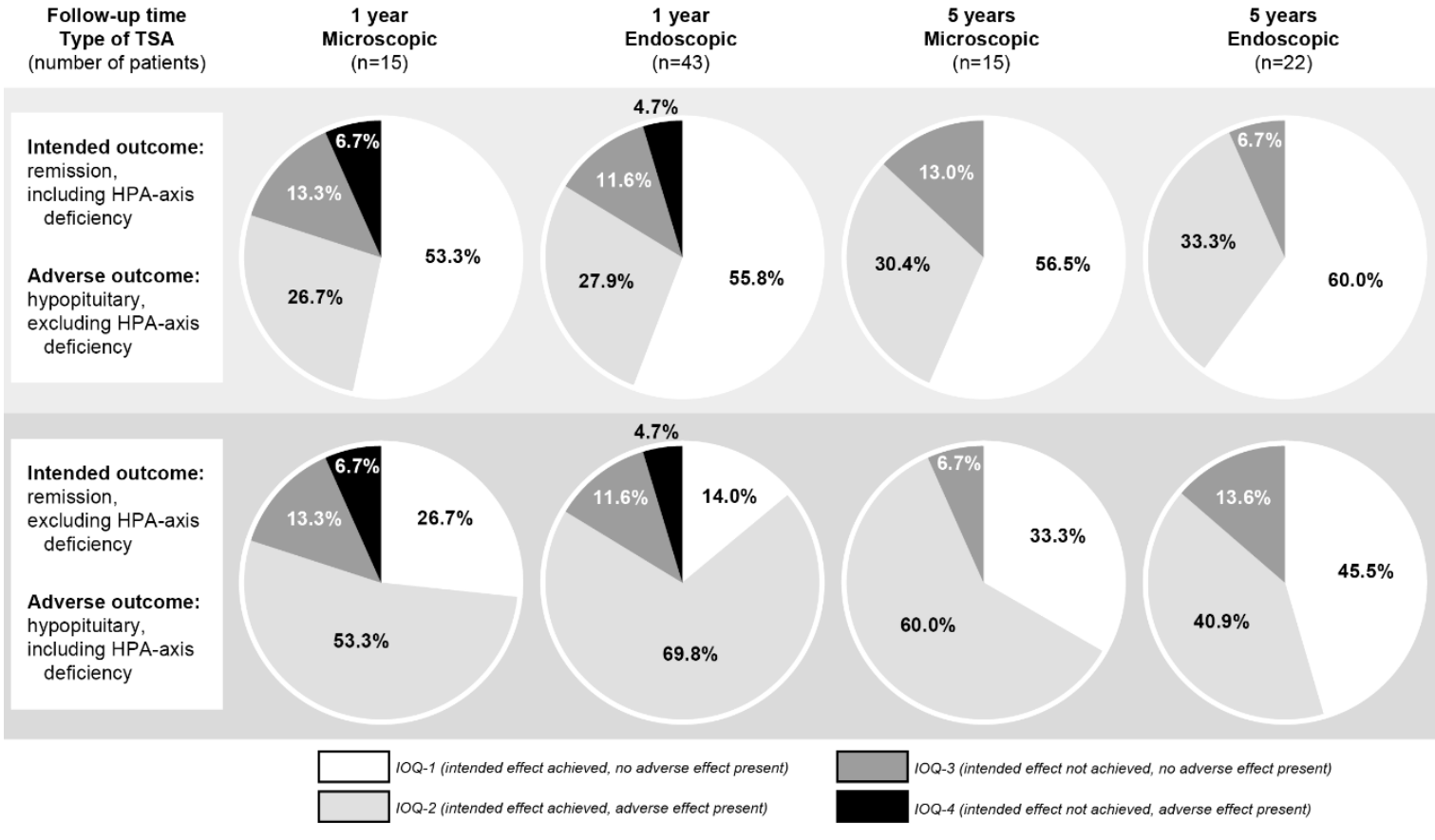

Figure 5. Outcome[2] integrated outcome quadrants in endoscopic versus microscopic transsphenoidal surgery in Cushing's disease patients.

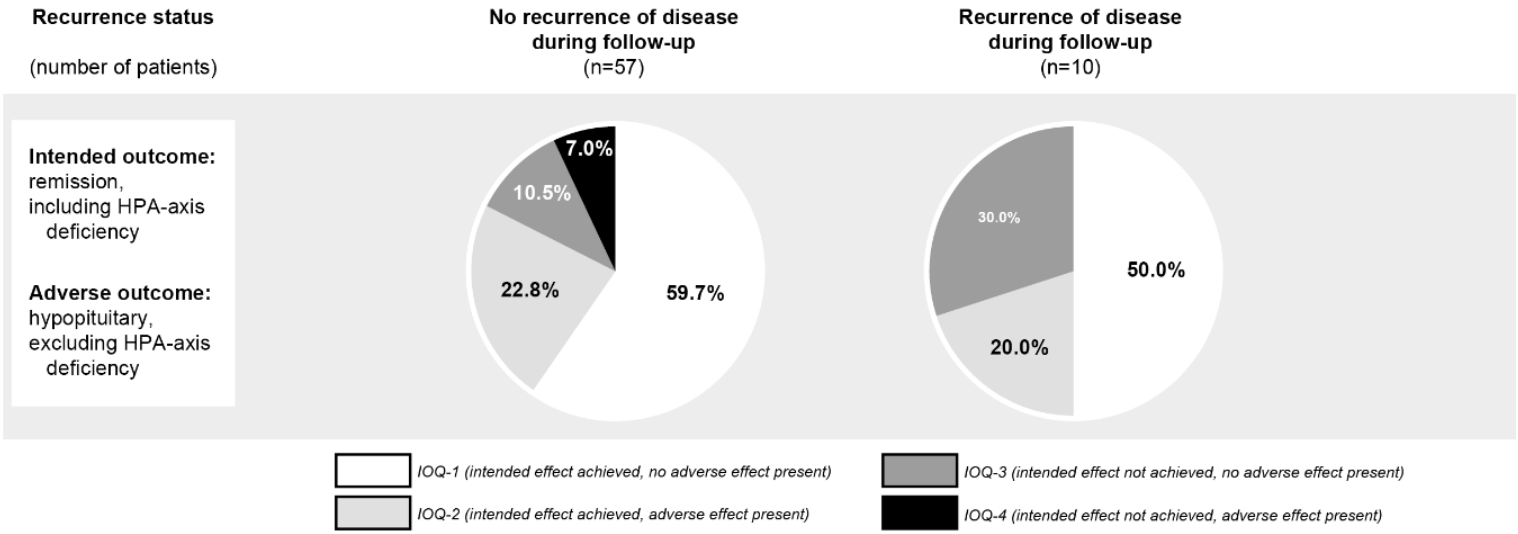

Figure 6. Outcome[2] integrated outcome quadrants in CD patients with eventual recurrence of disease versus patients without recurrence.

care team as a management approach to aim for remission without hypopituitarism, the outcome of the recurrence-of-disease group is of special interest. These patients flow through the different IOQs during the time course. During the five years of post-operative follow-up, ten patients (13.9%) manifested recurrence of disease (six macroadenoma patients, four microadenoma patients). In three of these ten patients (30.0%), recurrence of disease occurred within the first three to six months after surgery (after initial remission was biochemically confirmed), of which one was successfully re-operated within one year after first surgery.

The following re-interventions were performed: single transsphenoidal re-operation (n=2), multiple transsphenoidal re-operations (n=1), radiotherapy (n=2) or re-operation combined with radiotherapy (n=4). In one patient, no re-intervention was performed yet during follow-up time.

There were no patients with poor outcome (IOQ-4) in the recurrence-of-disease group at one year follow-up (e.g. all recurrence patients were in IOQ-3). Comparing these ten recurrence patients to the rest of de CD patients in our cohort at three months after the initial surgery, slightly less patients with an eventual recurrence were in IOQ-1 (50.0% versus 59.6% IOQ-1 in the non-recurrence group, figure 6). Of the ten patients with disease recurrence, seven were re-operated upon during follow-up time. In four of these seven patients, the re-operation was combined with radiotherapy, and one of these seven patients underwent re-operation more than once. Two patients with recurrent disease underwent radiotherapy alone in order to achieve biochemical remission. One recurrence patient did not receive any re-intervention yet during follow-up time. After re-intervention (n=9), remission was achieved in four patients (one radiotherapy alone, one multiple TSAs, two TSA combined with radiotherapy respectively), and in three patients no follow-up data was yet available as re-intervention had taken place by the end of the follow-up time. Of the four patients in remission after re-intervention, two patients were in IOQ-1 (both TSA combined with radiotherapy, with one of them having another recurrence five years after the first operation and four years after the re-intervention), two patients (one radiotherapy alone, one multiple TSAs) were in IOQ-2 at the end of follow-up. The two patients who were not in remission after re-intervention were in IOQ-3 (HPA-axis deficiency excluded as an adverse effect).

DISCUSSION

This study is the first to report on long-term outcome measures in patients with CD after transsphenoidal surgery, using Outcome2, a novel way to integrate treatment outcomes, which uniquely allows to show the delicate but clinically very important balance between treatment efficacy and safety. For CD this is of special interest as (transient) hypocortisolism can be either classified as intended, or adverse treatment effect, depending on whether the focus is on doctor's perspective or patients' perspective.

One year after surgery, good outcome was observed in 55% of the 72 included patients, whereas poor outcome was present in only 5%. When ongoing HPA-axis insufficiency was regarded as adverse outcome, only 17% of the patients showed good outcome after one year, whereas 68% was in remission with the presence of pituitary deficiencies. Over time, a gradually shift of patients to the good outcome category occurred, mainly due to recovery of the activity of the HPA-axis.

Five years after transsphenoidal surgery, the majority of patients were in remission, though a considerable proportion of patients had persistent hypopituitarism (partly explained by HPA-axis deficiency). For patients and physicians the awareness of the gradual course of recovery of HPA-axis functioning is needed when interpreting outcome, since HPA-axis deficiency is also a condition with morbidity and mortality, albeit less severe than active hypercortisolism.

Patients with microadenoma were more often in remission without new onset hypopituitarism compared to the macroadenoma patients. Also poor outcome was observed slightly more often in the macroadenoma patients, as might be expected due to the extent of the tumor and therefore the extensiveness of the surgical procedure. The results of patients with an invisible adenoma on preoperative imaging one year after surgery were not worse than the results of the rest of the cohort/microadenoma patients, despite a slightly more invasive surgical technique. These results should be taken into account in pre-operative patient counseling.

The results of the different operation techniques used in this cohort (e.g. microscopic versus endoscopic approach) did not differ significantly when comparing the IOQs. Comparing patients with eventual recurrent disease to the rest of the cohort three months after surgery, one could hypothesize more patients with eventual recurrence of disease would be in IOQ-1 direct postoperatively, due to less aggressive surgery. However, in our cohort slightly less recurrence-patients were in the good outcome group (IOQ-1) three months after surgery. The Outcome2 approach is very suitable to evaluate outcome of treatment strategies including multiple interventions instead of focusing on a single intervention. As was shown by the results of this study, even after multiple and combined interventions remission without adverse effects (IOQ-1) can be achieved and re-interventions can be considered as save. The poor outcome group (IOQ-4) is an interesting category both from patients' and doctors perspective. In our cohort, the poor outcome group is small. Macroadenoma patients and patients with an invisible/uncertain adenoma on preoperative MRI scan appeared to be at increased risk of poor outcome.

The remission rates obtained from this study are in line with previously published (small) studies on surgical outcome in CD.[38-40] An important difference between our study and other studies on surgical results in CD is that we included all surgeries, including those for giant/invasive adenomas, apoplexy and re-operations. The results of this large cohort study are theoretically generalizable to all CD patients treated by transsphenoidal adenomectomy, however, the generalizability may be reduced by the specific setting in our tertiary referral hospital, since all patients in this study were operated by experienced neurosurgeons and difficult procedures were not shunned, and the time period of inclusion.

In the current available literature, there are no other cohort studies presenting their surgical results against the important balance between the efficacy and safety of surgical treatment. This balance is of particular importance in the CD population, as the delicate balance between remission and ongoing pituitary gland injury is of major importance for the patient's wellbeing and quality of life.[8-12] Outcome2 can be used for uniform reporting of results and provide more accurate information at a glance for individualized patient counselling in the physician's office. With the presentation of results of this study, a care provider can easily recognize patient groups with good or adverse outcomes and modify treatment strategies accordingly if applicable. The use of Outcome2 can

provide comparisons of outcomes of different treatment strategies, for example medical therapy versus radiotherapy in persistent disease, and other outcomes relevant to patients and their quality of life (e.g. symptomatology, burden of disease, functional outcome) can be incorporated in the four outcome categories (IOQs) by adjusting the definitions of intended and adverse effects. The Outcome2 method for integrating outcome reveals specific elements of interest for each outcome group. For instance, the "intended effect at a cost" group (IOQ-2) shows which patients are paying a price for cure, and in the "no harm done" group (IOQ-3) an additional intervention may be useful to still achieve the treatment goal. It would be of interest to future research to include quality of life measures in the definitions of the four IOQs, to even further refine the outcome analysis. The Outcome2 approach is also very suitable to evaluate outcome of treatment strategies including multiple interventions instead of focusing on a single intervention. As was shown by the results of this study, even after multiple and combined interventions remission without adverse effects (IOQ-1) can be achieved and re-interventions can be considered as save. The form of outcome integration as depicted by the four IOQs can be used for the comparison between centres and studies, provided that identical definitions and outcome are used. In the pituitary field, we propose to use the Outcome2 approach, however, international consensus is needed. The advantages of the Outcome2 approach include a unified outcome measure regardless of the type of intervention or measurements used, taking the balance between efficacy and safety/adverse effects into account, actionable evaluation purposes providing insight in the shift of patients over different outcome categories over time, and insight in the impact of HPA-axis deficiency which is most relevant from a patients' perspective.

When interpreting the results of this study, a few limitations need to be taken into account. First of all, selective loss to follow-up could have led to selection bias. However, analysis of the distribution of patients lost to follow-up at five years after surgery over the IOQs after three months of follow-up showed these patients were similarly distributed over the four categories/IOQs as was the total group of patients at that point in time. Various reasons including both very poor health status as well as excellent health could have led to loss to follow-up, therefore the direction in which the results may have been biased could not be determined. Secondly, by dividing patients in four outcome categories, the number of patients per group were small in certain subgroup analyses. Furthermore, patients treated according to the microscopic surgical approach underwent surgery in a different time period compared to the patients treated by means of endoscopic surgery, since patients were not randomized to a certain operation technique, but were treated by the technique used at that time. This might have resulted in differences between these two subgroups. Baseline patients characteristics, such as the presence of comorbidities, size and invasiveness of the tumor, medical pretreatment and CSI score, did however not differ between the two groups. Over time, diagnostic imaging has changed with respect to the quality of MRI, resulting in better visualization of suspect lesions to target during surgery. Therefore, the effect of the imaging technique can theoretically not be separated from the effect of the surgical technique. Moreover, the volume of transsphenoidal operations per year in our center has increased over the years. It is well described that the surgeons volume of pituitary interventions is crucial to its outcomes.[41] Nonetheless, with the increase of the number of transsphenoidal operations in our center over the years, there was also an increase in

more difficult procedures, e.g. giant or invasive adenomas, which makes it challenging to determine the direction in which this might have biased our results. The determination of the goal/intended effect of treatment of each patient is discussed in our multidisciplinary pituitary care team, which plays a key role in pituitary care in the Leiden University Medical Center.

In conclusion, this study is the first to report on long-term outcome measures in a large cohort of CD patients after transsphenoidal surgery using the Outcome2 integrated outcome approach. This study shows that the majority of patients are in remission five years after transsphenoidal surgery, though in a considerable part of the patients at the expense of persistent failure of pituitary functions, including ongoing hypocortisolism. Application of the four different IOQs used provides an uniform, overall view at a glance of the important balance between efficacy and safety of the transsphenoidal adenomectomy in CD, and can be a helpful tool in individualised patient counselling.

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CHAPTER 10

GENERAL DISCUSSION AND SUMMARY

Stress is a psychophysiological response to difficult or challenging situations and to physical as well as psychological stressors.[1, 2] The endocrine system, and especially the stress hormone cortisol, play an important role in regulating the stress response.[3] Between 20 to 40 minutes after the initiation of a stressful event, cortisol secretion peaks, amongst others resulting in releasing energy stores by elevating blood glucose levels to provide metabolic 'fuel' for the body, and inhibiting non-essential functions such as aspects of immune system, digestive system, and reproductive system, permitting other systems like the sympathetic nervous system and certain psychological systems necessary to overcome threat to function effectively.[4] Cortisol is essential for survival, however prolonged stress and prolonged exposure to increased cortisol concentrations lead to tissue damage and disease, and consequently, adversely affect multiple vital organ systems.[2] In Cushing's syndrome (CS), cortisol is produced excessively, whereby prolonged and excessive hypercortisolism is associated with increased morbidity and mortality, and decreased quality of life.[5-8] Central serous chorioretinopathy (CSC), a specific form of macular degeneration, is another disorder in which stress and cortisol are believed to be involved in the pathophysiology, in this case by playing a possible role in triggering the development of the disease.[9-11] In CSC, thickening, hyperpermeability and choroidal congestion damage the retinal pigment epithelium, inducing serous subretinal fluid accumulation and subsequently detachment of the neuroretina.[9, 12, 13] Thus, both CS and CSC are rare conditions in which the activity of the hypothalamus-pituitary-adrenal (HPA)-axis and cortisol play a key role. This thesis addresses the pathophysiology of stress related diseases, taking the aforementioned rare diseases as a model for stress vulnerability of the brain (CS) and the eye (CSC). The second aim of this thesis was to describe the organization of thromboprophylaxis management, and the outcome evaluation and quality of care for patients treated for CS. **Chapter 1** provides a general introduction to the regulation of the stress response, the rare conditions of CS, and CSC, and discusses the underlying rationale for the studies presented in this thesis.

PART ONE

In **chapter 2**, a detailed ophthalmological screening with multimodal imaging including optical coherence tomography (OCT) was performed in a series of consecutive patients with active CS without visual complaints, in order to evaluate possible subclinical abnormalities within the CSC spectrum and the potential need for standardized ophthalmological evaluation of all CS patients. Of the 11 patients included, three patients showed abnormalities reminiscent of (subclinical) CSC. One patient was subsequently diagnosed with active CSC, including macular subretinal fluid on OCT, and was successfully treated with half-dose photodynamic therapy. In one other patient OCT revealed a unilateral pseudovitelliform lesion and on fluorescein angiography hyperfluorescent changes were seen, while the third patient showed unilateral leakage on fluorescein angiography. Therefore, retinal abnormalities resembling (subclinical) CSC in patients with CS may exist even in the absence of visual complaints, and might be more common than previously thought.

Clinical implications: Because of the therapeutical consequences aimed at prevention of loss of vision, clinicians/endocrinologists should actively question patients with CS about any visual complaints and apply a low threshold for referring patients for ophthalmological evaluation. We

advocate that routine screening of all newly diagnosed patients with CS for abnormalities within the CSC spectrum should be a topic of a future multi-center, prospective cohort study.

Vice versa, since a relationship is presumed between stress and the onset of CSC,[9-11] and CSC can be the presenting symptom of CS,[14] it is relevant to question whether patients with CSC should be screened for CS. In **chapter 3,** a systematic screening for the presence of CS in a large cohort of chronic CSC patients is presented, aiming to assess the prevalence of CS in patients with chronic CSC, and to assess whether chronic CSC is associated with hyperactivity of the HPA-axis. None of the 86 included chronic CSC patients met the clinical or biochemical criteria of CS. However, the activity of the HPA-axis was increased in patients compared to healthy controls, as reflected by higher 24 hour urinary free cortisol (within the normal range) with a mean difference of 32 nmol/24 hour, and accompanying higher waist circumference and diastolic blood pressure. Circadian, diurnal cortisol rhythm was preserved in CSC patients. Furthermore, in contrast to earlier studies suggesting an association between CSC and psychosocial stress,[11, 15-17] CSC patients did not report more stress or stress-related problems using validated questionnaires. In addition, no associations were found between HPA-axis activity, CSC activity and psychosocial stress.

Clinical implications: Based on these results, routine screening for CS in all CSC patients is clearly not indicated. Since the interpretation of the biochemical screening tests in light of the clinical features is often challenging and in order to minimize false positive test results, screening should be reserved for CSC patients with additional clinical signs and symptoms raising the suspicion of hypercortisolism. However, the results of the study clearly indicate increased activity of the HPA-axis in CSC patients when compared to controls, although this is not accompanied with the perception of more psychosocial stress. This observed higher activity of the HPA-axis is in concordance with the previously reported association between cortisol and CSC, yet further studies are needed to unravel the underlying pathophysiological mechanisms and the role of stress and stress-reducing interventions in the onset and clinical course of CSC.

The activity of the HPA-axis as a warrant of endogenous exposure to stress and cortisol can be determined with a number of different tests, all reflecting different aspects of secretion and exposure to cortisol. Concentrations of cortisol in scalp hair can be measured to estimate long-term cortisol exposure. In order to investigate the suspected relationship between cortisol and chronic CSC, hair cortisol concentrations in a large cohort of 48 chronic CSC patients, participating in the afore described study on HPA-axis evaluation in CSC, were evaluated and compared to the concentrations of hair cortisol of adult controls from the general population (**chapter 4**). Increased hair cortisol concentrations were present in 4% of patients with CSC and in 6% of the controls, and the mean hair cortisol concentration was not different between the two groups. This finding questions the previously reported suggestion of HPA-axis hyperactivity in CSC, however, the assessment method using hair cortisol concentrations most probably capture a different aspect of cortisol exposure then the biochemical evaluations used in other studies (e.g. 24 hour urinary free cortisol or salivary cortisol levels). We propose that either the hair cortisol concentrations technique is not sensitive enough to detect minor and perhaps short-term elevations in cortisol levels within

the normal range, keeping in mind the large individual variation in normal cortisol levels and glucocorticoid sensitivity, or minor increases in cortisol concentrations at tissue level leading to the specific CSC alterations are not reflected by increased concentrations of cortisol in hair. One could also argue that the long-term cortisol exposure is not increased in CSC, however a short peak or prolonged temporary elevation in cortisol levels in sensitive subjects may be sufficient to induce the retinal alterations characteristic of CSC. This hypothesis is supported by the described appearance of CSC after short-term steroid treatment.[10] Based on these novel observations, it is also plausible to assume that the relationship between CSC and cortisol is not as straightforward as previously thought. Furthermore, no correlation between hair cortisol concentrations and urinary free cortisol levels was seen in patients with chronic CSC, despite the reported strong correlations between hair cortisol concentrations and urinary free cortisol levels in patients with CS.[18, 19] Finally, no difference in hair cortisol concentrations was found between patients with active CSC disease compared to patients with inactive disease, indicating the absence of an association between disease severity and hair cortisol concentrations.

Clinical implications: Hair cortisol concentrations in patients with CSC are not elevated compared to population-based controls, and no association between hair cortisol concentrations and CSC severity was found. Therefore, hair cortisol concentrations are not useful in monitoring CSC disease activity.

Along with biochemical stress as reflected by HPA-axis hyperactivity, also psychosocial stress and 'type A' behavioural aspects are described to be associated with CSC. [11, 16, 20] In order to identify potentially modifiable psychosocial aspects in support to the current standard treatment, **chapter 5** reports on a cross-sectional study in a cohort of 86 patients with chronic CSC using validated questionnaires to capture the presence of possible maladaptive personality traits (i.e. traits related to type A behavioural pattern), apathy and irritability, and coping strategies. Patients' findings were compared to both Dutch population based reference data and data from patients treated for Cushing's disease. Psychological morbidity in the form of apathy and irritability was not increased in CSC patients. In addition, maladaptive personality traits such as type A behavioural characteristics were not more prevalent in patients with CSC compared to the general population. These are intriguing findings because they contradict what has been suggested in previous studies.[16, 21-23] However, in these studies, behavioural characteristics were mainly assessed using behavioural outcome measures, showing no correlation with personality characteristics and psychopathology[24], and type A behavioural characteristics were not strictly defined.

CSC patients make more use of certain coping strategies (e.g. passive coping, seeking social support, and in males also active coping). Remarkably, though not statistically significant, the personality profile, psychological morbidity, and coping characteristics of CSC patients were more comparable to features of treated Cushing's disease patients than to the population-based data. Because patients treated for Cushing's disease have been exposed long-term to excessive cortisol levels, these patients can be regarded as a human model to study the effects of cortisol excess, amongst others, on personality and behaviour. Maladaptive personality traits and psychological morbidity such as apathy and irritability have been well described in patients with

Cushing's disease.[25, 26] In line with the biochemical resemblance of an activated HPA-axis in both CSC (slightly activated HPA-axis) and Cushing's disease (excessive activation of the HPA-axis) as mentioned above, this study also showed a relative similarity regarding the spectrum of personality features.

Clinical implications: Ophthalmologists often assume and report stress-related and type A behavioural characteristics in CSC patients,[16, 21, 22] and therefore stress-reduction and interventions targeting personality traits are common in clinical management strategies.[27-29] However, based on the results of the present study, these interventions may not be useful. Yet, the use of certain coping strategies could be a point to address in psychosocial care and self-management programs.

PART TWO

The second part of this thesis focused on the organization, outcome, and quality of care for patients with CS. **Chapter 6** systematically reviewed the literature to investigate whether mortality remains increased in patients biochemically cured of Cushing's disease. In addition, a meta-analysis was performed, including follow-up studies reporting the standardized mortality ratio (SMR) for patients cured from Cushing's disease after initial treatment. A total of 766 patients that were included in eight studies were included in the meta-analysis. Seven out of the eight studies showed a SMR above 1.0, with a pooled SMR of 2.5 (95% CI 1.4 - 4.2) when including all studies. Also, when a sensitivity analysis excluding two outliers was performed, the SMR remained increased. This means that mortality remains increased in patients with Cushing's disease even after initial biochemical remission, suggesting that cure does not fully reverse the metabolic effects of long-term exposure to cortisol excess. Unfortunately, applying meta-regression techniques to assess potential causes of increased mortality was not possible due to the lack of individual patient characteristics stratified by cure status. However, it is plausible that adverse effects of the disease and/or its treatment such as hypopituitarism contribute to the persisting increased mortality risk, although the percentage of post-treatment hypopituitarism could not be extracted from the studies. Furthermore, the results of the meta-analysis are supported by evidence showing persistent multisystem morbidity after biochemical cure, since there is accumulating evidence that morbidity related to cortisol excess decreases after successful treatment of Cushing's disease, however does not normalize. A high prevalence of atherosclerosis and an increased cardiovascular risk are reported to maintain after curation, which are thought to be related to residual abdominal obesity and insulin resistance.[30] Also the risk for myocardial infarction and stroke in cured Cushing's disease patients is shown to remain increased during long-term follow-up,[31] and even an increased prevalence of psychopathology and cognitive impairments are documented.[25, 32]

Apart from the residual physical and psychological morbidity and increased mortality, patients biochemically cured from Cushing's disease also report persisting impairments in cognitive and executive functioning.[25, 33] Furthermore, a reduction in quality of life is reported to persist despite curation.[34] The question whether patients with remitted Cushing's disease also demonstrate altered performance and brain activity patterns with regard to cognitive planning and executive functioning, was assessed in **chapter 7** by means of functional magnetic resonance imaging, while

both patients and healthy controls complete a Tower of London task (parametric visuospatial planning task). Twenty-one cured Cushing's disease patients and an equal number of healthy gender-, age-, and level of education-matched controls were included. No differences were found in performance between the two groups, neither in number of correct trials, nor in response times per trial, or in the region of interest analysis. As previous studies revealed visuospatial impairments in active Cushing's disease patients,[35] our findings suggest that these impairments can improve after remission. Exploratory whole-brain analyses demonstrated increased brain activation in certain brain areas during the Tower of London task in remitted patients, indicating patients need to over-recruit these brain regions involved in higher cognitive processes to attain a similar performance level as healthy controls, and thus require more effort to successfully complete a visuospatial planning task.

Clinical implications: The persistently increased mortality risk despite remission of hypercortisolism suggests irreversible effects of long-term glucocorticoid excess exposure. It also seems that prolonged exposure of the brain to cortisol excess leads to permanent alterations in brain activation of certain regions, even after long-term remission. Cushing's disease may therefore result in long-term, irreversible, subtle scarring effects during (demanding) executive functioning tasks. These findings are important and of relevance to patients counseling in everyday clinical practice, but also to increase the awareness of the treating physician to provide good quality follow-up care with an eye for persisting complaints and comorbidity management.

During active hypercortisolism as well as in the postoperative period, and even after remission, an increased risk of venous thromboembolism has been consistently reported.[36-38] Evidence-based guidelines on prophylactic anticoagulation in these patients with such a rare condition are not available due to the absence of prospective treatment studies evaluating thromboprophylaxis effects on the occurrence of venous thromboembolism in CS. In the context of quality of care and evaluation of outcomes and complications, a clinical guideline addressing thromboprophylaxis management in patients with CS is currently a clear unmet need. A first step in conducting studies useful for guideline development, is to map the current clinical practice regarding this specific subject. **Chapter 8** aimed to map the current clinical thromboprophylaxis strategies in patients with CS across expert reference centers within the European Reference Network (ERN) on Rare Endocrine Conditions (Endo-ERN). These centers have specifically been endorsed as expert centers for the diagnosis and treatment of CS. The results of the online survey demonstrate that a large practice variation regarding thromboprophylaxis management in patients with CS still exists, even in the expert centers of Endo-ERN. Although the majority of the reference centers (23 out of 25) provided thromboprophylaxis to their patients, a standardized treatment protocol was available in only one center. Also the time of initiation and abrogation of thromboprophylaxis varied greatly between the centers.

Clinical implications: These results exemplify the need for a protocolled strategy for thromboprophylaxis in CS that will enable to assess the best, possibly even individualized, treatment options.

For quality and outcome evaluation purposes, an outcome evaluation method called Outcome Squared was recently developed by our center for patients surgically treated for pituitary tumors. This method unifies different outcome parameters in time, taking the balance between efficacy and safety into account, by including both remission and complications in a standard classification for multidimensional outcome evaluation.[39] Especially in Cushing's disease, for which transsphenoidal surgery is the first line treatment, both remission and complications determine the overall success of the procedure. Failure to achieve remission is potentially life threatening,[40] whereas complications such as hypopituitarism including adrenal insufficiency are also associated with comorbidities, increased mortality, and reduced quality of life.[41-44] **Chapter 9** reported on long-term integrated postoperative follow-up measures in patients with Cushing's disease using the Outcome Squared approach. Seventy-two consecutive patients treated by transsphenoidal resection were included. One year after surgery, 55.4% of the patients showed good outcome (remission without pituitary deficiencies excluding adrenal insufficiency), whereas in 4.6% poor outcome (no remission, pituitary deficiencies present) was observed. In 29.2% remission with pituitary deficiencies was observed, and 10.8% had persistent disease without pituitary deficiencies. When adrenal insufficiency was regarded as adverse outcome as well, in 17% good outcome after one year was reported, whereas 68% showed remission with the presence of pituitary deficiencies. With long-term follow-up, a gradual shift to the good outcome category occurred, mainly due to recovery of the HPA-axis. The results show that the majority of patients are in remission five years after transsphenoidal surgery (91%), some after successful re-interventions, though in a considerable number at the expense of persistent hypopituitarism (58% of patients in remission, partly explained by HPA-axis deficiency). Remission rates in this study are in line with previously published studies.[45-47] As might be expected due to the extent of the tumor and therefore a more extensive surgical procedure, macroadenoma patients more often showed new onset hypopituitarism or poor outcome when compared to patients with microadenomas. Also patients with an invisible/uncertain adenoma on preoperative MRI scan appeared to be at increased risk of poor outcome (no remission and persistent pituitary deficiencies).

Clinical implications: The four different integrated outcome quadrants used in the Outcome Squared method provide, for the first time, a uniform, patient-centred integrated overall view of the important balance between efficacy and safety of transsphenoidal surgery in Cushing's disease. The results of the subgroup analysis (e.g. microadenoma patients) can be used in the out-patient setting for individualised patient counselling. A health care provider can easily recognize patient groups with good or adverse outcomes and modify treatment strategies accordingly, if applicable. The results of this study also show that even after multiple and combined interventions remission without adverse effects can be achieved, and reinterventions thus also can be considered save procedures.

Future perspectives

Conducting prospective or randomized controlled clinical studies in rare diseases is often challenging, due to the small number of available patients, and the lack of available funding for these

trials. These studies however, are of utmost importance to improve insights in pathophysiology of the underlying condition and to improve treatments and quality of care, especially in an era in which evidence-based medicine has taken a flight, outcome evaluations are demanded by patients, society, and the profession, and evidence-based guidelines have become the cornerstone of clinical practice. And most importantly, the ultimate goal from a patients' perspective is to improve not only their prognosis, but first of all their quality of life. Because this thesis addresses two different rare diseases, as a model for stress vulnerability, suggestions for future research subjects with regard to both CS and CSC will be discussed below.

An increased activity of the HPA-axis as observed in CSC patients in our study, is in concordance with the previously reported association between cortisol and CSC. However, our study on hair cortisol concentrations in patients with CSC clearly demonstrated that the relationship between CSC and cortisol might not be as straightforward as previously thought. Further studies are required to unravel the underlying pathophysiological mechanisms, for example addressing inter-individual glucocorticoid sensitivity and differences in susceptibility to develop CSC, and the role of the mineralocorticoid receptor and glucocorticoid receptor, and their potential differential effects on gene expression in the pathogenesis of CSC. Furthermore, the studies described in this thesis did not find any rationale for interventions targeting personality features. It would, however, be of interest to future research whether altering coping mechanisms and reducing stress (and thereby potentially reducing HPA-axis activity) can improve the course of disease in CSC. Since it might be of clinical importance to routinely screen patients with CS for abnormalities within the CSC spectrum, further (prospective) studies on the prevalence and natural course of subclinical CSC are needed to assess whether incorporation of ophthalmological screening in the general clinical work-up of patients with CS is required.

This thesis has also shown that mortality remains increased in Cushing's disease, despite long-term biochemical remission. In order to improve survival in patients that have obtained long-term remission, future studies should focus on unraveling risk factors contributing to the increased mortality in these patients, and ultimately, studies on interventions addressing these risk factors should point out whether the increased mortality rates can be repulsed. With regard to alterations in brain activation of certain regions in remitted CS as was demonstrated in this thesis, longitudinal studies are needed to provide insight into the onset and time course of these alterations in brain activity patterns and cognition during active disease state and transition into remission, and to find out whether remission remits all (visuospatial) impairments. It would be of special clinical interest to find out whether the compensatory increased brain activity to normalize cognitive performance contributes to the reported persisting increased prevalence of mood related disorder, fatigue, decreased stress resilience in daily life, and reduced quality of life of patients with remitted CS. This should be captured by adding morbidity and quality of life related questionnaires to the longitudinal studies in order to associate the outcomes to the functional MRI data. In case a contribution of compensatory brain activity to morbidity is made plausible, the ultimate step would be to evaluate whether psychological interventions such as cognitive behavioral therapy addressing the balance between daily activities in need of the compensatory activities of certain brain areas and

activities that do not require these compensatory activities could be able to decrease complaints and increase quality of life.

With regard to thromboprophylaxis management in patients with CS, a first step in conducting studies required for guideline development on this topic was undertaken in this thesis by mapping the current clinical practice in expert centers endorsed for the diagnosis and treatment for this specific rare condition across Europe. Both patients and clinicians are in need for randomized controlled trials to establish the optimal prophylactic anticoagulant strategy (i.e. what would be the drug of first choice, when to start, when to stop, what to do with peri-operative thromboprophylaxis management, which patients are at increased risks of bleeding complications and how to adjust thromboprophylaxis treatment in these patients). Furthermore, future studies should assess additional risk factors to determine which patients are particularly at risk for venous thromboembolism and would benefit from thromboprophylaxis, in order to be able to carefully balance the known hypercoagulable state, potential additional risk factors for thrombosis, and increased risk of (perioperative) bleeding in daily clinical practice.

Treatment outcome evaluations should be performed to provide better insight in performance and quality of care, but also enable to identify predictors and moderators of outcome in order to develop the most effective treatments, gain insight into which treatment is best for which patient, and to provide the best individual patient counselling. The use of the Outcome Squared method can provide comparisons of outcomes of different treatment strategies, for example medical therapy versus radiotherapy in persistent disease, and other outcomes relevant to patients and their quality of life (e.g. symptomatology, burden of disease, functional outcome) can be incorporated in the four outcome categories by adjusting the definitions of intended and adverse effects. The method is also suitable to evaluate outcome of treatment strategies including multiple interventions rather than focusing on a single intervention. In future research, integrating outcomes using the Outcome Squared method can be used for the comparison between centres and studies, provided that identical definitions and outcome are used. In the field of pituitary diseases, we believe that the use of the Outcome Squared approach provides added value to the evaluation of the provided quality of care, however, international consensus is needed on the use of Outcome Squares in outcome evaluations to pursue comparability of studies that have a more detailed focus on outcome.

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CHAPTER 11

NEDERLANDSE SAMENVATTING

Stress is een psychofysiologische reactie op moeilijke of uitdagende situaties en zowel fysieke als psychologische stressoren.[1, 2] Het endocriene system, en het stress hormoon cortisol in het bijzonder, spelen een belangrijke rol in het reguleren van de stress respons.[3] Tussen de 20 en 40 minuten na het begin van een stressvolle gebeurtenis, piekt de cortisol secretie, wat onder andere resulteert in het vrijmaken van energie voorraden door verhoging van bloed suiker spiegels voor extra 'brandstof' voor het lichaam en in het remmen van lichaamsfuncties die op dat moment niet essentieel zijn, zoals bepaalde aspecten van het immuun systeem, het spijsverteringsstelsel en het voortplantingssysteem. Hierdoor kunnen andere systemen in het lichaam, zoals het sympatische zenuwstelsel en bepaalde psychologische systemen die nodig zijn om gevaar te overwinnen, effectief functioneren.[4] Cortisol is een essentieel hormoon voor overleving, echter langdurige stress en blootstelling aan verhoogde cortisol concentraties leidt tot weefselschade en ziekte, en beïnvloedt op deze manier verschillende vitale orgaansystemen nadelig.[2] Bij het syndroom van Cushing (CS) is er sprake van overproductie van cortisol, en het langdurige en excessieve hypercortisolisme is geassocieerd met een verhoogde morbiditeit en mortaliteit, alsmede met een verminderde kwaliteit van leven.[5-8] Centrale sereuze chorioretinopathie (CSC), een specifieke vorm van macula degeneratie, is een andere aandoening waarbij stress en cortisol verondersteld worden betrokken te zijn bij de pathofysiologie.[9-11] Bij CSC ontstaat er schade aan het retinale pigment epitheel, waardoor sereuze subretinale vochtophopingen ontstaan die leiden tot loslating van de neuroretina met verlies en vervorming van visus tot gevolg.[9, 12, 13] Derhalve zijn zowel CS als CSC zeldzame aandoeningen waarbij de activiteit van de hypothalamus-hypofyse-bijnier (HPA)-as en cortisol een belangrijke rol spelen. Dit proefschrift richt zich op de pathofysiologie van stress, waarbij de hierboven genoemde zeldzame aandoeningen gebruikt worden als een model voor de gevoeligheid voor stress van de hersenen (CS) en van het oog (CSC). Het tweede doel van dit proefschrift was het beschrijven van de organisatie, uitkomst evaluatie en kwaliteit van zorg voor patiënten met CS. **Hoofdstuk 1** geeft een algemene inleiding over de regulatie van de stressrespons, CS en CSC, en bespreekt de onderliggende rationale voor de onderzoeken die in dit proefschrift worden gepresenteerd.

DEEL 1

In **hoofdstuk 2** werd een oogheelkundige screening middels onder andere multimodale imaging technieken uitgevoerd in een serie patiënten met actief CS zonder visusklachten, met als doel om mogelijke subklinische afwijkingen binnen het spectrum van CSC op te sporen en om de noodzaak voor gestandaardiseerde oogheelkundige screening van alle patiënten met CS te evalueren. Van de 11 patiënten met nieuw gediagnostiseerde CS die geïncludeerd werden, werden bij 3 patiënten afwijkingen gevonden die zouden kunnen passen bij (subklinische) CSC. Eén patiënt werd gediagnostiseerd met actieve CSC (inclusief maculair, subretinaal vocht bij beeldvormend onderzoek) en werd succesvol behandeld met half-dose fotodynamische therapie. In een andere patiënt lieten de onderzoeken pseudovitelliforme afwijkingen en hyperfluorescente veranderingen aan één kant zien, terwijl bij een derde patiënt eenzijdige lekkage van vocht werd gezien. Daarom werd geconcludeerd dat retinale afwijkingen gelijkend op (subklinische) CSC kunnen voorkomen

bij patiënten met CS, zelfs als er geen visusklachten aanwezig zijn, en wellicht vaker voorkomen dan voorheen gedacht werd.

Klinische implicaties: Vanwege de therapeutische consequenties gericht op preventie van visusverlies is het belangrijk dat artsen/endocrinologen actief vragen naar visusklachten bij patiënten met CS en patiënten laagdrempelig verwijzen voor oogheelkundige evaluatie. Wij bepleiten dat routinematig screenen van alle nieuw gediagnostiseerde patiënten met CS op retinale afwijkingen in het CSC spectrum een onderwerp zou moeten zijn van een toekomstige multicenter, prospectieve cohort studie.

Andersom, gezien de veronderstelde relatie tussen stress en het ontstaan van CSC,[9-11] en gezien CSC het presenterende symptoom van CS kan zijn,[14] is het relevant om je af te vragen of patiënten met CSC gescreend zouden moeten worden op CS. In **hoofdstuk 3** werd een systematische screening voor de aanwezigheid van CS in een groot cohort van patiënten met chronische CSC beschreven, met als doel de prevalentie van CS bij patiënten met chronisch CSC vast te stellen en om te onderzoeken of chronisch CSC geassocieerd is met hyperactiviteit van de HPA-as. Geen van de 86 geïncludeerde CSC patiënten voldeed aan de klinische of biochemische criteria van CS. Echter, de activiteit van de HPA-as was verhoogd in patiënten vergeleken met gezonde controles, gezien het hogere vrije cortisol in de 24 uurs urine (wel binnen de normale range) met een mean verschil van 32 nmol/24 uur, en begeleidende grotere taille omtrek en hogere diastolische bloeddruk. Het circadiane, diurnale cortisol ritme was niet afwijkend in CSC patiënten. In tegenstelling tot eerdere onderzoeken die de suggestie hebben gewekt dat er een associatie bestaat tussen CSC en psychologische stress, [11, 15-17] rapporteerden CSC patiënten in ons cohort niet meer stress of stress-gerelateerde problemen op gevalideerde vragenlijsten. Bovendien werd er geen associatie gevonden tussen HPA-as activiteit, ziekteactiviteit van de CSC en psychologische stress.

Klinische implicaties: Op basis van deze resultaten is het routinematig screenen op CS van alle CSC patiënten niet geïndiceerd. Gezien de interpretatie van de beschikbare biochemische screeningstesten in het licht van de klinische kenmerken in de praktijk vaak uitdagend is en om het aantal vals positieve test resultaten te minimaliseren, zou screening moeten worden gereserveerd voor CSC patiënten met additionele klinische kenmerken en symptomen waardoor de verdenking op hypercortisolisme wordt gewekt. De resultaten van deze studie laten echter duidelijk zien dat er sprake is van hyperactiviteit van de HPA-as in CSC patiënten, hoewel dit niet gepaard gaat met de perceptie van meer psychosociale stress. Deze geobserveerde HPA-as hyperactiviteit is in overeenstemming met de eerder gerapporteerde associatie tussen cortisol en CSC. Er zijn echter verdere studies nodig om de onderliggende pathofysiologische mechanismen en de rol van stress en stress reducerende interventies bij het ontstaan en het klinisch beloop van CSC te ontrafelen.

De activiteit van de HPA-as als maat voor de endogene blootstelling aan stress en cortisol kan worden bepaald met een aantal verschillende testen. Alle beschikbare testen weerspiegelen verschillende aspecten van de secretie van en blootstelling aan cortisol. Concentraties van cortisol in hoofdhaar kunnen gemeten worden om de lange termijn blootstelling aan cortisol vast te stellen. Om de vermoedelijke relatie tussen cortisol en chronische CSC te onderzoeken, werden

haarcortisolconcentraties in een groot cohort van 48 chronische CSC patiënten gemeten en vergeleken met de haarcortisolconcentraties van volwassen controles uit de algemene populatie (**hoofdstuk 4**). Verhoogde haar cortisol concentraties waren aanwezig in 4% van de patiënten met CSC en in 6% van de controles, en de gemiddelde haar cortisol concentraties waren niet verschillend tussen de twee groepen. Deze bevinding zet vraagtekens bij de eerder gerapporteerde suggestie van hyperactiviteit van de HPA-as bij CSC, echter de verschillende meetmethodes weerspiegelen hoogstwaarschijnlijk verschillende aspecten van blootstelling aan cortisol. Mogelijk is ofwel de techniek van de haar cortisol concentraties niet gevoelig genoeg om kleine en wellicht kortdurende verhogingen van cortisolspiegels binnen het normale bereik te detecteren, rekening houdend met de grote individuele variatie in normale cortisol spiegels en gevoeligheid voor cortisol, ofwel worden milde verhogingen van cortisol concentraties op weefselniveau die leiden tot de specifieke CSC veranderingen niet weerspiegeld door verhoogde concentratie van cortisol in het haar. Men zou ook kunnen beargumenteren dat de langdurige blootstelling aan cortisol niet verhoogd is bij CSC, maar dat een korte piek of een wat langer durende maar tijdelijke verhoging van de cortisol spiegels bij gevoelige personen voldoende kan zijn voor het induceren van de retinale veranderingen die kenmerkend zijn voor CSC. Deze hypothese wordt ondersteund door het beschreven ontstaan van CSC na kortdurende behandeling met steroïden.[10] Gebaseerd op deze nieuwe observaties zou het ook aannemelijk kunnen zijn dat de relatie tussen CSC en cortisol niet zo eenvoudig is als eerder werd gedacht. Er werd geen correlatie gezien tussen de haar cortisol concentraties en de vrije cortisol spiegels in de 24 uurs urine bij patiënten met chronische CSC, ondanks dat eerdere studies wel een dergelijke sterke correlatie beschreven hebben bij patiënten met CS.[18, 19] Ten slotte werd er geen verschil in haar cortisol concentraties gevonden tussen patiënten met actieve CSC ziekte in vergelijking met patiënten met inactieve ziekte, wat aangeeft dat er geen verband is tussen de ernst van de ziekte en de haar cortisol concentraties.

Klinische implicaties: Haar cortisol concentraties in patienten met CSC zijn niet verhoogd vergeleken met haarcortisolconcentraties van volwassen controles uit de algemene populatie, en er werd geen associatie gevonden tussen haar cortisol concentraties en de ernst van de CSC. Daarom zijn haar cortisol concentraties niet bruikbaar voor het monitoren van CSC ziekte activiteit.

Naast biochemische stress zoals weerspiegeld door hyperactiviteit van de HPA-as, worden ook psychosociale stress en 'type A' gedragsaspecten geassocieerd met CSC.[11, 16, 20] Om potentiële modificeerbare psychosociale aspecten te identificeren ter ondersteuning van de huidige standaard behandeling, rapporteerde **hoofdstuk 5** over een cross-sectionele studie in een cohort van 86 patiënten met chronische CSC waarbij gebruik gemaakt werd van gevalideerde vragenlijsten om de aanwezigheid van mogelijke maladaptieve persoonlijkheidskenmerken (zoals kenmerken gerelateerd aan 'type A' gedragspatronen), apathie en prikkelbaarheid, en coping strategieën vast te stellen. De bevindingen van de patiënten werden vergeleken met zowel op de Nederlandse populatie gebaseerde referentiegegevens als met gegevens van patiënten die werden behandeld voor de ziekte van Cushing. Psychologische morbiditeit in de vorm van apathie en prikkelbaarheid was niet verhoogd in CSC patiënten. Bovendien kwamen maladaptieve persoonlijkheidskenmerken zoals 'type A' gedragskenmerken niet vaker voor bij patiënten met CSC in vergelijking met de

algemene populatie. Dit zijn intrigerende bevindingen omdat ze in tegenspraak zijn met wat in eerdere onderzoeken werd gesuggereerd.[16, 21-23] In deze onderzoeken werden gedragskenmerken echter voornamelijk beoordeeld met behulp van uitkomstmaten waarvan is aangetoond dat ze niet correleren met persoonlijkheidskenmerken en psychopathologie[24], en de 'type A' gedragskenmerken waren niet strikt gedefinieerd.

CSC patiënten maken meer gebruik van bepaalde coping strategieën (bijvoorbeeld passieve coping, sociale steun zoeken en bij mannen ook actieve coping). Opmerkelijk was, hoewel niet statistisch significant, dat het persoonlijkheidsprofiel, de psychologische morbiditeit en coping kenmerken van CSC patiënten meer vergelijkbaar was met de kenmerken van behandelde patiënten met de ziekte van Cushing dan met de gegevens van de algemene populatie. Omdat patiënten met ziekte van Cushing in remissie langdurig blootgesteld zijn aan excessieve cortisol spiegels, kunnen deze patiënten beschouwd worden als een menselijk model om de effecten van een teveel aan cortisol op onder andere persoonlijkheid en gedrag te bestuderen. Maladaptieve persoonlijkheidskenmerken en psychosociale morbiditeit zoals apathie en prikkelbaarheid zijn uitgebreid beschreven in patiënten met de ziekte van Cushing.[25, 26] In lijn met de biochemische gelijkenis van een geactiveerde HPA-as in zowel CSC (mild geactiveerd) als in de ziekte van Cushing (overmatig geactiveerd) zoals hierboven beschreven, toonde deze studie ook een relatieve overeenkomst met betrekking tot het spectrum van persoonlijkheidskenmerken tussen deze twee patiënten groepen.

Klinische implicaties: Oogartsen gaan vaak uit van en rapporteren stress gerelateerde en 'type A' gedragskenmerken bij CSC patiënten[16, 21, 22] en derhalve zijn stressvermindering en interventies gericht op persoonlijkheidskenmerken gebruikelijk in dagelijkse klinische management strategieën.[27-29] Echter op basis van de resultaten van deze studie lijken deze interventies niet nuttig. Het gebruik van bepaalde coping strategieën zou wel een aandachtspunt kunnen zijn in programma's voor psychosociale zorg en zelfmanagement.

DEEL 2

Het tweede deel van dit proefschrift was gericht op de organisatie, uitkomstevaluatie en kwaliteit van zorg voor patiënten met CS. **Hoofdstuk 6** besprak een systematisch onderzoek van de huidige literatuur om na te gaan of de mortaliteit verhoogd blijft bij patiënten die biochemisch zijn genezen van de ziekte van Cushing. Daarnaast werd een meta-analyse uitgevoerd, waarbij vervolgonderzoeken werden geïncludeerd die de gestandaardiseerde mortaliteitsratio (SMR) rapporteren voor patiënten die genezen zijn van de ziekte van Cushing na de eerste behandeling. In totaal werden 766 patiënten die deelgenomen hadden aan acht studies geïncludeerd in de meta-analyse. Zeven van de acht studies liet een SMR boven de 1.0 zien, met een gepoolde SMR van 2.5 (95% betrouwbaarheidsinterval 1.4-4.2) als alle studies werden meegenomen. Ook wanneer er een sensitiviteitsanalyse werd verricht waarbij twee uitbijters werden uitgesloten, bleef de SMR verhoogd. Dit betekent dat zelfs na genezing de mortaliteit verhoogd blijft bij patiënten met de ziekte van Cushing, wat suggereert dat genezing de nadelige metabole effecten van langdurige blootstelling aan een teveel aan cortisol niet volledig ongedaan maakt. Helaas was het vanwege

het ontbreken van patiënt kenmerken gestratificeerd naar genezingsstatus in de geïncludeerde studies niet mogelijk om meta-regressie technieken toe te passen om mogelijke oorzaken van de verhoogde mortaliteit te beoordelen. Het is echter aannemelijk dat nadelige effecten van de ziekte en/of behandeling, zoals hypopituitarisme, bijdragen aan het aanhoudende verhoogde sterfterisico, hoewel het percentage hypopituitarisme na behandeling niet uit de onderzoeken kon worden geabstraheerd. Bovendien worden de resultaten van de meta-analyse ondersteund door bewijs voor aanhoudende multi-systeem morbiditeit na biochemische genezing, aangezien er steeds meer studies aantonen dat de morbiditeit gerelateerd aan een teveel aan cortisol afneemt na succesvolle behandeling van de ziekte van Cushing, maar niet normaliseert. Een hoge prevalentie van atherosclerose en een verhoogd cardiovasculair risico zijn gerapporteerd na genezing, waarbij gedacht wordt dat dit gerelateerd is aan resterende abdominale obesitas en insuline resistentie.[30] Ook het risico op een hart infarct of beroerte in genezen patiënten met de ziekte van Cushing blijkt verhoogd te blijven tijdens langdurige follow-up,[31] en zelfs een persisterend verhoogde prevalentie van psychopathologie en cognitieve beperkingen zijn beschreven.[25, 32]

Afgezien van de resterende lichamelijke en psychische morbiditeit en verhoogde mortaliteit, rapporteren patiënten met de ziekte van Cushing ook aanhoudende stoornissen in het cognitief en executief functioneren.[25, 33] Daarnaast werd aangetoond dat tevens een verminderde kwaliteit van leven persisteert ondanks genezing.[34] De vraag of patiënten met de ziekte van Cushing in remissie ook veranderde patronen van prestaties en hersenactiviteit met betrekking tot cognitieve planning en executief functioneren vertonen, werd in **hoofdstuk 7** onderzocht door middel van functionele magnetische resonantie imaging (MRI) terwijl zowel patiënten als gezonde controles een Tower of London-taak (parametrische visueel-ruimtelijke planningstaak) uitvoerden. Eenentwintig patiënten die genezen waren van de ziekte van Cushing en eenzelfde aantal gezonde geslacht-, leeftijd- en opleidingsniveau-gematchte controles werden geïncludeerd. Er werden geen verschillen gevonden in de prestaties tussen de twee groepen, noch in het aantal correct uitgevoerde proeven, noch in responstijden per proef, of in de functionele analyses van de relevante hersenengebieden. Aangezien eerdere onderzoeken visuospatiale stoornissen aan het licht brachten bij patiënten met actieve ziekte van Cushing[35], suggereren onze resultaten dat deze beperkingen kunnen verbeteren na genezing. Verkennende analyses van de gehele hersenen toonden een verhoogde hersenactiviteit in bepaalde hersengebieden tijdens de Tower of London taak bij genezen patiënten, wat aangeeft dat patiënten deze hersengebieden, die betrokken zijn bij hogere cognitieve processen, veel meer moeten aanspreken om een vergelijkbaar prestatieniveau te bereiken dan gezonde controles, en dus meer inspanning nodig hebben om een visueel-ruimtelijke planningstaak met succes te kunnen voltooien.

Klinische implicaties: Het aanhoudende verhoogde sterfterisico ondanks genezing van hypercortisolisme suggereert dat er onomkeerbare effecten zijn van langdurige overmatige blootstelling aan cortisol. Het lijkt er tevens op dat langdurige blootstelling van de hersenen aan een teveel aan cortisol leidt tot blijvende veranderingen in de hersenactivatie van bepaalde hersengebieden, zelfs na langdurige remissie. De ziekte van Cushing kan daarom leiden tot onomkeerbare, subtiele veranderingen in de hersenen tijdens (veeleisende) executieve functies.

Deze bevindingen zijn van belang voor de begeleiding van patiënten in de dagelijkse klinische praktijk, maar ook voor het bewustzijn van de behandelend artsen om kwalitatief goede nazorg te kunnen leveren met oog voor de aanhoudende klachten en behandeling van co-morbiditeit.

Zowel tijdens actief hypercortisolisme als in de post-operatieve periode, en zelfs na genezing, is door meerdere studies een verhoogd risico op veneuze trombo-embolie gerapporteerd.[36-38] Evidence-based richtlijnen voor profylactische anticoagulatie bij deze patiënten met zo'n zeldzame aandoening zijn niet beschikbaar vanwege het ontbreken van prospectieve behandelstudies die de effecten van tromboseprofylaxe op het optreden van veneuze trombo-embolie bij CS evalueren. In de context van kwaliteit van zorg en evaluatie van uitkomsten en complicaties, is er momenteel behoefte aan een klinische richtlijn voor de omgang met tromboseprofylaxe bij patiënten met CS. Een eerste stap in het uitvoeren van studies die nodig zijn voor de ontwikkeling van een dergelijke richtlijn, is het in kaart brengen van de huidige klinische praktijk. Het doel van **hoofdstuk 8** was het in kaart brengen van de huidige klinische strategieën voor tromboseprofylaxe bij patiënten met CS in de referentiecentra van het European Reference Network (ERN) voor zeldzame endocriene aandoeningen (Endo-ERN). Deze centra zijn specifiek erkend als expertise centra voor de diagnose en behandeling van CS. De resultaten van de online enquête laten een grote praktijk variatie zien met betrekking tot de omgang met tromboseprofylaxe bij CS, zelfs in de expertise centra van de Endo-ERN. Hoewel de meerderheid van de referentiecentra (23 van de 25) hun CS patiënten tromboseprofylaxe gaf, was er slechts in één centrum een gestandaardiseerd behandelprotocol beschikbaar. Ook het tijdstip van aanvang en staken van de tromboseprofylaxe varieerde sterk tussen de centra.

Klinische implicaties: Deze resultaten illustreren de behoefte aan een geprotocolleerde strategie voor tromboseprofylaxe bij CS, waardoor optimalisatie en mogelijk individualisatie van deze behandeling mogelijk zal worden.

Voor kwaliteits- en uitkomstevaluatiedoeleinden is onlangs door ons centrum een uitkomstevaluatie methode genaamd Outcome Squared ontwikkeld voor patiënten die operatief zijn behandeld voor hypofysetumoren. Deze methode verenigt verschillende uitkomstparameters in de tijd, rekening houdend met de balans tussen effectiviteit en veiligheid.[39] Vooral bij de ziekte van Cushing, waarbij transsfenoïdale operatie de eerstelijns behandeling is, bepalen zowel remissie als complicaties het succes van de ingreep. Het niet bereiken van remissie is potentieel levensbedreigend,[40] terwijl complicaties zoals hypopituitarisme waaronder bijnierinsufficiëntie ook in verband worden gebracht met comorbiditeit en verminderde kwaliteit van leven.[41-44] **Hoofdstuk 9** rapporteerde over geïntegreerde postoperatieve lange-termijn follow-up van patiënten met de ziekte van Cushing met behulp van de Outcome Squared methode. Tweeënzeventig opeenvolgende patiënten die werden behandeld middels transsfenoïdale resectie werden geïncludeerd. Een jaar na de operatie had 55.4% van de patiënten een goede uitkomst (remissie zonder hypofysaire uitval exclusief bijnierinsufficiëntie), terwijl in 4.6% een slechte uitkomst werd waargenomen (geen remissie, wel hypofysaire uitval). Bij 29.2% werd remissie met hypofysaire uitval gezien, en 10.8% had persisterende ziekte zonder hypofysaire uitval. Wanneer bijnierinsufficiëntie ook als een nadelige uitkomst werd

beschouwd, werd bij 17% een goede uitkomst na één jaar gerapporteerd, terwijl 68% in remissie was met hypofysaire uitval. Bij langdurige follow-up trad een geleidelijke verschuiving op naar de goede uitkomst categorie, voornamelijk door herstel van de HPA-as. De resultaten laten zien dat de meerderheid van de patiënten vijf jaar na transsfenoïdale operatie in remissie is, sommigen na succesvolle re-interventies, hoewel dit in een aanzienlijk deel van de patiënten ten koste gaat van persisterende hypofyse uitval (deels verklaard door uitval van de HPA-as). De remissiepercentages in deze studie zijn in lijn met eerder gepubliceerde onderzoeken.[45-47] Zoals te verwachten was vanwege de omvang van de tumor en dus de uitgebreidere chirurgische ingreep, hadden patiënten met een macroadenoom vaker hypofyse uitval of een slechtere uitkomst in vergelijking met patiënten met een microadenoom. Ook patiënten met een onzeker/onzichtbaar adenoom op de preoperatieve MRI scan bleken een verhoogd risico te hebben op een slechte uitkomst.

Klinische implicaties: De vier verschillende geïntegreerde uitkomst kwadranten die in de Outcome Squared methode worden gebruikt, bieden een uniform, patiëntgericht totaalbeeld van het belangrijke evenwicht tussen effectiviteit en veiligheid van transsfenoïdale operaties bij de ziekte van Cushing. De resultaten van de subgroep analyse (bijvoorbeeld patiënten met microadenomen) kunnen worden gebruikt bij geïndividualiseerde patiënten begeleiding in de dagelijkse praktijk. Een zorgverlener kan gemakkelijk patiëntengroepen met goede of nadelige uitkomsten herkennen en, indien van toepassing, de behandelstrategieën dienovereenkomstig aanpassen. De resultaten van deze studie laten ook zien dat zelfs na meerdere en gecombineerde interventies remissie zonder nadelige effecten zoals hypopituitarisme kan worden bereikt, en re-interventies kunnen derhalve als veilig worden beschouwd.

TOEKOMSTPERSPECTIEVEN

Het uitvoeren van prospectieve en gerandomiseerde, gecontroleerde klinische studies naar zeldzame ziekten is vaak een uitdaging, vanwege het kleine aantal beschikbare patiënten en het gebrek aan financiering voor dergelijke onderzoeken. Deze onderzoeken zijn echter van het grootste belang om de inzichten in de pathofysiologie van de zeldzame aandoeningen te vergroten, alsmede de behandelingen en kwaliteit van zorg voor deze patiënten te verbeteren. Dit geldt zeker in het huidige tijdperk, waarin evidence-based medicine een vlucht heeft genomen, uitkomstevaluaties zowel door patiënten en de samenleving alsook door de beroepsgroep verwacht en geëist worden, en evidence-based richtlijnen de hoeksteen zijn geworden van het dagelijks klinisch handelen. Het belangrijkste hierbij is het uiteindelijke doel vanuit het perspectief van de patiënt, namelijk het verbeteren van niet alleen zijn of haar prognose, maar vooral van zijn of haar kwaliteit van leven. Omdat er twee verschillende zeldzame ziekten als model voor stress gevoeligheid besproken worden in dit proefschrift, zullen suggesties voor toekomstige onderzoeksonderwerpen met betrekking tot zowel CS als CSC hieronder besproken worden.

Hyperactiviteit van de HPA-as, zoals waargenomen werd bij patiënten met CSC in onze studie, bevestigt de eerder gerapporteerde associatie tussen cortisol en CSC. Echter, onze studie naar haar cortisol concentraties in patiënten met CSC toont dat deze relatie tussen CSC en cortisol wellicht niet zo eenvoudig is als eerder werd gedacht. Toekomstige studies zijn nodig om de onderliggende

pathofysiologische mechanismen te ontrafelen, bijvoorbeeld naar interindividuele cortisol gevoeligheid en verschillen in de ontvankelijkheid voor het ontwikkelen van CSC, en de rol van de mineralocorticoïd receptor en de glucocorticoïd receptor en hun effecten op genexpressie in de pathogenese van CSC.

De studies beschreven in dit proefschrift vonden geen enkele rationale voor interventies gericht op persoonlijkheidskenmerken bij CSC. Het zou echter interessant kunnen zijn voor toekomstig onderzoek om vast te stellen of het veranderen van coping-mechanismen en het reduceren van stress (en daarmee mogelijk ook het verminderen van activiteit van de HPA-as) het ziektebeloop van CSC zou kunnen verbeteren. Aangezien het van klinisch belang zou kunnen zijn om patiënten met CS routinematig te screenen op afwijkingen binnen het CSC-spectrum, is verder prospectief onderzoek nodig naar de prevalentie en het natuurlijke beloop van subklinische CSC om te beoordelen of oogheelkundige screening onderdeel zou moeten worden van de algemene klinische work-up van patiënten met CS.

Dit proefschrift heeft aangetoond dat de mortaliteit van patiënten met de ziekte van Cushing verhoogd blijft, ondanks langdurige biochemische remissie. Om de overleving van genezen patiënten te verbeteren, zou toekomstig onderzoek zich moeten richten op het identificeren van risicofactoren die bijdragen aan de verhoogde mortaliteit, en uiteindelijk zullen studies naar interventies die deze risicofactoren aanpakken uit moeten wijzen of de verhoogde sterftecijfers kunnen worden teruggedrongen. Met betrekking tot de veranderingen in hersenactivatie van bepaalde hersengebieden bij patiënten die genezen zijn van CS, zoals aangetoond in dit proefschrift, zijn longitudinale studies nodig om inzicht te krijgen in het ontstaan en het beloop van deze veranderingen in hersenactiviteitspatronen en cognitie tijdens actieve ziekte en tijdens de overgang naar remissie, en om na te gaan of alle (visuospatiale) beperkingen verdwijnen na remissie. Het is van speciaal klinisch belang om uit te zoeken of de compenserende verhoogde hersenactiviteit ter normalisatie van cognitieve prestaties bijdraagt aan de gerapporteerde aanhoudende verhoogde prevalentie van stemmingsstoornissen, vermoeidheid, verminderde stressbestendigheid in het dagelijks leven en de verminderde kwaliteit van leven na genezing van CS. Dit zou onderzocht kunnen worden door specifieke gevalideerde morbiditeit- en kwaliteit van leven gerelateerde vragenlijsten toe te voegen aan de longitudinale studies om deze uitkomsten te associëren met de functionele MRI data. En als een bijdrage van de compenserende hersenactiviteit aan persisterende morbiditeit aannemelijk wordt gemaakt, zou de ultieme stap zijn om te evalueren of psychologische interventies zoals cognitieve gedragstherapie gericht op de balans tussen dagelijkse activiteiten waarbij gebruik gemaakt wordt van die compenserende hersenactiviteit en activiteiten waarbij dit niet/minder het geval is, de persisterende klachten van patiënten zou kunnen verminderen en daarmee de kwaliteit van leven kunnen verbeteren.

Een eerste stap in de onderzoeken die nodig zijn voor het ontwikkelen van richtlijnen voor tromboseprofylaxe bij CS werd in dit proefschrift gezet door de huidige klinische praktijk in de expertise centra in Europa in kaart te brengen. Zowel patiënten als artsen hebben baat bij gerandomiseerde, gecontroleerde studies om de optimale profylactische anticoagulatie strategie vast te stellen. Vragen die beantwoord dienen te worden zijn onder andere: wat zou het medicament van eerste keus zijn, wanneer dient de medicatie gestart te worden, wanneer dient de medicatie

gestaakt te worden, wat te doen met tromboseprofylaxe perioperatief, welke patiënten hebben een verhoogd risico op bloedingscomplicaties en hoe om te gaan met de tromboseprofylaxe bij deze patiënten? Verder zouden toekomstige studies zich moeten richten op het identificeren van additionele risicofactoren voor het ontwikkelen van een veneuze trombo-embolie, om te bepalen welke patiënten zouden profiteren van tromboseprofylaxe en om een zorgvuldige afweging te kunnen maken tussen de bekende hypercoagulabiliteit, mogelijk aanvullende risicofactoren voor trombose en een verhoogd risico op (perioperatieve) bloedingscomplicaties.

Evaluaties van behandel uitkomsten moeten worden uitgevoerd om inzicht te verkrijgen in de prestaties en kwaliteit van zorg, maar ook om optimale individuele behandeling en begeleiding van patiënten mogelijk te maken. Door gebruik te maken van de Outcome Squared methode kunnen verschillende behandelstrategieën vergeleken worden (bijvoorbeeld medicamenteuze therapie versus radiotherapie bij persisterende ziekte). Ook kunnen andere uitkomsten die voor de kwaliteit van leven van patiënten relevant zijn (zoals symptomatologie, ziektelast, functionele uitkomsten) worden toegevoegd aan de vier uitkomst categorieën door de definities van beoogde en nadelige effecten van een behandeling aan te passen. De methode is daarnaast ook geschikt om de uitkomsten van behandelstrategieën waarin meerdere interventies zijn opgenomen te evalueren, in plaats van te concentreren op een enkele interventie. In toekomstig onderzoek kan het integreren van uitkomsten met behulp van de Outcome Squared methode worden gebruikt voor vergelijking tussen centra en studies, op voorwaarde dat identieke definities en uitkomsten worden gebruikt. Op het gebied van hypofyseaandoeningen zijn wij van mening dat het gebruik van de Outcome Squared methode van toegevoegde waarde kan zijn voor de evaluatie van de geleverde kwaliteit van zorg, echter internationale consensus met betrekking tot het gebruik van Outcome Squares in uitkomstevaluaties is noodzakelijk om de vergelijkbaarheid van onderzoeken met gedetailleerde focus op uitkomsten na te streven.

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APPENDIX &

LIST OF ABBREVIATIONS

CURRICULUM VITAE

LIST OF PUBLICATIONS

DANKWOORD

LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
ACTH	AdrenoCorticoTropic Hormone
aPTT	activated Partial Thromboplastin Time
AS	Apathy Scale
AT	Arterial Thrombosis
BAI	Beck Anxiety Inventory
BCVA	Best-Corrected Visual Acuity
BG	Blood Group
CD	Cushing's Disease
CFQ	Cognitive Failure Questionnaire
CHRPE	Congenital Hypertrophy of the Retinal Pigment Epithelium
CI	Confidence Interval
CLT	Cloth Lysis Time
CRH	Corticotropin-Releasing Hormone
CRVO	Central Retinal Vein Occlusion
cCSC	chronic Central Serous Chorioretinopathy
CS	Cushing's Syndrome
CSC	Central Serous Chorioretinopathy
CSI	Cushing's syndrome Severity Index
CT	Choroidal Thickness
DAPPsf	Dimensional Assessment of Personality Pathology short form
Dexa	Dexamethasone
DI	Diabetes Insipidus
DST	Dexamethasone Suppression Test
DVT	Deep Vein Thrombosis
EDI	Enhanced Depth Imaging
e.g.	Exempli Gratia
Endo-ERN	European Reference Network on Rare Endocrine Conditions
EPI	EchoPlanar Images
ERCUSYN	European Registry on Cushing's Syndrome
ERN	European Reference Network
Et al.	Et alii
ETDRS	Early Treatment of Diabetic Retinopathy Study
FA	Fluorescein Angiography
FAF	Fundus AutoFluorescence
fMRI	functional Magnetic Resonance Imaging
FOV	Field of View
FU	Follow Up
FQ	Fear Questionnaire

HAF	HyperAutoFluorescent
HC	HydroCortisone
HC	Healthy Control
HCC	Hair Cortisol Concentrations
HF	Hyperfluorescent
HPA-axis	Hypothalamus-Pituitary-Adrenal-axis
Hr	Hour
ICV	InterCranial Volume
IDS	Inventory of Depression Symptomatology
i.e.	Id Est
IOQ	Integrated Outcome Quadrant
IPSS	Inferior Petrosal Sinus Sampling
IS	Irritability Scale
Kg	Kilograms
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-MS/MS	Liquid Chromatography-tandem Mass Spectrometry
LC-tandem MS	Liquid Chromatography-tandem Mass Spectrometry
LMWH	Low-Molecular-Weight-Heparin
LUMC	Leiden University Medical Center
MADRS	Montgomery-Åsberg Depression Rating Scale
METC-LDD	Medical Ethics Committee Leiden Den Haag Delft
MRI	Magnetic Resonance Imaging
mSC	midnight Salivary Cortisol
N	Number
No	Number
NR	Not Reported
OCT	Optical Coherence Tomography
OD	Oculus Dexter
OS	Oculus Sinister
Outcome^2	Outcome Squared
PAI-1	Plasminogen Activator Inhibitor-1
PDT	PhotoDynamic Therapy
PE	Pulmonary Embolism
PT	Prothrombin Time
PTSD	Post-Traumatic Stress Disorder
PSS	Perceived Stress Scale
RC	Reference Center
RCD	Remitted Cushing's Disease
ROI	Region Of Interest
RPE	Retinal Pigment Epithelium
RSFC	Resting-State Functional Connectivity

rs-fMRI	resting-state functional Magnetic Resonance Imaging
RT	RadioTherapy
SD	Standard Deviation
SE	Standard Error
SMR	Standardized Mortality Ratio
SPH EQ	Spherical Equivalent of the manifest refraction
SRF	SubRetinal Fluid
TAFI	Thrombin Activatable Fibrinolysis Inhibitor
TE	Echo Time
TE	ThromboEmbolic
ToL	Tower of London
TR	Repetition Time
TSA	TransSphenoidal Adenomectomy
TSS	TransSphenoidal Surgery
UCS	Utrecht Coping Scale
UFC	Urinary Free Cortisol
VAS	Visual Analogue Scale
VTE	Venous ThromboEmbolism
vWF	von Willebrand Factor
XDP	crosslinked fibrin
xULN	x Upper Limit of Normal
YRS	Years

CURRICULUM VITAE

Femke Maria van Haalen werd geboren op 14 april 1985 te Leiden. Zij is samen met haar broers Bas en Koen opgegroeid in Eindhoven. In 2003 behaalde zij haar Gymnasium diploma aan het Lorentz Casimir Lyceum te Eindhoven. In datzelfde jaar startte zij met de studie Geneeskunde aan de Rijksuniversiteit Leiden. Tijdens haar studie was zij werkzaam als verzorgende in verpleeghuizen. Femke behaalde haar artsexamen in 2010 *cum laude*, waarna zij werkzaam was als arts assistent niet in opleiding op de afdeling Interne Geneeskunde van het Alrijne Ziekenhuis te Leiderdorp (toentertijd Rijnland Ziekenhuis). In 2011 begon zij met de opleiding tot internist in datzelfde ziekenhuis (opleider dr. MJFM Janssen), waarna zij in 2013 haar opleiding heeft voortgezet in het Leids Universitair Medisch Centrum (opleider prof. dr. JW de Fijter). Naast haar opleiding tot internist, begon zij in 2015 met wetenschappelijk onderzoek op de afdeling Endocrinologie van het Leids Universitair Medisch Centrum onder begeleiding van prof. dr. AM Pereira, prof. dr. OM Dekkers en prof. dr. NR Biermasz, waarvan de resultaten beschreven staan in dit proefschrift. Inmiddels heeft zij haar opleiding tot internist met aandachtsgebied Endocrinologie (opleider dr. NM Appelman-Dijkstra) afgerond en is zij bijna een jaar als Endocrinoloog werkzaam geweest bij de Noordwest Ziekenhuisgroep te Alkmaar en Den Helder. Momenteel werkt zij als Endocrinoloog in het Leids Universitair Medisch Centrum. Femke is getrouwd met Gerard Noppe, met wie zij drie kinderen heeft (Annemijn 2016, Job 2018 en Fiene 2021).

LIST OF PUBLICATIONS

FM van Haalen, M Kaya, ICM Pelsma, OM Dekkers, NR Biermasz, SC Cannegieter, MV Huisman, BJM van Vlijmen, RA Feelders, FA Klok, AM Pereira, on behalf of the Endo-ERN Cushing and Thrombosis study group. Current clinical practice for thromboprophylaxis management in patients with Cushing's syndrome across Reference Centers of the European Reference Network on Rare Endocrine Conditions (Endo- ERN). *Orphanet Journal of Rare Diseases* 2022 May 3;17(1):178.

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*shared first authorship

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