Clinical consequences of endogenous and exogenous glucocorticoid excess

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The studies described in this thesis were performed at the Department of Medicine, division of Endocrinology, Center for Endocrine Tumors Leiden, of the Leiden University Medical Center, Leiden, the Netherlands, and at the Department of Endocrinology, Diabetes and Nutrition, of the Charité Universitätsmedizin Berlin, Berlin, Germany

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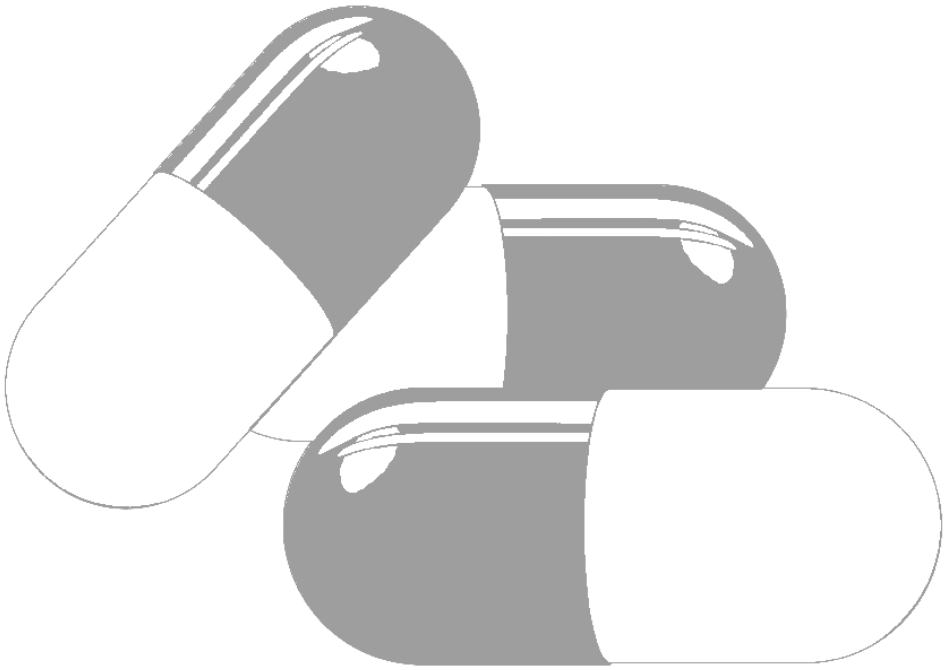
Chapter 1

General introduction and outline of the thesis



Introduction

Cortisol is a steroid hormone, which is produced in the cortex of the adrenal gland. Cortisol was first discovered by Edward Kendall, Tadeus Reichstein, and Philip Hench in the 1930s (1). After collecting 1,000 kg of adrenals from cattle, they extracted 25 g of active substance, in which they discovered 29 different steroids. Twenty-four of these were still undiscovered previously, including cortisol. In 1950, they shared the 'Nobel prize in physiology or medicine' for their discoveries relating to the hormones of the adrenal cortex, their structure, and biological effects (1).

The biological effects of cortisol are derived from the evolutionary perspective of stress response control, and consequently, these effects are widespread throughout the human body. These include immunosuppressive functions, e.g. reduction of an inflammatory response, and prevention of an allergic reaction, as well as the modern use of cortisol in the prevention of rejection of organ transplants (2, 3). In addition, cortisol co-regulates metabolic functions, e.g. control of blood pressure, glucose metabolism, bone mineral density, and lipid metabolism (4, 5). Finally, cortisol controls psychological functions, e.g. regulation of mood, well-being, and cognitive function (6). Therefore, cortisol is released in response both to physical and mental stress (7, 8).

In this chapter, the physiological and pathophysiological role of cortisol is explored, starting with the production and regulation of cortisol in healthy individuals. Subsequently, disruptions in the regulation of cortisol are discussed, including both states of cortisol excess (hypercortisolism) as well as cortisol deficiency (hypocortisolism), which is also known as adrenal insufficiency. Underlying causes, clinical features, and treatment options are presented.

The hypothalamus-pituitary-adrenal axis

Cortisol concentrations in the human body are controlled by the hypothalamus-pituitary-adrenal (HPA) axis (Figure 1). The central circadian oscillator (pacemaker), which is located in the hypothalamic suprachiasmatic nucleus in the brain, regulates this HPA-axis under basal, non-stressed conditions. This pacemaker ensures a daily rhythm in the production of cortisol (the so-called circadian rhythm), which is externally synchronized by the light-dark cycle as its most important input. The pacemaker mainly influences the hypothalamus, driving the release of corticotropin-releasing hormone (CRH) from the hypothalamic paraventricular nucleus. CRH stimulates the release of adrenocorticotropic hormone (ACTH) from the corticotroph

cells in the anterior pituitary gland. CRH also stimulates the production of new ACTH from its precursor POMC in the anterior pituitary, which is secreted after the initial fast release of pre-stored ACTH. ACTH is released from the anterior pituitary gland in around 40 pulses per 24 hours, with the highest amount of ACTH released in the morning around the time of waking. ACTH induces the release of cortisol from the adrenal cortex. Lowest concentrations of cortisol in the human body are found around midnight, and highest concentrations are found in the morning after the highest ACTH peak (9).

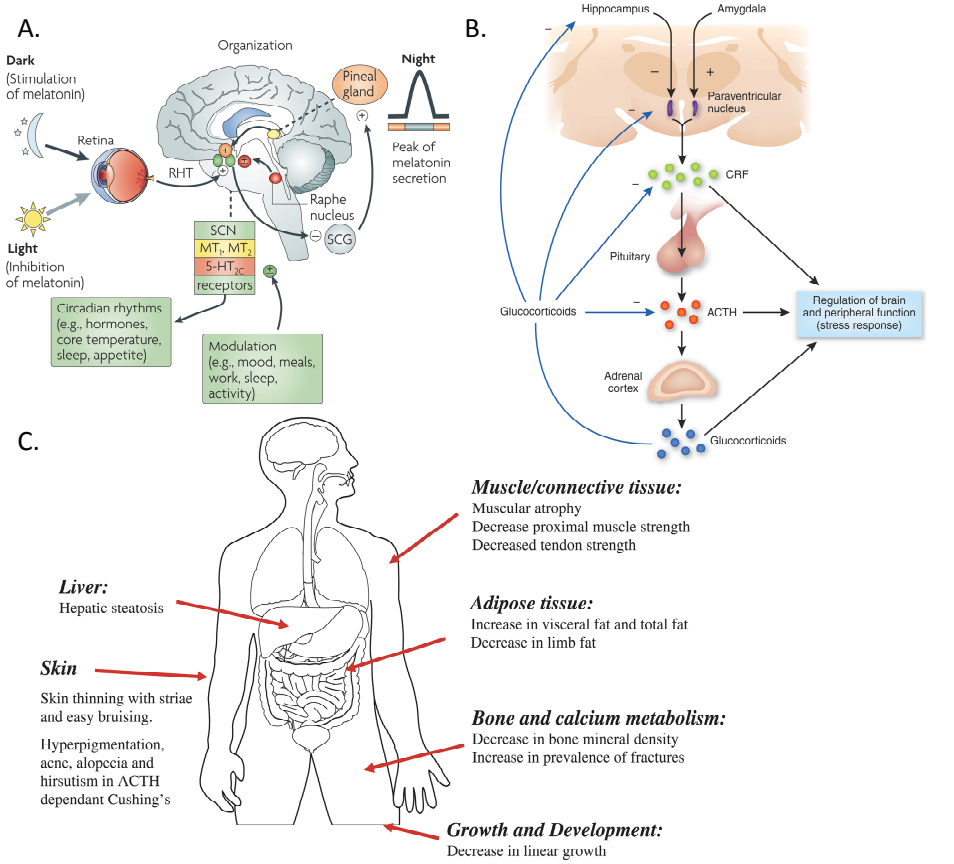


Figure 1: The hypothalamus-pituitary-adrenal (HPA) axis is regulated through the light-dark cycle via the retinohypothalamic tract (RHT), which in case of light inhibits the activity of the superior cervical ganglia (SCG). When dark, the SCG stimulates production of melatonin. Melatonin activates the suprachiasmatic nucleus (SCN) by the melatonin 1 and 2 receptors (MT1 and MT2), which in turn influences circadian rhythms including the HPA-axis. The SCN is modulated through daily behaviors and through serotonin from the raphe nucleus which acts at the serotonin receptor (5-HT2C) (A). The HPA-axis regulates production and release of glucocorticoids by excitatory control of the amygdala and inhibitory control of the hippocampus. The paraventricular nucleus in the hypothalamus produces corticotropin-releasing hormone (CRH), also known as corticotropin-releasing factor (CRF), which stimulates ACTH production and release from the pituitary, which in turn stimulates the adrenal cortex to synthesize and release glucocorticoids. Glucocorticoids inhibit the HPA-axis by negative feedback at various levels (B). Glucocorticoids have several metabolic influences in various tissues of the human body (C). Adapted from de Bodinat *et al.*, Hyman, and Fernandez-Rodriguez *et al.* (4, 11, 12).

Cortisol controls the abovementioned immunosuppressive, metabolic, and psychological functions by binding to the glucocorticoid receptor and also to the mineralocorticoid receptor. In addition, cortisol regulates its own secretion by a negative feedback mechanism through glucocorticoid and mineralocorticoid receptors in the brain and in the anterior pituitary gland (10).

Factors influencing cortisol concentrations

Circulating cortisol concentrations are lower in premenopausal women than in men of corresponding age, but there is no difference in cortisol concentrations between postmenopausal women and men older than fifty years. Therefore, there appears to be no sex difference, but there seems to be an influence of female hormones on cortisol concentrations. In older age, the morning cortisol peak occurs later than in younger age (13, 14). Food consumption rapidly increases concentrations of cortisol, mainly during the daytime (15). Differences in cortisol concentrations between different ethnic groups in relation to differences in social activity and living habits, suggest that these habits influence the synchronization of daily cortisol rhythms. E.g. in Chinese people, the cortisol peak occurs two hours earlier than in Caucasians, which may be related to their habits of early awakening and going to sleep early (16).

The morning cortisol peak after awakening is also known as the cortisol awakening response. This response seems to be influenced by both waking up as well as by daylight, as a smaller cortisol awakening response is seen after waking up in the afternoon than in the morning, and no cortisol awakening response is seen in the evening (17). Altered habits, such as lack of sleep, changes in meal pattern, and light-dark transitions, as may occur during travelling (jet lag) or shift work, affect the circadian rhythm in cortisol production, with complete inversion of cortisol rhythm when sleeping twelve hours out of phase with the usual sleep pattern (18). Circadian misalignment, by sleeping and eating at unusual times, reduces glucose tolerance in shift workers, increasing the risk of type 2 diabetes mellitus (19).

In reaction to unforeseen changes in the environment called ‘stressors’, which can be both external (e.g. temperature changes, food deprivation, physical trauma, infection) and internal (e.g. psychological stress), the stress system is activated. This stress system senses environmental changes through sensory organs and adjusts the central nervous system and peripheral organ activity to improve the chance of survival through restoration of internal homeostasis. The stress system includes both activation of the autonomic nervous system and the HPA-axis, and therefore leads to increased production of catecholamines and cortisol, which in turn increases metabolism (e.g. glucose concentrations), as needed to cope with the stressor at hand (the ‘fight-or-flight response’) (20).

Measurement of hormone concentrations and testing the HPA-axis

Approximately 95% of circulating cortisol is transported bound to cortisol-binding globulin (CBG). The remaining 5% is unbound, also called 'free cortisol', and is therefore available for executing the previously mentioned functions of cortisol by binding to the glucocorticoid and mineralocorticoid receptors (21). Cortisol bound to CBG can be released, depending on the saturation of the CBG (the percentage of CBG binding cortisol) and the affinity with which cortisol is bound to CBG. Cortisol-binding affinity is reduced locally in case of local inflammation and systemically in case of fever, leading to increased cortisol release from CBG (21).

Nowadays, cortisol can be measured by immunoassay or mass spectrometry in serum, saliva, urine, and hair. Serum total cortisol is usually measured in the morning to provide the peak cortisol concentration (22). Salivary cortisol on the other hand is collected at midnight, to capture the physiologically lowest cortisol concentration (23). Urinary free cortisol is usually measured in 24 h urine samples to provide the mean cortisol excretion (24). Hair cortisol can be examined to determine long-term cortisol exposure, which is until now only performed in research settings (25). Serum ACTH concentrations are measured by immunoassay, and they are often measured accompanying the morning serum total cortisol (26). Both salivary and hair cortisol reflect free cortisol measurements.

As cortisol concentrations vary during the day, single cortisol measurements provide limited information and are generally considered inadequate in determining normal functioning of the HPA-axis. Therefore, dynamic stimulation as well as suppression tests have been developed. In general, a rise in plasma glucocorticoids inhibits the ACTH secretion from the anterior pituitary gland, thereby suppressing the activity of the HPA-axis, whereas a fall in plasma glucocorticoids stimulates ACTH secretion from the anterior pituitary gland, thereby stimulating the activity of the HPA-axis. HPA-axis stimulation tests aim to maximize cortisol production from the adrenal cortex, whereas HPA-axis suppression tests aim to minimize cortisol production.

The most commonly used stimulation test in clinical practice is the ACTH stimulation test, which is used to test the maximum potential function of the adrenal glands when insufficient functioning is clinically suspected. During the ACTH stimulation test, usually a high dose (250 μg), or alternatively a low dose (0.5-1.0 μg), of ACTH is administered, and cortisol is measured after 30 (and sometimes also after 60) minutes and compared to the baseline cortisol measurement. Another stimulation test is the CRH test, in which 100 μg of CRH is administered, and cortisol and ACTH are measured for maximum increase compared to baseline (27). The insulin tolerance (stress) test can be used to induce the physiological stress response by administering insulin to induce hypoglycemia, leading to a rapid increase in catecholamines and

cortisol concentrations. However, as this is a potentially hazardous test in selected patients and uncomfortable for the patient, it is less commonly used (28). Finally, the overnight metyrapone stimulation test can be used to test the HPA-axis, in which patients are given a midnight metyrapone dose to inhibit the conversion of 11-deoxycortisol to cortisol, resulting in increased ACTH secretion due to loss of negative feedback in case of intact hypothalamic-pituitary function (29).

The low dose (1 mg) and high dose (8 mg) overnight dexamethasone suppression tests are available to test whether endogenous cortisol production can be suppressed. Because dexamethasone binds to the glucocorticoid receptor, both CRH and ACTH production and release are inhibited, and therefore also cortisol production is inhibited. Dexamethasone does not interfere with the assay used to measure cortisol concentrations. The high dose overnight dexamethasone suppression test is more potent in suppressing cortisol concentrations than the low dose test, but does not result in meaningful higher discriminatory power and is therefore not often necessary in the screening process of Cushing's syndrome (27).

Endogenous hypercortisolism

Cortisol overproduction by the human body itself is called endogenous hypercortisolism. The combination of clinical symptoms accompanying endogenous hypercortisolism is now known as Cushing's syndrome, and was first described in 1912 by Harvey Cushing (30):

"A syndrome of painful obesity, hypertrichosis, and amenorrhoea, with over-development of secondary sexual characteristics accompanying a low grade of hydrocephalus and increased cerebral tension. Pituitary, adrenal, pineal or ovary?"

An excess concentration of glucocorticoids induces specific metabolic changes and thereby alter body composition, including fat maldistribution, muscle wasting, insulin resistance, dyslipidemia, and hypercoagulability. Furthermore, risk of osteoporosis, hypertension, and neuropsychiatric disorders is increased by hypercortisolism (4, 31). Symptoms and signs associated with Cushing's syndrome include weight gain (with central obesity, rounded 'moon' face, and increased dorsal and supraclavicular fat pads called 'buffalo hump'), proximal muscle weakness, menstrual irregularities, loss of libido, depression, hirsutism, and thinness and fragility of the skin (with acne, purple striae, hyperpigmentation, and easy bruising) (Figure 2) (4, 32). Mortality is high in untreated severe Cushing's syndrome, with an estimated five-year survival rate of only 50% (33).

Endogenous hypercortisolism can be caused by a variety of etiologies (Table 1). The majority of these etiologies are ACTH-dependent, meaning that ACTH secretion is increased along with cortisol, and a minority of these etiologies are ACTH-independent, meaning that cortisol concentrations are increased without increase in ACTH concentrations (32). Cushing's disease is the most common underlying cause of endogenous hypercortisolism, with an incidence of 1.2-1.7 patients per million persons each year (35), which accounts for approximately 70% of cases of endogenous hypercortisolism (36). Cushing's disease is caused by an ACTH-secreting pituitary adenoma, which stimulates cortisol production by the cortex of the adrenal gland, and disrupts the negative feedback mechanism. Ectopic Cushing's syndrome results from a non-pituitary ACTH-producing source, which is a rare cause of ACTH-dependent Cushing's syndrome and occurs in approximately 5% of cases (36). ACTH-independent Cushing's syndrome is caused by an adrenal adenoma or carcinoma, which produces cortisol and thereby inhibits ACTH production by the pituitary gland, with an incidence of 0.2-0.6 patients per million persons each year (35). Adrenal Cushing's syndrome accounts for approximately 25% of cases of endogenous hypercortisolism (36). Other rare causes for Cushing's syndrome are ectopic CRH secretion, bilateral primary pigmented nodular adrenal hyperplasia, the ectopic actions of gastric-inhibitory peptide or catecholamines, and other adrenal-dependent processes associated with adrenocortical hyperfunction such as McCune-Albright syndrome and Carney's complex (32). These will not be further discussed in this thesis.

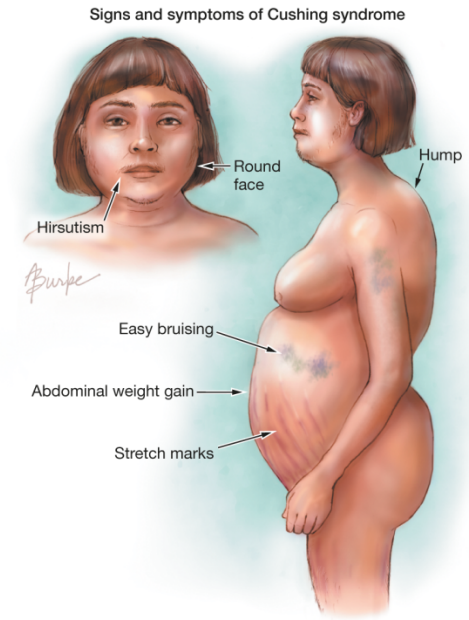


Figure 2: Signs and symptoms in Cushing's syndrome, derived from Pluta *et al.* (34).

Besides Cushing's syndrome, several other diseases are associated with hypercortisolism, formerly known as 'pseudo-Cushing states', now called 'non-neoplastic hypercortisolism'. These states need to be considered when diagnosing Cushing's syndrome, also because these patients show increased cortisol concentrations, as well as varying degrees of symptoms associated with Cushing's syndrome. Non-neoplastic hypercortisolism is related to excessive alcohol intake, depression and other psychiatric diseases, obesity, type 2 diabetes mellitus, insulin resistance, polycystic ovary syndrome, chronic kidney disease, anorexia nervosa, intense chronic exercise, and multiple sclerosis (37).

Treatment of Cushing's syndrome

Treatment of Cushing's syndrome is first aimed at reducing the disease burden of symptoms associated with hypercortisolism by normalizing cortisol secretion, or at least, reducing circulating cortisol concentrations. Further aims are restoring the metabolic changes and reducing the risk of complications, such as hypertension and diabetes mellitus, as well as reducing the increased mortality risk associated with untreated Cushing's syndrome. The ideal treatment leads to the highest percentage of patients in remission after treatment (effectiveness), with the lowest complication rate, and the lowest rate of disease recurrence (safety).

For Cushing's disease, the first-choice treatment method is transsphenoidal pituitary surgery. In 1907, Hermann Schloffer was the first to perform transsphenoidal pituitary surgery (38). Around 1970, this technique was re-discovered and improved due to the use of the operating microscope. This microscope allows selective removal of the ACTH-producing adenoma instead of complete hypophysectomy, as was performed early in the 20th century (39). Starting from 1992, the endoscope became available for transsphenoidal pituitary surgery, which can be used either separately or in combination with the microscope (40). Potential complications of transsphenoidal pituitary surgery are meningitis, cerebrospinal fluid leakage, bleeding, syndrome of inappropriate antidiuretic hormone release (SIADH), diabetes insipidus, and anterior pituitary deficiencies. Repeat transsphenoidal surgery can be performed in case of persistent or recurrent disease after first surgery. Radiotherapy is recommended for patients with an invasive pituitary adenoma, and can be used in case of persistent or recurrent disease. Although radiotherapy is generally highly effective, a potential complication of radiotherapy is hypopituitarism, and it usually takes several months before the first beneficial effects of radiotherapy are evident (41).

First-choice treatment for ectopic Cushing's syndrome is removal of the ACTH-producing tumor. Adrenalectomy is the first-choice treatment in adrenal Cushing's syndrome if one adrenal gland is involved, removing the adrenal gland with the cortisol-producing adenoma, or carcinoma. Bilateral adrenalectomy can also be

Table 1: Overview depicting the different types of hypercortisolism.

	Cushing's disease	**Adrenal Cushing's syndrome**	**Ectopic Cushing's syndrome**	**Pseudo-Cushing states**	**Exogenous hypercortisolism**
Cortisol	High	High	High	High	High
ACTH	High	Low	High	High	Low
Cause	Pituitary adenoma	Adrenal adenoma or carcinoma	Ectopic ACTH-producing tumor	Other diseases, e.g. excessive alcohol intake, depression, obesity, chronic kidney disease	Use of corticosteroids
Treatment	1: Transsphenoidal pituitary surgery 2: Radiotherapy, bilateral adrenalectomy and/or cortisol lowering medication	1: Adrenalectomy 2: Cortisol lowering medication	1: Ectopic tumor resection 2: Cortisol lowering medication	Treatment of the underlying disease	Withdrawal of corticosteroids

ACTH=adrenocorticotropic hormone

performed in Cushing's disease or ectopic Cushing's syndrome when a contra-indication for the first-choice surgical procedure exists (e.g. unidentified tumor, metastatic ectopic tumor), or in case of persistent or recurrent disease after first surgery. Bilateral adrenalectomy has the disadvantage of causing primary adrenal insufficiency, necessitating life-long hydro-cortisone and fludrocortisone replacement therapy. Medical treatment aimed at reducing cortisol concentrations (steroido-genesis inhibitors) is recommended as second-line treatment in patients with Cushing's disease when surgery is unsuccessful, as primary treatment in patients with ectopic Cushing's syndrome with unidentified or metastatic tumor, and as adjunctive therapy to reduce cortisol concentrations in adrenocortical carci-noma. Potential side effects vary per medical agent (41). A complete summary of cortisol-lowering drugs tested in Cushing's disease is provided by Ciato *et al.*, including site and mechanism of action and development stage (42).

Criteria for diagnosis and remission of Cushing's syndrome

Cushing's syndrome is diagnosed based on both clinical grounds as well as bio-chemical test results. Biochemical criteria are dependent on the assay used. Therefore, reference values should be determined per laboratory for all used biochemical parameters. The following biochemical tests are considered relevant for establishing the diagnosis of Cushing's syndrome: 24 h free urinary cortisol excretion, midnight salivary cortisol, and the 1 mg overnight dexamethasone suppression test (43). To differentiate

between ACTH-dependent and ACTH-independent Cushing's syndrome, ACTH concentrations are measured. In case of ACTH-dependent Cushing's syndrome, pituitary imaging by magnetic resonance imaging (MRI) can identify a pituitary adenoma in the majority of cases. Computed tomography (CT) can be performed if a contraindication for MRI exists, but its discriminatory power to detect intrasellar pathology is very limited. If pituitary imaging remains inconclusive, bilateral simultaneous sampling of the inferior petrosal sinuses (IPSS) can be performed to confirm the pituitary origin of ACTH overproduction. If IPSS is negative, additional imaging of the thorax and abdomen are performed to identify a potential ectopic source of ACTH secretion. In case of ACTH-independent Cushing's syndrome, adrenal imaging by MRI or CT is used to identify an adrenal adenoma or carcinoma (44).

Remission status is determined based on clinical and biochemical criteria three months after surgery. Patients are considered in remission if there is dependence on hydrocortisone replacement, or hydrocortisone independence without any biochemical signs of hypercortisolism, in combination with regression of clinical signs (41). Patients are considered to have persistent Cushing's syndrome if there is absence of remission after the first operation and before a second intervention for hypercortisolism is performed. Patients have recurrent disease if, after a period of remission, there is re-occurrence of clinical signs as well as biochemical recurrence of the hypercortisolism as measured by the aforementioned tests.

Exogenous hypercortisolism

When cortisol is administered as a medical agent, it is called hydrocortisone (1). A variety of synthetic corticosteroids, which act similar to hydrocortisone, has been developed, which can be administered in various ways, e.g. orally, via inhalation, intranasally, topical application (e.g. skin, eye), intravenously, intra-muscularly, and intra-articularly. Each has different characteristics, which results in a different choice of agent per disease with varying treatment schedules (45).

Hypercortisolism due to the administration of corticosteroids as medication is called exogenous hypercortisolism, which can lead to iatrogenic Cushing's syndrome. Corticosteroids are used in the treatment of inflammatory diseases (e.g. asthma, rheumatic diseases), malignancies, and after organ transplantation, in order to prevent an inflammatory response (2, 46). Both oral and inhalation corticosteroids are used by approximately 1% of the adult population at any moment (46, 47). Potential side effects of corticosteroid treatment are all symptoms listed for endogenous hypercortisolism because corticosteroids bind to the same receptors as cortisol. Additionally, use of corticosteroids as medical treatment can suppress the

HPA-axis by binding to the glucocorticoid and mineralocorticoid receptors in the hypothalamus and the anterior pituitary gland, lowering ACTH concentrations and thereby suppressing adrenal function (45).

Iatrogenic Cushing's syndrome can only be treated by reducing the dose of corticosteroids. However, due to the potential suppression of the adrenal function during the state of hypercortisolism, corticosteroid dose reductions should be executed with caution to prevent adrenal insufficiency. Additionally, patients with both exogenous and endogenous hypercortisolism may experience corticosteroid withdrawal syndrome to a variable degree when corticosteroid doses are tapered too quickly. Patients with corticosteroid withdrawal syndrome may experience a range of symptoms that are difficult to interpret, e.g. fatigue, mood changes, diffuse aches, and weakness, resulting from a decrease in cortisol concentrations, even though adrenal function is sufficient (48).

Adrenal insufficiency

The state of hypocortisolism is known as adrenal insufficiency, because the adrenal cortex is incapable of producing adequate cortisol concentrations. In primary adrenal insufficiency, this is due to a disease of the adrenal gland itself, whereas in secondary adrenal insufficiency lack of ACTH production leads to insufficient cortisol production. The most common cause of adrenal insufficiency is exogenous corticosteroid use. Secondary adrenal insufficiency, resulting from e.g. congenital hypopituitarism, a pituitary tumor, or pituitary surgery, is the next most common cause of adrenal insufficiency, with a prevalence of 1 in 3,000 persons. The most common cause of primary adrenal insufficiency is Addison's disease, due to autoimmune adrenalitis, leading to destruction of the adrenal cortex. Other causes of primary adrenal insufficiency are congenital adrenal hyperplasia (of which 21-hydroxylase deficiency is the most common form), or bilateral adrenalectomy. All-cause primary adrenal insufficiency is a rare condition with a prevalence of 1 in 8,000 persons, of which 85% is caused by an autoimmune disease (49, 50). Patients with Cushing's syndrome using cortisol-lowering medication can also experience adrenal insufficiency if cortisol concentrations are suppressed excessively by the cortisol-lowering medication without concurrent hydrocortisone replacement.

Patients with adrenal insufficiency generally present with fatigue, weight loss, postural dizziness, anorexia and/or abdominal discomfort. Hyperpigmentation of the skin can be present to a variable degree in primary adrenal insufficiency, as well as low blood pressure with increased postural drop, and failure to thrive in children. Furthermore, laboratory testing can show hyponatremia, hyperkalemia, and

uncommonly hypoglycemia and hypercalcemia (51). Adrenal insufficiency is diagnosed if cortisol remains under the test-specific cut-off value after a stimulation test using ACTH, CRH, insulin-induced hypoglycemia, or metyrapone. As most symptoms are nonspecific, diagnosis is often delayed. Mortality is increased in both treated and untreated patients with adrenal insufficiency (50).

The most feared complication of adrenal insufficiency is a potentially life-threatening situation called adrenal crisis, which represents an acute and severe state of glucocorticoid deficiency. An adrenal crisis can occur after any situation with increased demand for cortisol, e.g. any illness or psychological stress. There is no consensus on the definition of adrenal crisis, and various definitions are used in both clinical and research settings. The following definition may be most useful for clinical practice: a combination of 1) Major impairment of general health with at least two of the following signs and symptoms: hypotension, nausea or vomiting, severe fatigue, fever, somnolence, hyponatremia or hyperkalemia, hypoglycemia, and 2) Clinical improvement following parenteral glucocorticoid administration (52). Importantly, patients with adrenal crisis can have many other nonspecific symptoms, e.g. weight loss, dizziness, abdominal pain, fever, back and leg cramps, which can complicate a correct and timely diagnosis of adrenal crisis.

Patients with adrenal insufficiency are treated with supraphysiological glucocorticoid replacement to control symptoms of adrenal insufficiency and to prevent occurrence of an adrenal crisis. On the other hand, overtreatment with glucocorticoids should be avoided, to prevent symptoms of hypercortisolism. Typically, patients with adrenal insufficiency receive 15-25 mg of hydrocortisone daily, divided into a larger dose in the morning and two smaller doses at lunch and dinner time, to represent the normal circadian cortisol secretion (49). An adrenal crisis is treated by an immediate bolus injection of 100 mg of hydrocortisone intravenously or intramuscularly, followed by continuous infusion of 200 mg hydrocortisone per 24 hours intravenously. Furthermore, rehydration with a rapid intravenous administration of 1 L of isotonic saline should take place within the first hour (53).

To prevent (recurrent) adrenal crisis, an endocrinologist and a specialist endocrine nurse should periodically evaluate all patients with adrenal insufficiency, at least once every 6-12 months. Patients should be educated about the risk of adrenal crisis, including recognition of symptoms of adrenal crisis, and about correct dose adjustment of glucocorticoid replacement. As a rule, the glucocorticoid replacement dose should be doubled during illness with fever that requires bed rest or antibiotics. Furthermore, glucocorticoids should be administered intravenously or intramuscularly during longer periods of vomiting or diarrhea, during preparation for colonoscopy, and in case of acute trauma or surgery. The patient with adrenal insufficiency and

partner or parents should know how to (self-)administer intramuscular hydrocortisone, and they should be in possession of a hydrocortisone emergency injection kit. Finally, patients with adrenal insufficiency should carry a steroid emergency card (Figure 3) (53).

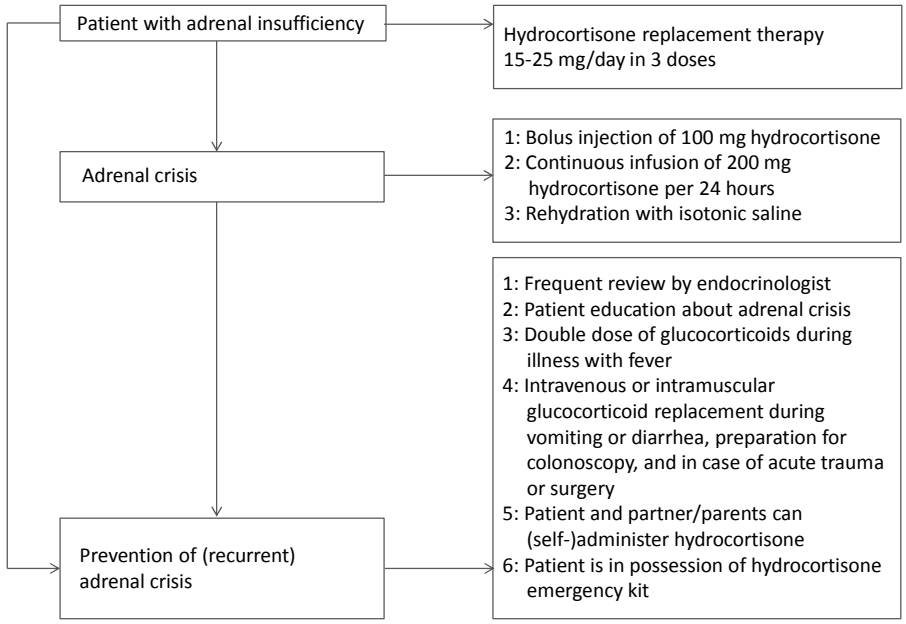


Figure 3: Management of patients with adrenal insufficiency.

Outline of the thesis

Endogenous Cushing's syndrome is rare, but hypercortisolism due to use of exogenous corticosteroids is not. Severe complications due to exogenous hypercortisolism are underestimated and not well-recognized in clinical practice, including mortality and adrenal insufficiency. Endogenous Cushing's syndrome on the other hand causes high morbidity and mortality rates, and therefore treatment of this rare syndrome should be optimized. Furthermore, Cushing's syndrome can serve as a model for long-term exposure to stress, which also leads to high concentrations of cortisol. Research on the topic of Cushing's disease before and after treatment will therefore inform us about potential consequences of long-term exposure to stress, both during periods of stress as well as after abrogation of the stress-inducing situation.

Part I: Complications of corticosteroid use

In part I of this thesis, the complications of corticosteroid use will be explored. Adrenal insufficiency is a well-known complication of corticosteroids use. However, it is unknown in which patients, or under which circumstances, this complication occurs. In **Chapter 2** and in **Appendix I**, the literature is systematically reviewed and, by use of meta-analysis, the proportion of patients with adrenal insufficiency after use of corticosteroids is estimated per route of administration, underlying disease, treatment dose, and treatment duration, to identify patients with high risk of adrenal insufficiency. Mortality may be increased in patients using corticosteroids as medication. However, both corticosteroid use, and mortality, depend on underlying disease. Therefore, in **Chapter 3**, we examined all patients with incident perforated diverticular disease in Denmark, and compared mortality rates between patients that used corticosteroids and patients that did not use corticosteroids to find if use of corticosteroids causes excessive mortality in patients with perforated diverticular disease.

Part II: Treatment outcome in Cushing's syndrome

For the first-choice treatment in Cushing's disease, selective transsphenoidal adenomectomy, two surgical techniques are in use: microscopy and endoscopy. Until now, only few small cohort studies have compared both techniques, so insufficient evidence exists to recommend use of one technique over the other. In **Chapter 4**, we compare both techniques in the large Leiden cohort of patients treated at our referral center, aiming to find differences in surgical outcome, e.g. remission rate and recurrence rate, and in complication rates, e.g. cerebrospinal fluid leakage and anterior pituitary deficiencies. This subject is further investigated in **Chapter 5**, where we perform a systematic review of the literature regarding microscopic and endoscopic transsphenoidal surgery. We use meta-analysis to find differences between both techniques that indicate a preference for either technique based on surgical outcome or complication rates. Medical treatment aimed at reducing cortisol concentrations is used regularly in patients with contraindications to surgery or in case of persistent or recurrent disease. To explore effectiveness and potential side effects of this treatment method, we systematically reviewed the literature and performed meta-analyses per cortisol-lowering drug in **Chapter 6**.

Part III: Clinical outcome in Cushing's syndrome

Cushing's disease occurs more often in females than in males, and males are thought to be at higher risk for ectopic Cushing's syndrome than females. In **Chapter 7**, we study a cohort of patients from Leiden and Berlin to determine whether there are differences between both sexes regarding clinical presentation, diagnostic strategy, and treatment outcome in ACTH-dependent Cushing's syndrome. A potential serious complication of treatment of Cushing's syndrome is adrenal crisis due to inadequately

treated adrenal insufficiency. However, it is unknown how often this complication occurs, and which patients may be at increased risk of developing adrenal crisis after treatment for Cushing's syndrome. We determine the incidence of adrenal crisis in a population of successfully treated patients with Cushing's syndrome from Leiden and Berlin and explore patient characteristics for potential risk factors for adrenal crisis in **Chapter 8**. Hypercortisolism also negatively affects cognition and quality of life, which may even be present in the long-term. In **Chapter 9**, we perform a systematic review of the literature and we determine by meta-analysis if patients improve in quality of life and cognitive functioning after treatment. We also determine if patients normalize regarding quality of life and cognitive functioning by comparing their outcomes to scores from a healthy control population.

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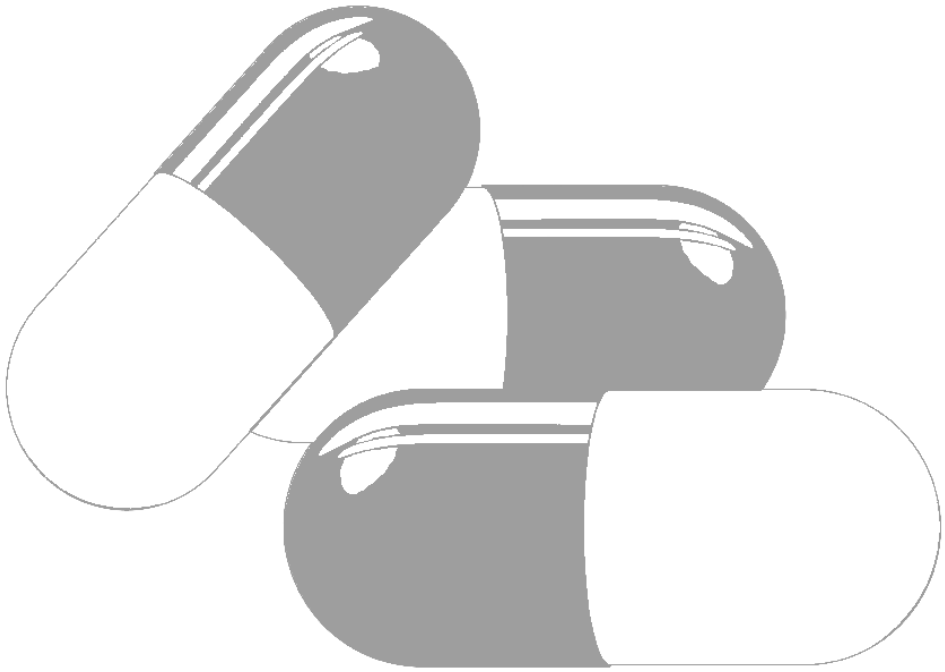
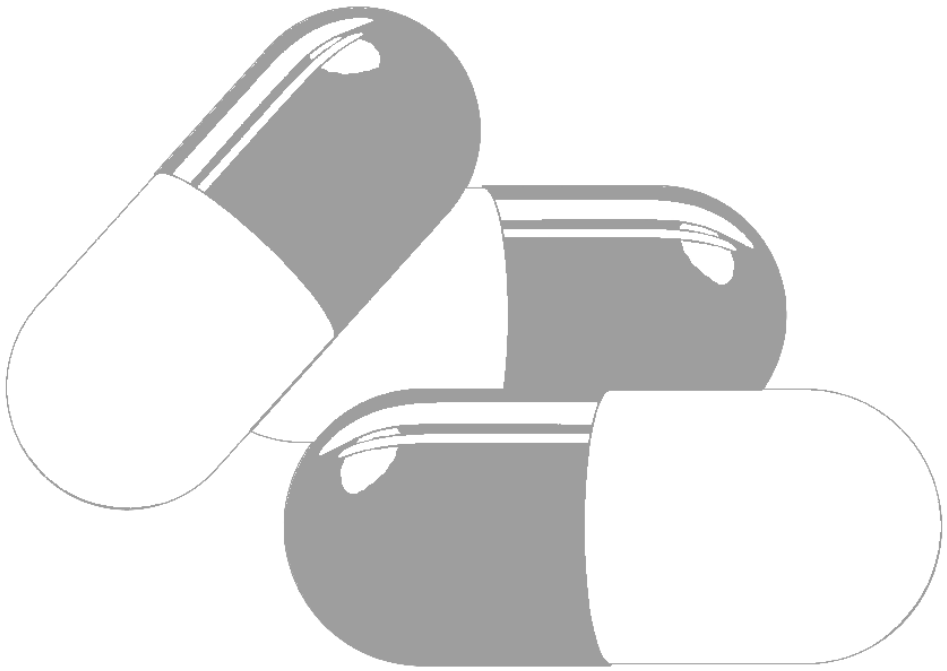
Part I

Complications of corticosteroid use



Chapter 2

Adrenal insufficiency in corticosteroids use: systematic review and meta-analysis



Leonie H. A. Broersen, Alberto M. Pereira, Jens Otto L. Jørgensen, and Olaf M. Dekkers

J Clin Endocrinol Metab. 2015 Jun;100(6):2171-80

Abstract

Objective
We aimed to estimate pooled percentages of patients with adrenal insufficiency after treatment with corticosteroids for various conditions in a meta-analysis. Secondly, we aimed to stratify the results by route of administration, disease, treatment dose, and duration.

Methods
We searched seven electronic databases (PubMed, MEDLINE, EMBASE, COCHRANE, CENTRAL, Web of Science, and CINAHL/Academic Search Premier) in February 2014 to identify potentially relevant studies. Original articles testing adult corticosteroid users for adrenal insufficiency were eligible.

Results
We included 74 articles with a total of 3,753 participants. Stratified by administration form, percentages of patients with adrenal insufficiency ranged from 4.2% for nasal administration (95% confidence interval [CI]: 0.5%-28.9%) to 52.2% for intra-articular administration (95% CI, 40.5%-63.6%). Stratified by disease, percentages ranged from 6.8% for asthma with inhalation corticosteroids only (95% CI: 3.8%-12.0%) to 60.0% for hematological malignancies (95% CI: 38.0%-78.6%). The risk also varied according to dose from 2.4% (95% CI: 0.6%-9.3%) (low dose) to 21.5% (95% CI: 12.0%-35.5%) (high dose), and according to treatment duration from 1.4% (95% CI: 0.3%-7.4%) (<28 days) to 27.4% (95% CI: 17.7%-39.8%) (>1 year) in asthma patients.

Conclusions
1) Adrenal insufficiency after discontinuation of glucocorticoid occurs frequently; 2) There is no administration form, dosing, treatment duration, or underlying disease for which adrenal insufficiency can be excluded with certainty, although higher dose and longer use give the highest risk; 3) The threshold to test corticosteroid users for adrenal insufficiency should be low in clinical practice, especially for those patients with nonspecific symptoms after cessation.

Introduction

Corticosteroids are widely used for the treatment of various inflammatory conditions and malignancies and after organ transplantation. Therapy with corticosteroids is targeted toward inhibition of an inflammatory response (1–3). However, the use of corticosteroids is associated with numerous side effects and is considered to be the most common cause of adrenal insufficiency (4, 5). Chronic use of corticosteroids inhibits the function of the hypothalamic-pituitary-adrenal axis by negative feedback, which may cause adrenal insufficiency also after the cessation of corticosteroid treatment (4, 6). Adrenal insufficiency is a serious, potentially life-threatening side effect of corticosteroid use. Therefore, patients may require glucocorticoid replacement therapy after chronic use of corticosteroids in periods of stress, such as trauma, surgery, or acute illness, until full recovery of adrenal function. In some cases, chronic replacement with physiological doses of glucocorticoid therapy is indicated (7–9).

Neither treatment dose and duration, nor administration form, nor random serum cortisol measurements seem to accurately predict the development of adrenal insufficiency after the use of corticosteroids (10, 11). The magnitude of the risk of developing this side effect is unclear. Given the high prevalence of corticosteroid users, it is of great clinical relevance to try to obtain knowledge about the risk of developing adrenal insufficiency.

Objective of the study

The aim of this study is to perform a systematic review and meta-analysis of the percentage of patients that develops adrenal insufficiency after the use of corticosteroids. Secondary aims are to stratify the results by route of administration, underlying disease, treatment dose, and duration, and to perform a separate analysis for the studies that repeated the test for adrenal insufficiency.

Methods

Eligibility criteria

Original studies assessing adrenal insufficiency in adult human corticosteroid users were eligible for inclusion. The diagnosis of adrenal insufficiency had to be established by one of the following tests: the insulin tolerance test, ACTH stimulation tests (0.5, 1, or 250 μg), CRH test, or metyrapone test. There were no restrictions in dose, duration, or type of corticosteroid therapy. Eligible administration forms of

corticosteroids were oral, inhalation, topical, nasal, intra-articular injection, and intra-muscular injection.

Articles were excluded if the examined population was not at risk of adrenal insufficiency secondary to the use of corticosteroids (e.g., corticosteroid replacement therapy for primary or secondary adrenocortical failure, if not all patients used corticosteroids, or if patients included in the study were selected on the basis of having adrenal insufficiency). Articles were also excluded if no data or insufficient data were presented to analyze adrenal insufficiency after corticosteroid use.

Inclusion of articles was restricted to those in English and to articles that included at least 10 subjects to minimize the risk of selection bias. Articles containing the following populations were excluded: pregnant women, intensive care patients, and patients receiving corticosteroids perioperatively. Because we aimed to include studies in individuals aged 12 years or older, no dose corrections for body surface area were deemed necessary. If an article presented data for multiple study groups, of which some were eligible for inclusion, eligible study groups were included if the pertinent data could be extracted. Articles were also excluded if they were duplicates from already included articles or if they examined the same population as an already included article. Articles that were not retrievable online were requested by contacting the authors.

Definition of adrenal insufficiency

The cutoff value for serum cortisol used to define adrenal insufficiency was ≤500 nmol/L or higher (such as ≤550 nmol/L) to include as many articles as possible that have a low false-negative rate for adrenal insufficiency (12–14). Two articles were included that used a cutoff of 2 standard deviation (SD) values below the cortisol value from a reference group within the study population, and one article was included that had a different cutoff value due to assay technique (≤370 nmol/L). After using the metyrapone test, cortisol had to be at least 200 nmol/L (12). If no cutoff had been provided or if the cutoff used was not 500 nmol/L but individual data were presented, a cutoff of 500 nmol/L was employed to identify patients with adrenal insufficiency. A separate sensitivity analysis was performed for articles testing adrenal insufficiency at least 24 hours after the last use of corticosteroids (12).

Search strategy

In February 2014, PubMed, MEDLINE, EMBASE, COCHRANE, CENTRAL, Web of Science, and CINAHL/Academic Search Premier were searched in cooperation with a specialized librarian to identify potentially relevant articles (see Supplemental Data 1 for complete search string). References of key articles were also assessed to identify

potentially eligible articles. Only articles published from 1975 to the present were searched because radioimmunoassay (RIA) for cortisol became available shortly before the start of that year (15). Randomized controlled trials, cohort studies, and cross-sectional studies were considered, whereas case-control studies and case series are not suitable to estimate absolute risks (16).

Data extraction

All identified articles were entered in Reference Manager version 12 (Thomson Reuters) and were first screened on title and abstract. Potentially relevant articles were then reviewed in detail before inclusion into this meta-analysis. Two different reviewers performed both the screening of the title and abstract and the review in detail for potentially relevant articles. Articles containing more than one study group had multiple entries in this meta-analysis. The Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines were used for reporting (17).

Risk of bias assessment

Study elements that could potentially bias an association between corticosteroid use (exposure) and the development of adrenal insufficiency (outcome) were assessed for all included articles. Risk of selection bias was considered low if consecutive exposed patients or a random sample of exposed patients was included (thereby preventing selection bias) and if eligibility criteria were reported. Ascertainment of exposure to corticosteroids was considered adequate if this was done by protocol or medical record. Measurement of adrenal insufficiency was considered adequate if RIA was used for measuring cortisol concentrations (15). Loss to follow-up <5% was considered a low risk of bias for randomized controlled trials and cohort studies. Studies not following these criteria harbor a higher risk of bias. We did not exclude these articles from analyses because this would result in a very low number of studies available for systematic review and meta-analyses.

Statistical analysis

The main outcomes of this meta-analysis were the pooled percentages of patients with adrenal insufficiency after corticosteroid use, stratified by administration form, disease, treatment dose, and treatment duration. Percentages were pooled in a random-effects logistic regression model. A fixed logistic regression model was used when the number of studies in a particular subgroup was less than five. Analyses were performed with Stata version 12.1 (StataCorp).

Analysis stratified by administration form was based on administration forms used at the time of adrenal testing. If studies included patients using multiple types of corticosteroids (for example, use of inhalation corticosteroid next to oral corticosteroids), this was classified as multiple administration forms. Disease groups

are: asthma (including chronic obstructive pulmonary disease) with only inhalation corticosteroids, asthma (including chronic obstructive pulmonary disease) with other administration forms (including multiple administration forms) of corticosteroids, allergic rhinitis and rhinosinusitis, dermatological disorders (psoriasis, atopic dermatitis, and lichen planus), rheumatic diseases (including osteoarthritis and rheumatoid arthritis), renal transplant, hematological cancers (including myeloma, lymphoma, acute lymphoblastic leukemia, and Hodgkin's disease), nasal polyposis, cystic fibrosis, and Crohn's disease. Diseases that were studied in one study only were not included in the analysis of adrenal insufficiency after the use of corticosteroids stratified by condition.

Treatment duration was categorized as follows: <1 month use, short term; 1 month to 1 year, medium term; and >1 year, long-term use. Treatment dose was categorized according to recommended doses, with the doses between the lower and upper bounds of the recommendation coded as medium dose, doses below the lower bound as low dose, and doses above the upper bound as high dose. Because the most used doses were supraphysiological, doses were not grouped according to physiological and supraphysiological dose. Limits used for the aim of categorization of dose groups and references can be found in Supplemental Data 2. For categorization, the average dose and duration were used. Studies not reporting treatment dose or duration could not be included in the respective stratified analysis. Not included in the treatment duration analysis were articles with multiple short courses of corticosteroids spread out over a period of time longer than 1 month. Analysis of the percentage of patients with adrenal insufficiency by treatment dose and by treatment duration was performed in asthma patients only, as opposed to the entire population of corticosteroids users, to provide a homogeneous patient population.

Separate analysis of study groups that performed repeated tests after discontinuation of corticosteroids was performed. Retesting 4 weeks after cessation of corticosteroid therapy was predominantly performed after a short-term, high-dose corticosteroid treatment regimen, whereas retesting 6 months after cessation of corticosteroid therapy predominantly occurred after long-term corticosteroid use in a medium-dose regimen. These two groups were therefore separated in the analysis. The percentage of patients with adrenal insufficiency at the retest was calculated as the number of patients with adrenal insufficiency at the retest divided by the total number of patients that were measured at time of the first test.

Sensitivity analyses were performed for studies using the ACTH 250-µg test only to exclude test heterogeneity and for studies using the RIA only because this is the preferred assay to detect cortisol. All sensitivity analyses were performed in asthma patients only, to minimize patient heterogeneity. No sensitivity analysis for insulin

tolerance test use only was performed because there were only three studies using this test, and none of them included asthma patients.

Results

Study selection

The initial search provided 3,600 unique articles. By assessment of references of key articles, another 16 articles were found, yielding a total of 3,616 articles. After screening titles and abstracts, 365 articles remained for detailed review. Reasons for exclusion are shown in Supplemental Data 3. Finally, 74 articles were included in this meta-analysis, containing a total of 136 study groups. Although in principle articles containing patients below the age of 12 years were excluded, two articles including patients from 9 to 11 years old were included because most of the patients in these articles were above the age of 12. One article could not be retrieved even after contacting the first author (18).

Study characteristics

Study characteristics are shown in Supplemental Data 4. Included studies were published from 1975 to 2014. Of the 74 articles, 36 were clinical trials (19–54), 23 were cohort studies (1, 2, 8, 11, 55–73), and 15 were cross-sectional studies (10, 74–87). The 136 study groups contained a total of 3,753 participants, of which 124 were healthy volunteers. There were 68 studies on asthma patients, eight studies on rhinitis or rhinosinusitis patients, 12 studies on patients with dermatological conditions (psoriasis, atopic dermatitis, and lichen planus), eight studies on patients with rheumatological disorders (including rheumatoid arthritis and osteoarthritis), eight studies on renal transplant patients, four studies on patients with hematological malignancies, two studies on patients with nasal polyposis, three studies on patients with cystic fibrosis, two studies on patients with Crohn's disease, and one study each on patients with glaucoma, kidney and pancreas transplantation, bronchiectasis, various carcinomas, and giant cell arteritis, respectively. There were eight studies on patients with various conditions.

Risk of bias assessment

Inclusion of consecutive exposed patients or use of a random sample of exposed patients was explicitly stated in 17 articles (23%). Eligibility criteria were reported in 48 articles (65%). In 38 articles (51%), it was unclear how exposure to corticosteroids was ascertained. The remaining 36 articles did this by the use of a protocol or by retrieving data from medical records. In 14 articles (24%), loss to follow-up was reported. Reported loss to follow-up in these articles was 0 to 12.7%. Loss to follow-

up exceeded 5% in one cohort study and two clinical trials. Details of risk of bias analysis at the level of individual studies are shown in Supplemental Data 5.

Study outcomes
Of the 3,753 participants, 1,190 were diagnosed with adrenal insufficiency. The ACTH 250-µg test was used by 103 study groups, and the time between the last dose of corticosteroids and the test for adrenal insufficiency was reported to be 24 hours or longer in 79 study groups. In seven study groups including 199 patients, use of other corticosteroids was allowed as co-medication. Details of study outcomes and tests used at the level of individual studies are shown in Supplemental Data 6.

Adrenal insufficiency and symptoms of adrenal insufficiency
In only 10 study groups, symptoms of adrenal insufficiency were reported. In total, 10 of 521 patients reported symptoms of adrenal insufficiency. Symptoms were not scored systematically in either of the articles. After testing, 98 patients appeared to have adrenal insufficiency within these study groups. Consequently, 88 patients would have been missed when only patients with symptoms of adrenal insufficiency had been tested.

Pooled analysis: adrenal insufficiency by administration form (Figure 1)
The percentage of adrenal insufficiency was 48.7% (95% confidence interval [CI]: 36.9%–60.6%) after oral administration of corticosteroids. The results for other administration forms were: 7.8% (95% CI: 4.2%–13.9%) for inhalation, 4.7% (95% CI: 1.1%–18.5%) for topical administration, 4.2% (95% CI: 0.5%–28.9%) when administered intranasally, and 52.2% (95% CI: 40.5%–63.6%) in patients that used intra-articular corticosteroids. The use of multiple administration forms of corticosteroids resulted in a pooled percentage of adrenal insufficiency of 42.7% (95% CI: 28.6%–58.0%).

Pooled analysis: adrenal insufficiency per condition (Figure 2)
Pooled percentages of adrenal insufficiency per condition are presented in Figure 2 for conditions with at least two studies. Pooled percentages ranged from 6.8% to 60.0%. Asthma patients had an overall percentage adrenal insufficiency of 11.1% (95% CI: 6.8%–17.7%). This was lower for patients with asthma using inhaled corticosteroids (6.8%; 95% CI: 3.8%–12.0%), than for asthma patients using other administration forms including oral (43.7%; 95% CI: 27.3%–61.6%).

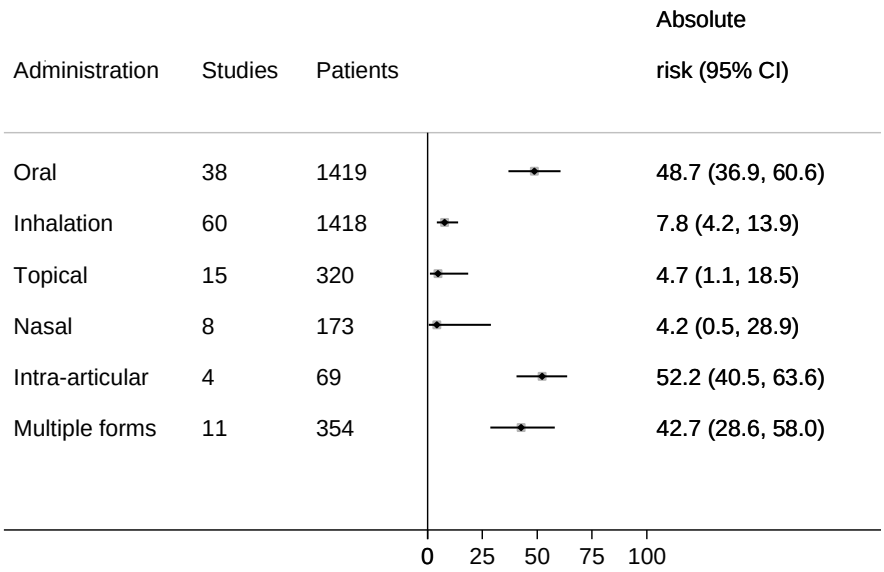


Figure 1: Meta-analysis, adrenal insufficiency after corticosteroids use by administration form.

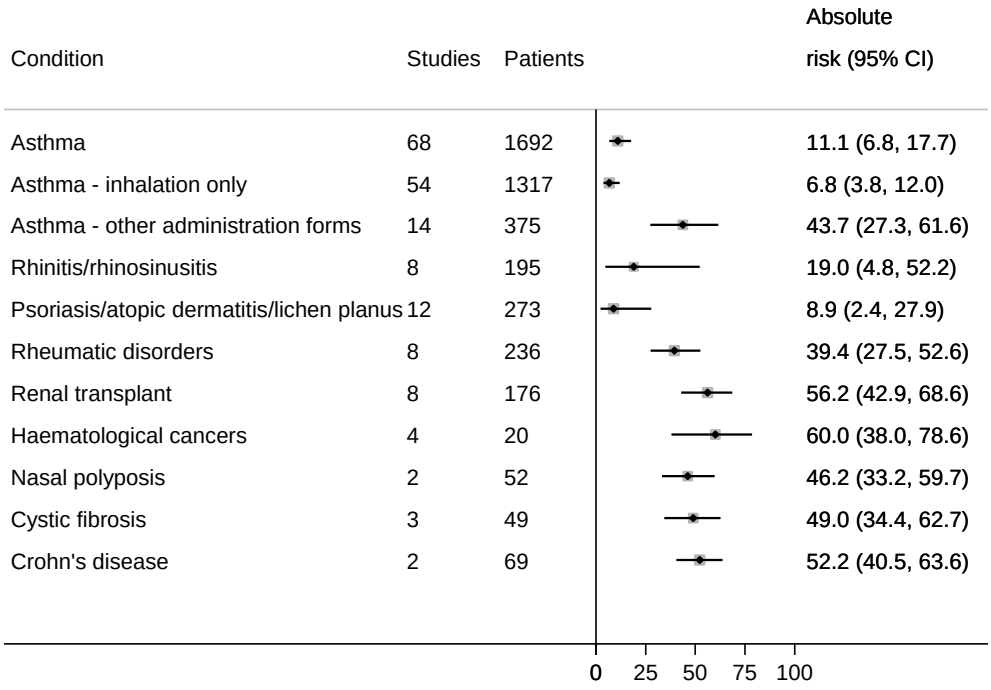


Figure 2: Meta-analysis, adrenal insufficiency after corticosteroids use per condition.

Adrenal insufficiency by treatment dose and treatment duration (Figure 3)
Analysis per treatment dose and treatment duration was performed in asthmatic patients only for reasons of population homogeneity. Use of corticosteroids in low, medium, or high doses resulted in a percentage of adrenal insufficiency of 2.4% (95% CI: 0.6%–9.3%), 8.5% (95% CI: 4.2%–16.8%), and 21.5% (95% CI: 12.0%–35.5%), respectively. Use of corticosteroids for a short, medium, or long term resulted in a percentage of adrenal insufficiency of 1.4% (95% CI: 0.3%–7.4%), 11.9% (95% CI: 5.8%–23.1%), and 27.4% (95% CI: 17.7%–39.8%), respectively.

If performed in asthma patients using inhaled corticosteroids only, the percentages of adrenal insufficiency in low, medium, and high doses were 1.5% (95% CI: 0.2%–9.4%), 5.4% (95% CI: 2.7%–10.4%), and 18.5% (95% CI: 8.7%–35.2%), respectively. In short-, medium-, and long-term treatment duration groups, percentages of adrenal insufficiency were 1.3% (95% CI: 0.2%–7.2%), 9.0% (95% CI: 4.3%–17.9%), and 20.3% (95% CI: 12.4%–31.6%), respectively (data not presented in figure).

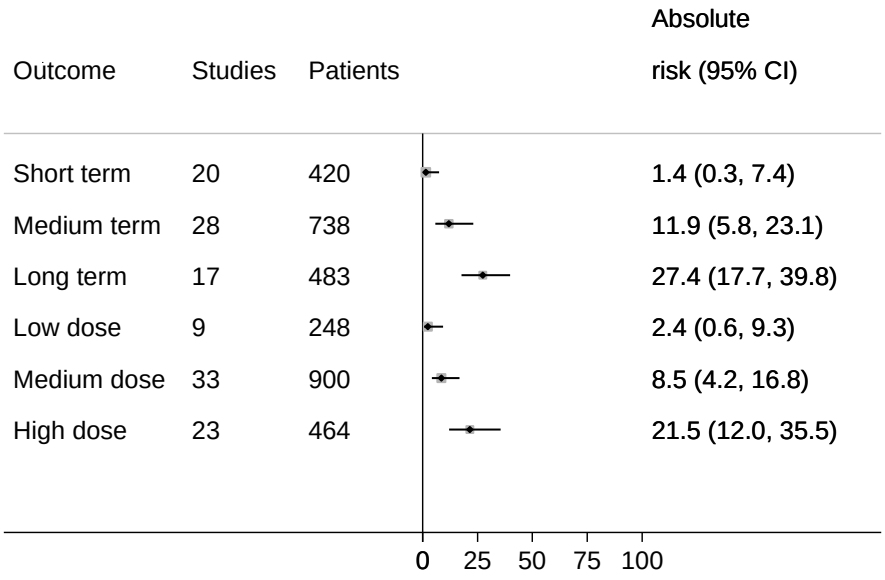


Figure 3: Meta-analysis, adrenal insufficiency per dose and duration in asthma patients.

Adrenal insufficiency after retesting (Figure 4)

Analysis of retests was split into studies that retested after 4 weeks, using mainly short-term, high-dose corticosteroids, and studies that retested after 6 months, using mainly long-term, medium-dose corticosteroids. Studies retesting after 4 weeks had a percentage of adrenal insufficiency after their first test of 38.7% (95% CI: 21.7%–58.8%). After 4 weeks, retesting showed a percentage of adrenal insufficiency of 14.9% (95% CI: 6.8%–29.5%). Studies retesting after 6 months had a percentage of adrenal insufficiency after their first test of 56.4% (95% CI: 38.2%–72.9%). After 6 months, the percentage of patients with adrenal insufficiency was still 25.3% (95% CI: 19.4%–32.3%).

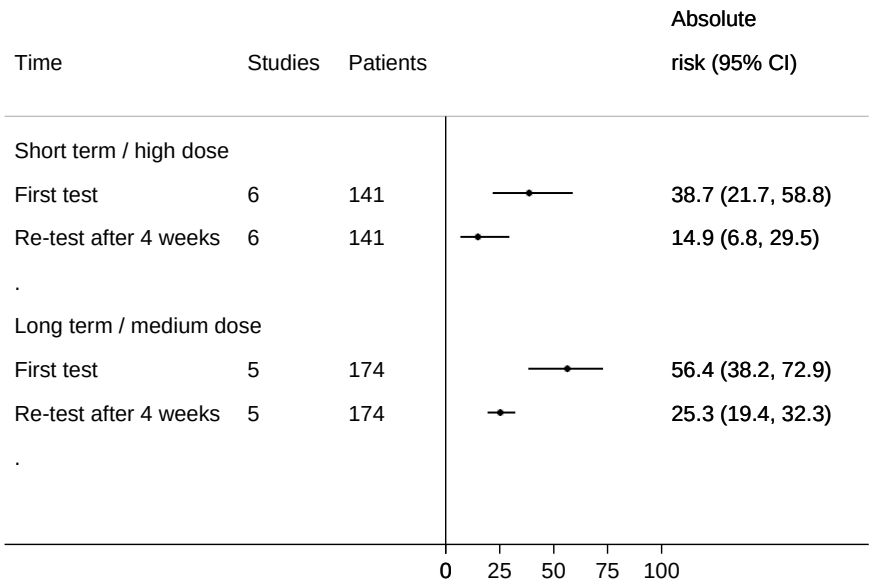


Figure 4: Meta-analysis, adrenal insufficiency after corticosteroids use by time of test.

Sensitivity analysis

For the sensitivity analysis, we combined all studies with asthma patients, which resulted in a percentage of adrenal insufficiency of 11.1% (95% CI: 6.8%–17.7%) as a reference group. When only studies were included that explicitly tested for adrenal insufficiency at least 24 hours after the last corticosteroid dose, the percentage of adrenal insufficiency was slightly lower (6.6%; 95% CI: 2.2%–18.3%). When performed on studies using the ACTH 250-µg test only, a percentage of 8.5% (95% CI: 4.7%–14.8%) was found. When performed on studies using RIA only, a percentage of 14.7% (95% CI: 7.1%–27.9%) was found.

Discussion

We performed a systematic review and meta-analysis to estimate the percentage of patients that develop adrenal insufficiency after the use of corticosteroids. Depending on administration form, the percentage of patients with adrenal insufficiency varied from 4.2% for nasal corticosteroids to 52.2% for intra-articular corticosteroids. Stratified by disease, percentages ranged from 6.8% for asthma patients with inhalation corticosteroids only to 60.0% for patients with hematological malignancies. According to dose, the percentage of adrenal insufficiency varied from 2.4% (low dose) to 21.5% (high dose), and according to treatment duration from 1.4% (<28 days) to 27.4% (>1 year) in asthma patients. This means that there is no administration form, disease, dose group, or treatment duration for which the risk of adrenal insufficiency can be safely excluded. Although the percentage of patients with adrenal insufficiency after corticosteroids use declines over time, a substantial number of patients remained adrenal insufficient after 6 months.

This is the first meta-analysis providing a broad view on the risk of adrenal insufficiency after use of various types of corticosteroids for several diseases. To the best of our knowledge, only one meta-analysis (88) has been published on appropriately tested adrenal insufficiency in asthma, reporting percentages of adrenal insufficiency ranging from 5.5% to 13.3%. In the current meta-analysis, we found a percentage of 6.8% of adrenal insufficiency in asthmatic patients using inhaled corticosteroids, which is in line with results from the meta-analysis mentioned.

Included studies displayed heterogeneity in the type of corticosteroid used, underlying condition, treatment dose, treatment duration, and route of administration. It is important to consider that this heterogeneity reflects clinical practice. It should also be kept in mind that condition, treatment dose, treatment duration, and route of administration are clearly related. In our stratified analysis, we did not adjust for all mutually dependent factors, mainly because these factors are related in clinical practice as well, but also because meta-regression techniques would fall short in the absence of individual patient level data to disentangle these clearly related factors. Differences in the percentage of patients with adrenal insufficiency per condition may partly be explained by treatment dose and duration, partly by administration form, and partly by the nature of the disease. Higher treatment dose and longer treatment duration give higher systemic levels of corticosteroids, and therefore higher percentages of adrenal insufficiency (89). This might explain the low risk of adrenal insufficiency in nasal corticosteroids use and the high risk of adrenal insufficiency in rheumatic diseases, after renal transplant, in hematological malignancies, and when multiple administration forms are used. The

use of oral corticosteroids results in higher systemic levels of corticosteroids than in cases of inhalation, topical, and nasal corticosteroids use, and consequently leads to higher percentages of adrenal insufficiency (32). The use of nasal as well as oral and inhalation corticosteroids in rhinitis and rhinosinusitis patients might have contributed to the higher percentage of adrenal insufficiency than in patients using nasal corticosteroids only. The use of only topical corticosteroids in patients with psoriasis, atopic dermatitis, or lichen planus may explain the low percentage of patients with adrenal insufficiency. The different administration forms in asthma patients may largely explain the low percentage of adrenal insufficiency in patients with asthma using only inhalation corticosteroids and the high percentage of adrenal insufficiency in asthma patients using other administration forms of corticosteroids. Intra-articular corticosteroids are administered at high doses and are known to suppress serum cortisol levels within 24–48 hours, recovering only after 1–4 weeks (90). This might explain the high percentage of adrenal insufficiency after the use of intra-articular corticosteroids. The high rate of adrenal insufficiency is probably also a reflection of the fact that these injections are depot formulations. The presence of adrenal insufficiency in such situations may in part be due to the continued presence of corticosteroids in the body, whereas reduction of steroid levels will be gradual rather than abrupt. Most studies did not provide data on treatment adherence, and assessing the impact of (non-)adherence on risk of adrenal insufficiency was therefore not possible. Because all included studies were observational, the results of our meta-analyses are likely to reflect clinical practice.

Included studies also showed heterogeneity in cortisol assay and in the type of cortisol tests performed. The sensitivity analysis did not reveal any material difference in overall percentage of adrenal insufficiency if only articles using a RIA were included, or if only articles using the ACTH 250-µg test were included. Although the diagnostic performance of RIA in the routine evaluation of adrenocortical function is considered superior to other competitive protein-binding analytical methods, like fluorimetry (91, 92), the chemiluminescence immunoassay seems to have comparable diagnostic performance and accuracy to RIA (93). It should be kept in mind that test criteria for adrenal insufficiency available in clinical practice have a high sensitivity rather than a high specificity, and therefore the number of false-positive test results is not negligible. None of the studies retrieved by our literature search used the more modern tandem mass spectrometry (94).

Several pathophysiological pathways may be involved in the development of adrenal insufficiency after the use of corticosteroids. It is certainly relevant to disentangle these different pathways and address the question of whether differences in dosage, treatment duration, and type of corticosteroid differentially affect the activity of the hypothalamic-pituitary-adrenal axis. However, in our review we aimed to evaluate

the effect of corticosteroids on adrenal function in clinical practice instead of disentangling the exact mechanisms of adrenal insufficiency.

There was no sensitivity analysis performed for low risk of bias articles only, because there was only one article with low risk of bias (based on the inclusion of patients and loss to follow-up) within the group of studies with asthma patients only. If only studies with a time gap of at least 24 hours between the last dose of corticosteroids and the test for adrenal insufficiency were included, the percentage of adrenal insufficiency decreased only slightly. In our main analyses, we included articles irrespective of the time between last corticosteroid use and time of test. It is important to keep in mind that the percentage of patients with adrenal insufficiency would have been slightly higher than estimated in this meta-analysis had all articles used a time gap of at least 24 hours between the last dose of corticosteroids and the time of the test.

Corticosteroids are used by at least 1% of the population (3). The risk of developing adrenal insufficiency in these patients is 1.4% to 60.0%, and symptoms of mild to moderate adrenal insufficiency, like fatigue and abdominal discomfort, are nonspecific and therefore difficult to ascribe to adrenal insufficiency. In addition, accurate predictors are not available to distinguish between the patients that will become adrenal insufficient and those that will not. Also there is insufficient evidence to prove any withdrawal scheme after steroid use to be efficient or safe (95). Therefore, we recommend that all patients with unexplained symptoms after steroid withdrawal be tested for possible adrenal insufficiency. In case of insufficient response, treatment should be initiated with physiological doses of hydrocortisone.

In conclusion, this study demonstrates that all patients using corticosteroid therapy are at risk for adrenal insufficiency. This implies that clinicians should: 1) Inform patients about the risk and symptoms of adrenal insufficiency; 2) Consider testing patients after cessation of high-dose or long-term treatment with corticosteroids; and 3) Display a low threshold for testing, especially in those patients with nonspecific symptoms after cessation.

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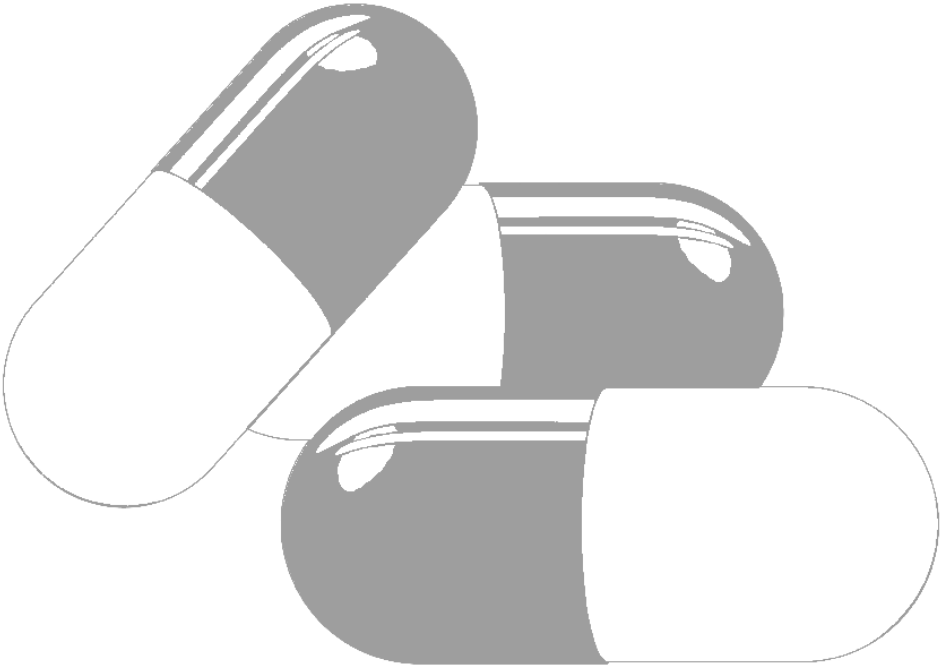
Chapter 3

Corticosteroid use and mortality risk in patients with perforated colonic diverticular disease: a population-based cohort study



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Abstract

Background
Corticosteroids are a potential risk factor for mortality in patients with perforated diverticular disease, due to blinding of disease severity, hampered wound healing or adrenal insufficiency. We examined mortality in corticosteroid users and non-users among patients with perforated diverticular disease.

Methods
A cohort study based on medical databases including all patients ≥18 years in Denmark (source population 5,289,261 inhabitants) admitted to a hospital with incident perforated diverticular disease between 2005 and 2013. 7-day, 1-month, 3-month and 1-year mortality risks in corticosteroid users and non-users were calculated using the Kaplan–Meier method, and compared with Cox proportional hazard regression adjusted for age, sex and comorbidities.

Results
The study included 4,640 patients with perforated diverticular disease. Of these, 3,743 (80.7%) had not used corticosteroids in the year before admission and 725 (15.6%) had been exposed to systemic corticosteroid treatment. The remaining 172 patients had been exposed to either inhaled or intestinal acting corticosteroid therapy. Mortality risk in non-users was 4.4% after 7 days and 15.6% after 1 year. This risk was doubled for corticosteroid users who filled their last prescription during the 90 days before admission, with mortality risks ranging from 14.2% after 7 days to 47.6% after 1 year. 1-year mortality risk was even higher for corticosteroid users with a first filled prescription ≤90 days before admission: 52.5%.

Conclusions
Corticosteroid use was associated with clearly increased mortality risk after perforated diverticular disease. Thus, use of corticosteroids should be regarded as an important clinical prognostic factor for mortality in patients with this condition.

Introduction

In Western countries, the prevalence of colonic diverticular disease reaches 50% among persons aged 60 and older (1, 2). Although most patients remain asymptomatic, the lifetime risk for developing diverticulitis is ~10%–25% (3), which is known to present with perforation (4, 5). The incidence of perforated diverticular disease is ~4 per 100,000 persons each year, reported in hospital records in the UK of Great Britain and Northern Ireland and the USA (6). Mortality after perforated diverticular disease requiring operative intervention has been reported to be as high as 12%–36% in small cohort studies (78–121 cases) (7, 8). Given this high mortality risk, a better understanding of prognostic factors associated with mortality is clearly needed.

Use of corticosteroids is a potential prognostic factor affecting mortality in patients with perforated diverticular disease. Corticosteroids are widely prescribed for inflammatory diseases, malignancies and after organ transplantation, in order to inhibit an inflammatory response (9–11). At the same time, corticosteroids have been associated with bowel perforation in patients with diverticular disease. Three case-control studies reported increased odds ratios of 1.7 to 28.3 and one cohort study reported a relative risk of 2.2 (12–15). A recent meta-analysis showed odds ratios for perforation of diverticular disease after use of corticosteroids of 2.17 to 31.90 (16).

There are several reasons why mortality may be increased in patients with perforated diverticular disease who use corticosteroids. Disease severity might be masked by use of corticosteroids (17), leading to incorrect staging of disease severity and, consequently, inadequate treatment (6). Corticosteroid use might hamper wound healing as well through its anti-inflammatory effects, by suppressing cellular wound responses and by inducing diabetes, and thus increasing the risk of wound infection (18). A systematic review also showed that corticosteroid use may increase the risk of anastomotic leakage after colorectal surgery (19). Use of corticosteroids is associated with an increased risk of venous thromboembolism (20). In perforated diverticulitis, undiagnosed and untreated adrenal insufficiency may lead to an Addisonian crisis, which increases mortality risk in affected patients (21).

Study aims

The primary aim of this study was to examine absolute mortality risks and compare 7-day, 1-month, 3-month and 1-year mortality rates in patients with perforated diverticular disease who were corticosteroid users versus non-users. The secondary aim was to examine mortality rates in patients with perforated diverticular disease who used corticosteroids administered in different forms and with different cumulative doses.

Methods

Source population

The source population for this study was the entire population of Denmark (5,289,261 inhabitants) between 2005 and 2013. The study period began in 2005 because a full record of medications sold at community pharmacies and hospital-based outpatient pharmacies became available starting in 2004 in the *National Health Service Prescription Database* (22). The study was based on data from the *Danish National Patient Registry*, which has recorded all acute care hospital discharges since 1977 and all outpatient specialist clinic and emergency room visits since 1995 (23). Data from the *Danish Civil Registration System* were used to determine vital status (24).

Study population and follow-up

The study included all adult patients (≥18 years), with and without use of corticosteroids, hospitalized with incident perforated diverticular disease between 2005 and 2013. Identification of perforated and non-perforated diverticular disease was based on International Classification of Diseases (ICD) codes (see Supplemental Data 1 for specification of the codes). The codes used for perforated diverticular disease have a positive predictive value ranging from 0.73 to 0.75 (25). There were no restrictions in corticosteroid dose or treatment duration. Patients diagnosed during an emergency room visit were excluded because of the low predictive value of emergency room diagnoses; diagnostic accuracy improves with more extensive diagnostic procedures (26). The year 2005 as start of follow-up was chosen in order to provide a 1-year prediagnosis period in which exposure to corticosteroids could be assessed similarly in all patients. Patient follow-up began on the hospital admission date for perforated diverticular disease and ended on death, emigration or end of follow-up on 30 November 2013, whichever came first.

Classification of corticosteroid use and surgical procedures

Corticosteroid use was categorized as follows, based on the *National Health Service Prescription Database* (22):

I. *Non-users*: patients not prescribed corticosteroids during the year prior to hospital admission.

II. *Current users*: patients using corticosteroids at time of admission, that is, the last prescription for corticosteroids was filled during the 90 days before admission. The group of current users was subdivided into new users, defined as patients who filled their first prescription ≤90 days before admission, and chronic users, which included all other current users.

III. *Recent users*: patients who used corticosteroids until shortly before admission, that is, their last corticosteroid prescription was filled between 91 and 365 days before admission.

Analyses were performed separately for patients using systemic corticosteroids regardless of use of other forms of corticosteroids, for patients using systemic corticosteroids only, for patients using inhaled corticosteroids only, and for patients using intestinal acting corticosteroids only. There was one patient using both inhaled and intestinal acting corticosteroids.

The analysis of cumulative dose was based on categorizing the 1-year cumulative dose of systemic corticosteroids among current users, calculated using prednisone dose equivalents. The cumulative dose cut-offs were ≤625 mg, >625 mg to 2000 mg, >2000 mg to 3500 mg and >3500 mg, to provide four groups of approximately equal size.

Surgical procedures related to diverticular perforation (including a 30-day period around date of admission for diverticular disease) were categorized as explorative surgery including lavage; stoma with or without resection; and resections with primary anastomosis (see Supplemental Data 2 for surgical codes).

Statistical analysis

Descriptive contingency tables were prepared, showing demographic characteristics and medical history (age, sex, calendar year of perforated diverticular disease diagnosis, comorbidities) of corticosteroid users and non-users.

For time-to-event analyses, the Kaplan-Meier method and Cox regression were used. Absolute mortality risks in corticosteroid users and non-users were calculated using the Kaplan-Meier method. Cox proportional hazard regression was used to compare mortality rates among the predefined categories of corticosteroid users, providing hazard ratios (HRs) for 7-day, 1-month, 3-month and 1-year mortality. Stratified analyses were performed according to type of surgery within 30 days before or after diagnosis of perforated diverticular disease.

Subgroup analyses were performed to compare mortality risks among patients taking different forms of corticosteroids (inhaled only, intestinal only and systemic only) and with different 1-year cumulative doses of systemic corticosteroid use. Also, sensitivity analyses were performed after excluding patients with malignancies, patients with inflammatory bowel disease, patients with a previous code for diverticular disease and patients with rheumatic diseases including mixed connective tissue disease.

In addition to crude analyses, we performed adjusted analyses including the following potential confounders: age, sex, use of cardiovascular medications (anticoagulants, non-steroidal anti-inflammatory drugs, ACE/angiotensin 2 receptor inhibitors, statins,

ß blockers, calcium channel blockers, diuretics, selective serotonin reuptake inhibitors, and nitrates) (16) during the 90 days before admission, hypertension, chronic obstructive pulmonary disease (COPD; as a proxy for smoking), liver disease or chronic pancreatitis, alcoholism-related diseases other than those affecting the liver or pancreas, inflammatory bowel disease, rheumatoid arthritis, connective tissue disease, malignancies and modified Charlson Comorbidity Index score (see Supplemental Data 1 for specification of the codes). The modified Charlson Comorbidity Index score was calculated after exclusion of COPD, liver diseases, connective tissue disease and malignancies. These characteristics and conditions are associated with corticosteroid use and constitute potential risk factors for mortality.

We used SAS V.9.2 (SAS Institute, Cary, North Carolina, USA) for our statistical analyses. Permission from the Danish Data Protection Board was granted.

Results

Study population

A total of 4,640 patients with perforated diverticular disease were included in the study. Of these, 3,743 (80.7%) had not used corticosteroids in the year before hospital admission (Table 1). Of the 897 patients using corticosteroids in the prior year, 725 (80.8%) were exposed to systemic corticosteroid treatment (current and recent users combined). Of systemic corticosteroid users, 88.8% used prednisone or prednisolone, 6.9% methylprednisolone, 13.4% ß-methasone, 1.5% triamcinolone and 1.0% hydrocortisone. Since the number of patients using only intestinal acting corticosteroids was small (n=28), this patient group was not analyzed further. Corticosteroid users were on average older than non-users and more often female than non-users (64.8% vs 54.2%). Patients using systemic corticosteroids were more often hypertensive, more often had COPD, chronic bronchitis, emphysema or asthma, and more often had malignancies, rheumatoid arthritis, connective tissue disease or inflammatory bowel disease. Almost all patients using only inhaled corticosteroids had a diagnosis of COPD, chronic bronchitis, emphysema or asthma, but were otherwise comparable to non-users. Charlson Comorbidity Index scores were higher for all groups of corticosteroid users compared with non-users.

Mortality risk in patients with perforated colonic diverticular disease

A total of 889 patients (19.2%) with perforated diverticulitis died within 1 year following their diagnosis (Table 2). Mortality risk in non-users of corticosteroids was 4.4% after 7 days, 8.8% after 30 days, 11.9% after 90 days and 15.6% after 1 year. This risk was doubled for current users of corticosteroids (adjusted HRs ranging from 1.96 (30-day mortality) to 2.10 (7-day mortality)), with mortality risks reaching 14.2%

after 7 days and 47.6% after 1 year (Figure 1). Mortality risk was even higher for new corticosteroid users, increasing from 15.7% 7 days post diagnosis to 52.5% after 1 year. Among chronic corticosteroid users, mortality risks also were almost doubled, with adjusted HRs of 1.89 after 7 days and 1.74 after 1 year, and absolute mortality risks reaching 13.7% after 7 days and 45.9% after 1 year.

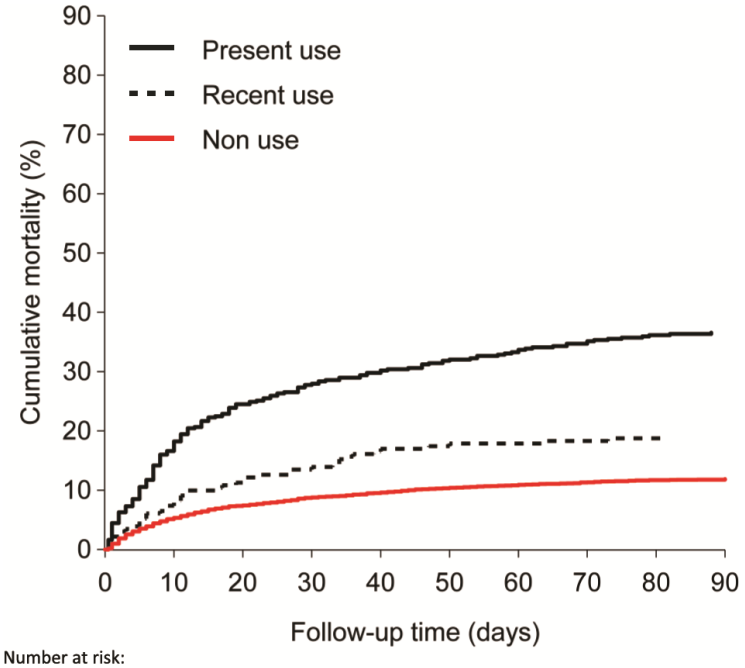

Figure 1: Cumulative mortality in patients with systemic corticosteroid use, regardless of other use.

Mortality risk according to 1-year cumulative dose

In our study population, 124 patients had a 1-year cumulative dose ≤625 mg, 144 patients had a 1-year cumulative dose >625–2000 mg, 140 patients had a 1-year cumulative dose >2000–3500 mg and 86 patients had a 1-year cumulative dose >3500 mg (Table 3). A higher 1-year cumulative dose was not clearly associated with higher mortality risk in patients with perforated diverticular disease.

Abdominal surgical procedures

In total, 2,497 (53.8%) of 4,640 patients had a diverticulitis-related surgical procedure (Table 1). The percentage of patients with surgery within 30 days before or after incident perforated diverticular disease was higher among systemic corticosteroid users (62.9%) compared with users of inhalation corticosteroids only (56.6%) and non-users (52.1%). Patients using systemic corticosteroids had a surgical

procedure resulting in a stoma (33.8%) more often than patients not using corticosteroids (22.6%).

Within categories of surgery (explorative surgery including lavage, stoma, resection with primary anastomosis), mortality risk was increased when comparing current users to non-users. After explorative surgery, mortality risk after 1 year was 14.8% for non-users and 43.5% for current users (adjusted HR 1.12 [95% CI 0.69 to 1.83]). For patients who received a stoma, mortality risk after 1 year was 26.5% for non-users and 48.8% for current users (adjusted HR 1.37 [95% CI 1.03 to 1.82]). After a resection with primary anastomosis, mortality risk after 1 year was 15.2% for non-users and 54.1% for current users (adjusted HR 2.88 [95% CI 1.57 to 5.28]).

Subgroup and sensitivity analyses

The mortality risk among patients currently using systemic corticosteroids only (13.9% after 7 days and 48.0% after 1 year) was comparable to that among patients using systemic corticosteroids regardless of other corticosteroid use (Figure 2). Compared with non-users, patients who currently used only inhaled corticosteroids also had an increased mortality risk (6.2% after 7 days and 22.3% after 1 year), with an adjusted HR ranging from 1.44 to 1.85 (Table 4).

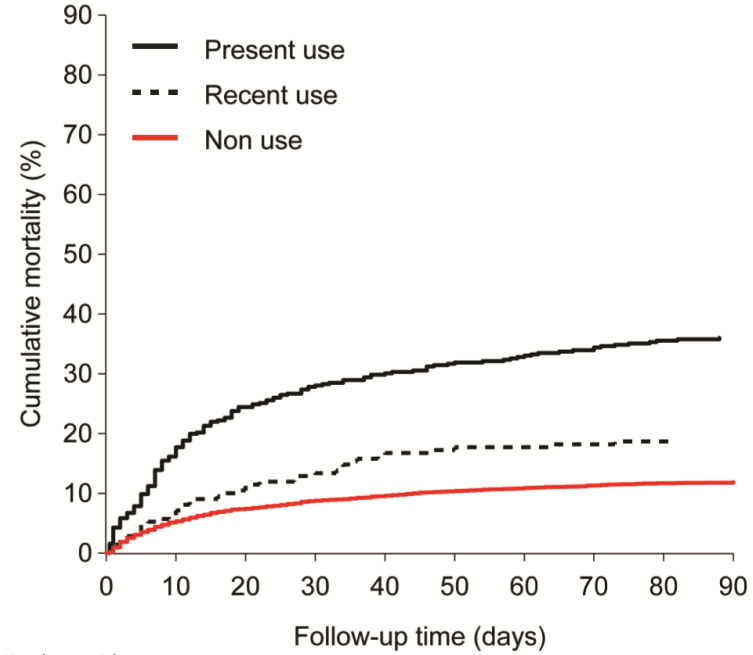

Figure 2: Cumulative mortality in patients with systemic use of corticosteroids only.

Table 1: Baseline characteristics of corticosteroid users and non-users among patients with perforated diverticular disease.

	Non-use		Systemic use regardless of other uses		Systemic use only		Inhaled use only	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Total	3743	100.0	725	100.0	656	100.0	145	100.0
Current use	-	-	494	68.1	446	68.0	81	55.9
New use	-	-	121	16.7	109	16.6	7	4.8
Chronic use	-	-	373	51.4	337	51.4	74	51.1
Recent use	-	-	231	31.9	210	32.0	64	44.1
Age, years								
18-59	1431	38.2	119	16.4	113	17.2	42	29.0
60-69	860	23.0	146	20.1	133	20.3	37	25.5
70-79	777	20.8	239	33.0	213	32.5	41	28.3
80+	675	18.0	221	30.5	197	30.0	25	17.2
Sex (male)	1713	45.8	256	35.3	232	35.4	56	38.6
Calendar year of perforated diverticular disease diagnosis								
2005-2008	1556	41.6	328	45.2	294	44.8	60	41.4
2009-2013	2187	58.4	397	54.8	362	55.2	85	58.6
Comorbidities								
Diverticular disease of the colon without perforation	897	24.0	173	23.9	151	23.0	36	24.8
Hypertension	844	22.5	263	36.3	247	37.7	39	26.9
COPD, chronic bronchitis, emphysema and asthma	682	18.2	334	46.1	278	42.4	137	94.5
Liver disease and/or chronic pancreatitis	111	3.0	25	3.4	24	3.7	5	3.4
Alcoholism-related diseases other than those affecting the liver or pancreas	310	8.3	42	5.8	38	5.8	13	9.0
Cancer	503	13.4	201	27.7	189	28.8	19	13.1
Rheumatoid arthritis / connective tissue disease	138	3.7	200	27.6	186	28.4	4	2.8
Inflammatory bowel disease	71	1.9	40	5.5	32	4.9	4	2.8
Charlson Comorbidity Index score								
0	2659	71.0	371	51.2	334	50.9	99	68.3
1-2	888	23.7	261	36.0	241	36.7	36	24.8
≥3	196	5.2	93	12.8	81	12.3	10	6.9
Surgery within 30 days before or after diagnosis of diverticulitis (total)	1949	52.1	456	62.9	425	64.8	82	56.6
Explorative surgery including lavage	616	16.5	115	15.9	108	16.5	24	16.6
Stoma	845	22.6	245	33.8	228	34.8	38	26.2
Resection with primary anastomosis	488	13.0	96	13.2	89	13.6	20	13.8

COPD=chronic obstructive pulmonary disease, N=number of patients.

Table 2: Mortality among patients with systemic corticosteroid use, regardless of use of intestinal acting or inhaled corticosteroids compared with non-use.

Corticosteroid use	Period	Absolute risk (95% CI)	Rate per 1000 PYRs (95% CI)	Crude HR (95% CI)	Adjusted HR* (95% CI)
Non-use	7 days	4.4 (3.8 to 5.1)	6.46 (5.47 to 7.44)		
	30 days	8.8 (7.9 to 9.7)	3.12 (2.78 to 3.45)		
	90 days	11.9 (10.9 to 12.9)	1.46 (1.33 to 1.60)		
	1 year	15.6 (14.4 to 16.8)	0.51 (0.47 to 0.55)		
Current use	7 days	14.2 (11.4 to 17.6)	21.81 (16.70 to 26.92)	3.38 (2.56 to 4.47)	2.10 (1.52 to 2.90)
	30 days	27.9 (24.2 to 32.1)	11.53 (9.60 to 13.45)	3.56 (2.92 to 4.35)	1.96 (1.56 to 2.47)
	90 days	36.6 (32.5 to 41.0)	5.71 (4.87 to 6.54)	3.59 (3.02 to 4.27)	1.97 (1.62 to 2.41)
	1 year	47.6 (43.2 to 52.1)	2.22 (1.93 to 2.50)	3.75 (3.22 to 4.38)	2.05 (1.72 to 2.45)
New use	7 days	15.7 (10.3 to 23.5)	24.20 (13.32 to 35.09)	3.75 (2.33 to 6.03)	2.88 (1.77 to 4.70)
	30 days	31.4 (23.9 to 40.5)	13.46 (9.18 to 17.73)	4.09 (2.92 to 5.73)	2.72 (1.92 to 3.85)
	90 days	46.3 (37.9 to 55.6)	7.87 (5.81 to 9.93)	4.75 (3.60 to 6.28)	3.06 (2.29 to 4.09)
	1 year	52.5 (43.9 to 61.7)	2.83 (2.13 to 3.53)	4.43 (3.41 to 5.75)	2.89 (2.21 to 3.79)
Chronic use	7 days	13.7 (10.6 to 17.6)	21.04 (15.27 to 26.81)	3.26 (2.38 to 4.46)	1.89 (1.30 to 2.74)
	30 days	26.8 (22.6 to 31.6)	10.93 (8.79 to 13.07)	3.40 (2.71 to 4.25)	1.68 (1.29 to 2.19)
	90 days	33.4 (28.8 to 38.4)	5.08 (4.18 to 5.97)	3.23 (2.65 to 3.94)	1.58 (1.25 to 2.00)
	1 year	45.9 (40.9 to 51.2)	2.05 (1.74 to 2.36)	3.54 (2.98 to 4.21)	1.74 (1.42 to 2.14)
Recent use	7 days	6.1 (3.6 to 10.0)	8.97 (4.27 to 13.67)	1.39 (0.81 to 2.40)	1.11 (0.63 to 1.95)
	30 days	13.9 (10.0 to 19.1)	5.12 (3.34 to 6.89)	1.63 (1.13 to 2.34)	1.15 (0.79 to 1.68)
	90 days	19.2 (14.7 to 24.9)	2.53 (1.78 to 3.28)	1.68 (1.24 to 2.30)	1.17 (0.84 to 1.61)
	1 year	26.3 (21.0 to 32.6)	0.95 (0.71 to 1.20)	1.78 (1.36 to 2.33)	1.23 (0.93 to 1.63)

*HR adjusted for age, sex, use of cardiovascular medication, hypertension, COPD, liver disease or chronic pancreatitis, alcoholism-related diseases other than those affecting the liver or pancreas, inflammatory bowel disease, rheumatoid arthritis, connective tissue disease, malignancies and modified Charlson Comorbidity Index score.
CI=confidence interval, COPD=chronic obstructive pulmonary disease, HR=hazard ratio, PYR=person year.

Table 3: Mortality among patients with current use of systemic corticosteroids, regardless of other corticosteroid use, according to 1-year cumulative dose compared with non-use.

Cumulative dose of corticosteroids*	Period	Absolute risk (95% CI)	Rate per 1000 PYRs (95% CI)	Crude HR (95% CI)	Adjusted HR† (95% CI)
Non-use	7 days	4.4 (3.8 to 5.1)	6.46 (5.47 to 7.44)		
	30 days	8.8 (7.9 to 9.7)	3.12 (2.78 to 3.45)		
	90 days	11.9 (10.9 to 12.9)	1.46 (1.33 to 1.60)		
	1 year	15.6 (14.4 to 16.8)	0.51 (0.47 to 0.55)		
≤625 mg	7 days	15.3 (10.1 to 23.0)	23.54 (12.96 to 34.13)	3.65 (2.27 to 5.87)	2.38 (1.44 to 3.92)
	30 days	26.6 (19.7 to 35.3)	10.95 (7.22 to 14.69)	3.38 (2.37 to 4.84)	1.92 (1.32 to 2.79)
	90 days	37.9 (30.0 to 47.1)	5.93 (4.24 to 7.63)	3.73 (2.76 to 5.04)	2.10 (1.53 to 2.87)
	1 year	40.5 (32.4 to 49.7)	1.80 (1.30 to 2.30)	3.18 (2.38 to 4.24)	1.81 (1.34 to 2.45)
>625 to 2000 mg	7 days	13.2 (8.6 to 19.9)	20.36 (11.21 to 29.52)	3.15 (1.96 to 5.06)	1.76 (1.06 to 2.94)
	30 days	31.2 (24.4 to 39.5)	13.15 (9.31 to 16.99)	4.03 (2.95 to 5.50)	2.08 (1.48 to 2.93)
	90 days	39.6 (32.1 to 48.1)	6.41 (4.75 to 8.07)	3.97 (3.01 to 5.23)	2.08 (1.48 to 2.93)
	1 year	53.4 (45.4 to 61.8)	2.68 (2.08 to 3.28)	4.36 (3.43 to 5.54)	2.24 (1.72 to 2.91)
>2000 to 3500 mg	7 days	16.4 (11.2 to 23.7)	25.67 (15.18 to 36.16)	3.98 (2.57 to 6.16)	2.74 (1.62 to 4.64)
	30 days	30.0 (23.2 to 38.4)	12.46 (8.69 to 16.23)	3.87 (2.81 to 5.33)	2.24 (1.52 to 3.30)
	90 days	38.2 (30.6 to 46.8)	6.12 (4.47 to 7.76)	3.80 (2.85 to 5.05)	2.17 (1.54 to 3.06)
	1 year	48.9 (40.9 to 57.6)	2.37 (1.80 to 2.94)	3.92 (3.05 to 5.06)	2.18 (1.61 to 2.97)
>3500 mg	7 days	10.5 (5.6 to 19.1)	15.71 (5.45 to 25.97)	2.44 (1.24 to 4.76)	1.55 (0.75 to 3.24)
	30 days	20.9 (13.8 to 31.2)	8.30 (4.47 to 12.14)	2.59 (1.61 to 4.16)	1.31 (0.78 to 2.19)
	90 days	26.8 (18.7 to 37.6)	3.80 (2.25 to 5.35)	2.50 (1.64 to 3.80)	1.21 (0.77 to 1.90)
	1 year	46.2 (36.1 to 57.7)	1.91 (1.30 to 2.52)	3.33 (2.40 to 4.63)	1.59 (1.11 to 2.29)

*Cumulative dose defined as the 1-year cumulative dose of systemic corticosteroids among current users, calculated as prednisone dose equivalents.
†HR adjusted for age, sex, use of cardiovascular medication, hypertension, COPD, liver disease or chronic pancreatitis, alcoholism-related diseases other than those affecting the liver or pancreas, inflammatory bowel disease, rheumatoid arthritis, connective tissue disease, malignancies and modified Charlson Comorbidity Index score.
CI=confidence interval, COPD=chronic obstructive pulmonary disease, HR=hazard ratio, PYR=person year.

When we excluded patients with malignancies, inflammatory bowel disease, a previous code for diverticular disease, or rheumatic diseases including mixed connective tissue disease, the results of the analysis of current users of systemic corticosteroids regardless of use of other corticosteroids did not materially change for mortality risk after 7 days, 30 days, 90 days or 1 year.

Discussion

We examined mortality risks between corticosteroid users and non-users among patients with perforated diverticular disease. One-year mortality risk for current users of corticosteroids was 47.6%, double that of non-users after adjustment for confounders. One-year mortality risk was even higher for new corticosteroid users: 52.5%. Thus, corticosteroid use was clearly associated with an increased mortality risk for patients with perforated diverticular disease, continuing until at least 1 year after diagnosis, regardless of corticosteroid dose.

This is the first large cohort study comparing mortality risks between corticosteroid users and non-users among patients with perforated diverticular disease. The advantage of the cohort approach is the ability to calculate absolute mortality risks, which can inform clinical practice. Our study showed that corticosteroid use is a strong indicator for 1-year mortality. An earlier study of patients with perforated peptic ulcer disease reported a 30-day mortality ratio of 2.1 after corticosteroid use (27). This is in line with the increased mortality risk (adjusted HR of 1.96 for 30-day mortality) that we found among current users of systemic corticosteroids in the present study.

The following study limitations need to be taken into account in interpreting our results. Determination of exposure was based on redeemed prescriptions, which may not always coincide completely with medication taken by patients (28). We also did not consider use of corticosteroids for longer than a year before hospital admission. Also, since prednisolone and prednisone were the most frequently used systemic steroids (89%), the results in first line apply to this patient category; whether our results also apply to patients using steroids with high first pass metabolism needs further study. Finally, perforated diverticular disease may be misclassified (6, 17), as the sensitivity of relevant ICD codes is not 100% accurate. Since misclassification is probably more likely in corticosteroid users (due to blinding of symptoms and severity of the condition), mortality risk is potentially overestimated if less severe cases in corticosteroid users go undetected (25). However, in analyses stratified by type of surgery, which most likely harmonizes the diagnostic category, a similar increased risk was found.

Table 4: Mortality among current corticosteroid users: subgroup and sensitivity analyses compared with non-use.

Restrictions	Period	Absolute risk (95% CI)	Rate per 1000 PYRs (95% CI)	Crude HR (95% CI)	Adjusted HR* (95% CI)
Non-use	7 days	4.4 (3.8 to 5.1)	6.46 (5.47 to 7.44)		
	30 days	8.8 (7.9 to 9.7)	3.12 (2.78 to 3.45)		
	90 days	11.9 (10.9 to 12.9)	1.46 (1.33 to 1.60)		
	1 year	15.6 (14.4 to 16.8)	0.51 (0.47 to 0.55)		
Inhaled only	7 days	6.2 (2.6 to 14.2)	9.16 (1.13 to 17.18)	1.42 (0.58 to 3.45)	1.55 (0.60 to 3.97)
	30 days	17.3 (10.6 to 27.4)	6.46 (3.08 to 9.84)	2.04 (1.20 to 3.49)	1.85 (1.04 to 3.27)
	90 days	21.0 (13.6 to 31.6)	2.83 (1.48 to 4.17)	1.87 (1.15 to 3.04)	1.72 (1.03 to 2.89)
	1 year	22.3 (14.7 to 33.0)	0.79 (0.43 to 1.16)	1.54 (0.96 to 2.46)	1.44 (0.88 to 2.37)
Systemic use only	7 days	13.9 (11.0 to 17.5)	21.30 (16.00 to 26.60)	3.30 (2.47 to 4.42)	2.02 (1.44 to 2.82)
	30 days	28.0 (24.1 to 32.5)	11.53 (9.51 to 13.55)	3.56 (2.90 to 4.38)	1.96 (1.55 to 2.48)
	90 days	36.0 (31.7 to 40.7)	5.60 (4.73 to 6.47)	3.52 (2.94 to 4.22)	1.92 (1.56 to 2.36)
	1 year	48.0 (43.4 to 52.8)	2.23 (1.93 to 2.53)	3.77 (3.22 to 4.42)	2.04 (1.70 to 2.45)
Systemic use, regardless of other uses	7 days	14.2 (11.4 to 17.6)	21.81 (16.70 to 26.92)	3.38 (2.56 to 4.47)	2.10 (1.52 to 2.90)
	30 days	27.9 (24.2 to 32.1)	11.53 (9.60 to 13.45)	3.56 (2.92 to 4.35)	1.96 (1.56 to 2.47)
	90 days	36.6 (32.5 to 41.0)	5.71 (4.87 to 6.54)	3.59 (3.02 to 4.27)	1.97 (1.62 to 2.41)
	1 year	47.6 (43.2 to 52.1)	2.22 (1.93 to 2.50)	3.75 (3.22 to 4.38)	2.05 (1.72 to 2.45)
Excluding patients with malignancies	7 days	14.3 (11.1 to 18.4)	21.94 (15.92 to 27.97)	4.03 (2.91 to 5.59)	2.22 (1.50 to 3.28)
	30 days	26.3 (22.1 to 31.2)	10.80 (8.62 to 12.99)	4.04 (3.18 to 5.13)	2.00 (1.50 to 2.67)
	90 days	32.9 (28.3 to 38.0)	5.00 (4.10 to 5.91)	3.87 (3.13 to 4.78)	1.93 (1.50 to 2.48)
	1 year	42.3 (37.3 to 47.7)	1.85 (1.55 to 2.15)	3.93 (3.26 to 4.74)	2.03 (1.62 to 2.55)
Excluding patients with inflammatory bowel disease	7 days	14.3 (11.4 to 17.8)	21.91 (16.63 to 27.20)	3.35 (2.52 to 4.46)	2.03 (1.46 to 2.83)
	30 days	28.1 (24.2 to 32.4)	11.58 (9.59 to 13.57)	3.54 (2.89 to 4.34)	1.89 (1.49 to 2.40)
	90 days	36.6 (32.4 to 41.2)	5.72 (4.86 to 6.59)	3.55 (2.97 to 4.24)	1.87 (1.52 to 2.29)
	1 year	48.1 (43.6 to 52.8)	2.25 (1.95 to 2.54)	3.76 (3.21 to 4.39)	1.96 (1.64 to 2.36)
Excluding patients with a previous code for diverticular disease	7 days	15.7 (12.4 to 19.7)	24.47 (18.28 to 30.66)	3.43 (2.53 to 4.65)	2.06 (1.45 to 2.93)
	30 days	29.8 (25.5 to 34.6)	12.57 (10.26 to 14.88)	3.66 (2.94 to 4.56)	2.03 (1.58 to 2.63)
	90 days	38.5 (33.8 to 43.6)	6.15 (5.16 to 7.15)	3.67 (3.03 to 4.45)	2.01 (1.61 to 2.51)
	1 year	49.3 (44.4 to 54.5)	2.35 (2.01 to 2.68)	3.80 (3.20 to 4.51)	2.07 (1.70 to 2.53)
Excluding patients with rheumatic diseases including mixed connective tissue disease	7 days	15.3 (11.8 to 19.6)	23.73 (17.22 to 30.25)	3.72 (2.71 to 5.10)	2.13 (1.51 to 3.01)
	30 days	28.5 (23.9 to 33.6)	11.87 (9.48 to 14.25)	3.71 (2.95 to 4.67)	1.95 (1.52 to 2.51)
	90 days	37.8 (32.9 to 43.3)	5.98 (4.93 to 7.02)	3.79 (3.10 to 4.62)	1.95 (1.57 to 2.42)
	1 year	48.4 (43.1 to 54.0)	2.31 (1.95 to 2.67)	3.91 (3.28 to 4.67)	2.06 (1.70 to 2.49)

*HR adjusted for age, sex, use of cardiovascular medications, hypertension, COPD, liver disease or chronic pancreatitis, alcoholism-related diseases other than those affecting the liver or pancreas, inflammatory bowel disease, rheumatoid arthritis, connective tissue disease, malignancies and modified Charlson Comorbidity Index score.

CI=confidence interval, COPD=chronic obstructive pulmonary disease, HR=hazard ratio, PYR=person year.

The central causal question is whether increased mortality risk in corticosteroid users is actually caused by corticosteroid use prior to diagnosis. Clinical arguments underlying the rationale for a causal association are masked disease severity (17), impaired wound healing and increased infection risk (18), increased risk of venous thromboembolism (20) and unrecognized adrenal insufficiency (29). The risk was doubled for corticosteroid users in our fully adjusted statistical model, but residual confounding (no perfectly valid data on actual disease severity status) might still be an issue. For instance, high mortality among patients using corticosteroids may be explained partially by the underlying diseases for which corticosteroids were prescribed. However, our subgroup analyses showed no clear differences when high-risk subgroups were excluded. Regardless of the causal question, our study indicated that corticosteroid use was an important prognostic factor for mortality.

Two explanations are possible for the lack of an association observed between mortality risk and dose. First, the risk may be associated with corticosteroid use per se, so that the maximum increase in risk is already reached at a low (but still supraphysiological) dose. Second, our classification did not distinguish between short-term, high-dose corticosteroid use and long-term, low-dose use, because the prescription database used does not provide certainty whether a drug is prescribed in high doses for short-term use, or in lower doses for longer duration. Although clinically short-term, high-dose corticosteroid use and long-term, low-dose corticosteroid use are very heterogeneous groups of patients, in our analysis according to dose, both translate into the same 1-year cumulative dose.

In conclusion, this study demonstrated that corticosteroid use was associated with increased mortality risk after diagnosis of perforated diverticular disease. Clinically, use of systemic corticosteroids should be regarded as an important risk marker for mortality among patients with this disease.

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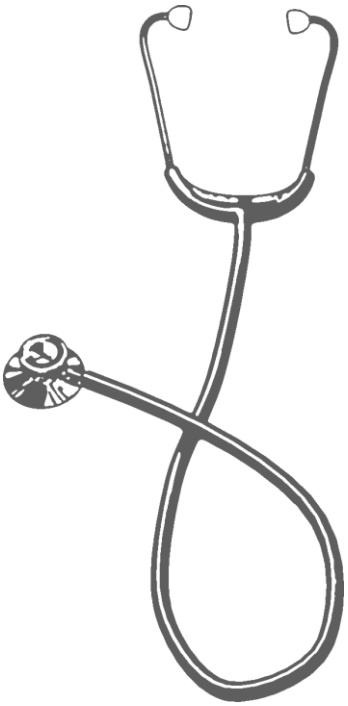
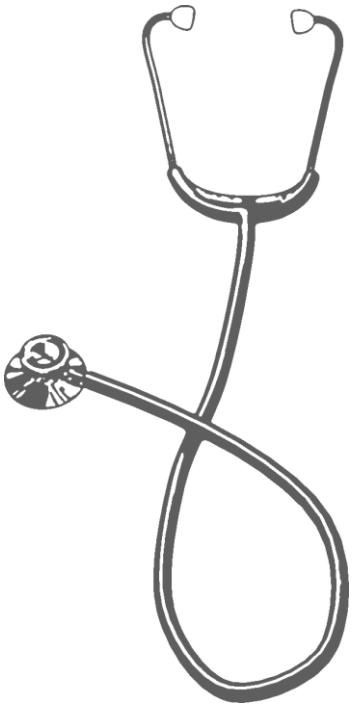
Part II

Treatment outcome in Cushing's syndrome



Chapter 4

Microscopic versus endoscopic transsphenoidal surgery in the Leiden cohort treated for Cushing's disease: surgical outcome, mortality, and complications



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Abstract

Background
First-choice treatment for Cushing's disease is transsphenoidal adenomectomy. Since its introduction in the 1970s, many centers have now switched from microscopic to endoscopic surgery. We compared both techniques for the treatment of Cushing's disease at the Leiden University Medical Center, a European reference center for pituitary diseases.

Methods
Cohort study with inclusion and follow-up of consecutive patients with Cushing's disease primarily treated by transsphenoidal surgery at the Leiden University Medical Center between 1978 and 2016. We compared remission rates (primary endpoint), mortality, and complications between microscopic (performed up to 2005) and endoscopic (performed from 2003 onwards) surgery. Subgroup analyses were performed by tumor size, surgical experience, and preoperative imaging techniques. Additionally, surgeons' intraoperative findings regarding presence and removal of the adenoma were related to surgical outcome.

Results
Of 137 included patients, 87 were treated microscopically and 50 endoscopically. Three months after microscopic surgery, 74 patients (86%) were in remission. Five-year recurrence-free survival was 89% (95% confidence interval [CI]: 82%-96%), and ten-year recurrence free survival was 84% (95% CI: 75%-93%). After endoscopic surgery, 39 patients (83%) were in remission. Both five-year and ten-year recurrence-free survival were 71% (95% CI: 55%-87%). Hazard ratio for recurrence was 0.47 (95% CI: 0.19-1.14), and for mortality 2.79 (95% CI: 0.35-22.51), for microscopic versus endoscopic surgery. No learning curve was found for endoscopy, nor an influence of preoperative imaging technique for microscopy. In addition, we did not find a clear relation between the surgeons' intraoperative findings and surgical outcomes.

Conclusions
This study did not identify a clear advantage of microscopic or endoscopic transsphenoidal surgery for the treatment of Cushing's disease based on clinical outcome. The transition to endoscopic surgery at our center was not accompanied by transient worsening of outcomes, which may be reassuring for those considering transitioning.

Introduction

Cushing's disease is caused by an adrenocorticotropic hormone (ACTH)-secreting pituitary adenoma, resulting in endogenous glucocorticoid excess. The incidence is estimated to be 1.2-1.7 per million each year (1). Glucocorticoid excess causes osteoporosis, central obesity, insulin resistance, dyslipidemia, hypertension, hypercoagulability, and neuropsychiatric disorders (2, 3). First-choice treatment remains transsphenoidal pituitary surgery, selectively removing the corticotroph adenoma (4, 5). Despite biochemical cure, mortality risk remains increased in patients with Cushing's disease (6).

Two main techniques have been used for transsphenoidal pituitary surgery: microscopic and endoscopic surgery. The endoscope has also been used to confirm findings during microscopic surgery, which is called endoscopy assisted surgery. Microscopic surgery used to be the established method for transsphenoidal surgery. However, since 1992, when the endoscope was first used as a tool to assist in microscopic surgery for a pituitary adenoma (7), many centers have switched to a full endoscopic technique, which was first described in 1997 for treatment of Cushing's disease (8). However, proper evaluation of outcomes in a single center has yet to be performed. A limited number of small cohort studies (13-25 patients per study group) have compared both the microscopic and endoscopic techniques for treatment of Cushing's disease performed in the same center (9-12). None showed a significant difference in remission rate or surgical morbidity between microscopic and (transition to) endoscopic surgery. Additionally, significant differences in remission rates based on tumor size could not be determined (9). Several systematic reviews concerning transsphenoidal microscopic versus endoscopic surgery have evaluated outcomes for all pituitary adenomas and found comparable results for remission and recurrence rates, and inconclusive results regarding complication rates (13-15). Theoretically, endoscopic surgery has the advantage of achieving better tumor visualization, mainly in laterally invasive or large tumors. This increased visibility enables improved resectability, leading to potentially higher remission rates. However, these advantages have also led to a higher risk of cerebrospinal fluid (CSF) leakage, most likely due to a more aggressive surgical approach in an attempt to obtain complete adenoma removal (16). Several studies have reported on a learning curve for the endoscopic technique (17-20), showing that outcomes improve with increased surgical experience, which possibly leads to biased results in series when comparing between years of experience in the microscopic surgical technique and the newer endoscopic technique.

Due to the lack of comparability of available studies, we present our long-term data on transsphenoidal surgery for Cushing's disease, reporting on both surgical outcome,

with as primary endpoint remission rate, as well as complication rates for both techniques. To date, there are unresolved questions regarding long-term remission rates and surgical morbidity after both treatments, overall as well as in relation to tumor size. Furthermore, differences in long-term mortality, endocrinological complications, and morbidity between the two surgical techniques need to be explored to be able to properly evaluate both treatment options. Finally, in order to establish the predictive value of intraoperative findings, it is important to relate the surgeons' intraoperative findings regarding presence and removal of the adenoma to surgical outcome.

Study aims

The primary study aim was to compare microscopic and endoscopic transsphenoidal surgery for Cushing's disease regarding: 1) Surgical outcome (remission rate = primary endpoint, recurrence, and hydrocortisone dependency), 2) Long-term mortality risk, and 3) Short- and long-term surgical and endocrinological complications. Secondary study aims were to stratify these results based on 1) Tumor size and 2) Surgical experience for endoscopy, and 3) To relate surgeons' intraoperative findings regarding presence and removal of the adenoma to surgical outcome.

Methods

Study population

Consecutive patients with Cushing's disease primarily treated with transsphenoidal surgery at the tertiary referral center Leiden University Medical Center, the coordinating center of the newly established European Reference Network for rare Endocrine conditions, and a European reference center for pituitary diseases, were included in this cohort study. Inclusion started in 1978, when the first transsphenoidal operation in the Netherlands was performed in Leiden. Microscopic surgery was performed from 1978 to 2005, whereas endoscopic surgery has been performed since 2003. Patients were included if surgery was performed before January 1st 2017. Patients were excluded if they had undergone a unilateral adrenalectomy and/or pituitary irradiation prior to transsphenoidal surgery. There were no restrictions in presurgical medical treatment with cortisol lowering agents or in adjuvant therapy in case of persistent disease or for recurrence of disease.

The diagnosis of Cushing's disease was made based both on clinical grounds and biochemical tests: increased morning serum cortisol (>500 nmol/L), increased 24-h urinary free cortisol (UFC) excretion (>220 nmol until 2010, after which the cut-off level was >150 nmol), increased midnight salivary cortisol excretion (>5.7 nmol/L, measured since 2004), insufficient suppression of morning serum cortisol after low-

dose dexamethasone (1 mg in the evening), as well as a non-suppressed ACTH. Until 1988, urinary 17-KgS was measured in some patients instead of UFC (increased if >16 mg/24h for women and >22 mg/24h for men). All patients underwent pituitary imaging either by computed tomography (CT, until 1994, and one patient in 2009 due to a contraindication for magnetic resonance imaging [MRI]), or MRI. Three patients who were operated before 1980 underwent imaging by sellar planigraphy, with or without basal cisternography (X-ray). If pituitary imaging yielded inconclusive results, bilateral simultaneous sampling of the inferior petrosal sinuses (IPSS) was performed. Subsequently, pituitary surgery with exploration of the sella was performed when the results of IPSS were consistent with a pituitary source of ACTH overproduction. Otherwise, imaging studies (CT of the thorax and octreotide or gallium DOTATATE PET scan) were performed to identify an ectopic source of ACTH overproduction. In case no ectopic ACTH-producing tumor was found, diagnostic tests were repeated after a period of watchful waiting, and surgical treatment was performed only after imaging or IPSS indicated a pituitary adenoma.

Interventions and postoperative evaluation

The primary treatment was either microscopic or endoscopic transsphenoidal pituitary adenomectomy for all included patients. A direct transnasal transsphenoidal microscopic approach was performed solely until 2002. From 2003 onwards, the endoscopic procedure was introduced in our center for the surgical treatment of pituitary tumors, including Cushing's disease. At first, an endoscopic assisted approach was used to confirm findings of the microscope. In this study, endoscopic assisted surgery was analysed in the study group of microscopic surgery, because the surgery was primarily performed using the microscope, which was therefore the main technique to be considered for treatment success and complication rates. From 2006 onwards, purely endoscopic transnasal transsphenoidal approaches were the only surgical treatment modality. All surgical procedures were performed by three dedicated pituitary neurosurgeons. Postoperative biochemical evaluation was always performed within two weeks. Three to six months postoperatively, remission was defined based on both clinical criteria (dependency on hydrocortisone replacement, or hydrocortisone independency without any biochemical signs of hypercortisolism and regression of clinical signs) and biochemical criteria (morning cortisol suppression after 1 mg dexamethasone to below 50 nmol/L and normal 24-h UFC excretion or midnight salivary cortisol excretion in two consecutive samples, if not on hydrocortisone replacement) (21). Persistent Cushing's disease was defined as the absence of remission after first surgery. Disease recurrence was defined as biochemical recurrence according to the aforementioned criteria, and re-occurrence of clinical signs, after a period of remission of at least three months.

Endpoints and follow-up

Study endpoints were surgical outcomes, mortality, and short- and long-term morbidity. Surgical outcomes considered were the following: 1) Remission rate (primary endpoint), 2) Persistent disease, 3) Recurrent disease, and 4) Hydrocortisone dependency three months after surgery (divided in three categories: a) Absolute deficiency [insufficient cortisol response to corticotropin-releasing hormone (CRH)-test or equivalent], b) Hydrocortisone to treat withdrawal symptoms despite normal cortisol response to CRH stimulation, and c) Pragmatic hydrocortisone replacement without stimulation test).

Short-term morbidity (≤3 month after first surgery): 1) Postoperative CSF-leakage, 2) Bacterial meningitis (or start of antibiotic treatment due to suspicion of meningitis), 3) All intra- and postoperative bleedings (considered were internal carotid artery injury, epistaxis, severe venous blood loss), 4) Severe bleeding (requiring surgical intervention or described as severe in patient file), 5) Syndrome of inappropriate antidiuretic hormone secretion (Na <135 mmol/L), 6) Diabetes insipidus (transient, requiring medication at least once), 7) Anterior pituitary deficiency other than ACTH requiring medication, 8) Corticosteroid withdrawal syndrome (complaints and/or requirement of increase in hydrocortisone replacement dose) (22), and 9) Cardiovascular morbidity (thrombosis, pulmonary embolism, cerebrovascular accident, transient ischemic attack, and myocardial ischemia).

Long-term morbidity (>3 months after first surgery): 1) Anterior pituitary deficiency other than ACTH requiring medication (measured one year after surgery), 2) Hydrocortisone dependency for >3 years (for patients with >3 years follow-up), 3) Diabetes insipidus (permanent: >3 months), 4) Cardiovascular morbidity, 5) Hypertension (de novo as well as persisting after surgery), 6) Diabetes mellitus (de novo as well as persisting after surgery), and 7) Neuropsychiatric morbidity (complaints/symptoms as well as consultation of psychologist or psychiatrist).

Follow-up was defined as time between date of surgery and death, loss to follow-up, or December 31st 2016, whichever came first. For time-to-event analyses, follow-up was defined as time between date of surgery and outcome event (mortality or recurrence), death, loss to follow-up, or December 31st 2016, whichever came first. Presurgical information was collected regarding diagnosis (including Cushing's syndrome severity index score [CSI score] (23)), comorbidities (hypertension, diabetes mellitus, dyslipidemia), and medical treatment prior to surgery. In case of loss to follow-up, data collection was completed to our best ability by contacting both patient and current health care provider.

Tumor characteristics: tumor size on preoperative imaging divided tumors into microadenomas (≤10 mm) and macroadenomas (>10 mm). Giant adenomas (>3 cm) were not analysed separately. Intraoperative findings were classified into three categories: complete adenomectomy (surgeon reported complete removal of the adenoma), (at least) partial adenomectomy (surgeon reported partial removal of the adenoma, but is uncertain whether there is residual tumor), and residual tumor (surgeon is certain there is residual tumor).

Risk of bias

Consecutive patients were included in this study to minimize selection bias, although selection could still be a cause of bias in our data. Patients were assigned to a treatment method based on the availability of the treatment method in our center at time of inclusion, although this does not guarantee comparability of both study groups, e.g. due to time trends. To assess potential differences through time, we plotted percentage macroadenomas per period of five calendar years. Selective loss to follow-up could have led to selection bias (24), although this was minimized by our attempts at complete data collection, reducing the number of patients lost to follow-up by 21. Selective referral of patients from other centers, mainly patients with (difficult and/or invasive) macroadenomas, could potentially have led to selection bias if referral was different for microscopy and endoscopy. Interventions were unlikely to be misclassified. Confounding is a potential source of bias and was assessed by comparing baseline patient characteristics between the study groups. Furthermore, follow-up time was assessed as a potential source of bias.

Statistical analysis

To describe homogeneity between the surgical groups as well as between microadenomas and macroadenomas, various parameters were analysed by bivariate test. Contingency tables were presented, comparing characteristics of microscopic and endoscopic surgery. A table was prepared with short- and long-term morbidity according to tumor size, comparing microscopic to endoscopic surgery. All variables in Tables 1 and 2 were compared between the two surgical techniques for the entire cohort as well as for microadenomas and macroadenomas separately as this is considered an effect modifier. We used the unpaired T-test for continuous outcomes, and the two-sample test of proportions for categorical variables (in case of >2 categories, we performed this test separately per category to calculate the difference between the groups). Additionally, the Chi-squared test was used to calculate one p-value for categorical variables with >2 categories. If data were missing for ≥5% of patients per parameter, this was marked in the tables. An “as treated” analysis was performed, excluding patients with missing data for confounding variables from the adjusted analyses.

Table 1: Demographic characteristics.

	Microscopic trans-sphenoidal adeno-mectomy		Endoscopic trans-sphenoidal adeno-mectomy		Tested difference (95% CI; p-value)	Tested difference (95% CI; p-value) (Microadenoma only)	Tested difference (95% CI; p-value) (Macroadenoma only)
	N	**%**	**N**	**%**			
Total	87	100.0	50	100.0		Sample size: 103	Sample size: 34
*Age at diagnosis, years**	38.9	15.4	44.4	15.1	5.5 (0.1 to 10.9; p=0.045)	3.4 (-2.6 to 9.3; p=0.263)	9.0 (-3.5 to 21.4; p=0.152)
Sex (female)	69	79.3	34	68.0	11.3 (-4.2 to 26.8; p=0.140)	7.7 (-10.4 to 25.8; p=0.391)	20.8 (-8.5 to 50.1; p=0.170)
Calendar year of transsphenoidal surgery°	1992	1978-2005	2011	2003-2016	19.2 (17.1 to 21.2; p=0.000)	20.4 (18.1 to 22.6; p=0.000)	14.7 (10.1 to 19.3; p=0.000)
Duration of follow-up (years)$	16.9	11.7-26.7	4.7	2.0-7.4	13.4 (11.0 to 15.8; p=0.000)	14.0 (11.2 to 17.7; p=0.000)	11.3 (6.9 to 15.6; p=0.000)
Comorbidities at diagnosis							
Hypertension	64	73.6	38	76.0	2.4 (-12.6 to 17.4; p=0.756)	3.3 (-15.2 to 21.8; p=0.723)	15.3 (-11.0 to 41.6; p=0.271)
Diabetes mellitus	14	16.1	15	30.0	13.9 (-1.0 to 28.8; p=0.055)	13.5 (-4.1 to 31.1; p=0.110)	14.5 (-14.0 to 43.0; p=0.320)
Dyslipidemia	8	9.1	8	16.0	6.9 (-4.9 to 18.7; p=0.225)	3.0 (-8.0 to 14.0; p=0.569)	9.0 (-20.7 to 38.7; p=0.553)
*Cushing's syndrome Severity Index score**	6.9	2.3	6.8	2.8	-0.10 (-0.98 to 0.79; p=0.832)	0.09 (-1.00 to 1.17; p=0.877)	-0.72 (-2.31 to 0.86; p=0.357)
0-4	13	15.1	10	20.0			
5-8	53	61.6	27	54.0			
9-16	20	23.2	13	26.0			
Tumor size					11.3	-	-
Microadenoma	69	79.3	34	68.0	(-4.2 to 26.8;		
Macroadenoma	18	20.7	16	32.0	p=0.140)		
Cavernous sinus invasion	5	27.8	8	50.0			
Referral by other neurosurgeon or university medical center	4	4.6	6	12.0	7.4 (-2.6 to 17.4; p=0.109)	3.0 (-5.9 to 11.9; p=0.459)	13.9 (-11.8 to 39.6; p=0.389)
Medical treatment prior to surgery	57	65.5	47	94.0	28.5 (16.5 to 40.5; p=0.000)	34.8 (23.6 to 46.0; p=0.000)	14.6 (-14.4 to 43.6; p=0.335)
Metyrapone	8	9.2	16	32.0			
Ketoconazole	51	58.6	32	64.0			
Pasireotide	0	0.0	2	4.0			

*mean + standard deviation, °mean + range, $median + interquartile range; CI=confidence interval

Table 2: Diagnostic strategy and results.

	Microscopic trans-sphenoidal adeno-mectomy		Endoscopic trans-sphenoidal adeno-mectomy		Tested difference (95% CI; p-value)	Tested difference (95% CI; p-value) (Microadenoma only)	Tested difference (95% CI; p-value) (Macroadenoma only)
Inferior petrosal sinus sampling	30	34.5	13	26.0	8.5 (-7.2 to 24.2; p=0.302)	2.4 (-17.6 to 22.4; p=0.815)	^
Radiologic imaging					p=0.000	p=0.000	p=0.003
CT or X-ray	27	31.0	1	2.0	29.0 (18.5 to 39.5)	31.9 (19.3 to 44.5)	16.7 (-0.5 to 33.9)
MRI 1.5 Tesla	60	69.0	18	36.0	33.0 (16.5 to 49.5)	38.7 (20.1 to 57.3)	27.0 (-2.8 to 56.8)
MRI 3 Tesla	0	0.0	31	62.0	62.0 (48.5 to 75.4)	70.6 (55.3 to 85.9)	43.8 (19.5 to 68.1)
Radiology results					p=0.144	p=0.359	p=0.339
Adenoma	56	64.4	40	80.0	15.6 (0.6 to 30.6)	14.1 (-5.2 to 33.4)	5.6 (-5.0 to 16.2)
No adenoma	24	27.6	7	14.0	13.6 (1.6 to 27.0)	12.7 (-4.9 to 30.3)	5.6 (-5.0 to 16.2)
Inconclusive	7	8.0	3	6.0	2.0 (-6.7 to 10.7)	1.3 (-10.6 to 13.2)	#
Histology results					p=0.784	p=0.940	p=0.339
Adenoma	67	77.9	41	82.0	4.1 (-9.7 to 17.9)	0.0 (-18.2 to 18.2)	5.6 (-5.0 to 16.2)
No adenoma	16	18.6	7	14.0	4.6 (-8.1 to 17.3)	1.5 (-15.3 to 183)	5.6 (-5.0 to 16.2)
Inconclusive	3	3.5	2	4.0	0.5 (-6.2 to 7.2)	1.5 (-7.8 to 10.8)	#
Immunohisto-chemistry results: ACTH-positive	57	66.3	45	90.0	23.7 (10.7 to 36.7; p=0.002)	23.5 (6.9 to 40.1; p=0.015)	16.7 (-0.5 to 33.9; p=0.087)
Surgeon's intraoperative findings					p=0.123	p=0.231	p=0.447
Complete adenomectomy	48$	58.5	25	50.0	8.5 (-9.0 to 26.0)	9.8 (-10.9 to 30.5)	8.4 (-24.9 to 41.7)
(At least) partial adenomectomy	15$	18.3	18	36.0	17.7 (2.0 to 33.4)	18.2 (-0.8 to 37.2)	19.5 (-7.9 to 46.9)
Residual tumor	4$	4.9	2	4.0	0.9 (-6.3 to 8.1)	1.5 (-1.5 to 4.5)	5.1 (-19.2 to 29.4)
No tumor identified	15$	18.3	5	10.0	8.3 (-3.5 to 20.1)	6.8 (-8.7 to 22.3)	5.9 (-5.3 to 17.1)

ACTH=adrenocorticotropic hormone, CI=confidence interval, CT=computed tomography, MRI=magnetic resonance imaging

^Inferior petrosal sinus sampling was considered only for patients with uncertain pituitary tumor, and therefore never in macroadenomas, $Data were missing for ≥5% of patients, #No patients with a macroadenoma with this result, therefore, no analysis could be performed.

To compare mortality and remission rates time-to-event analyses were performed. Kaplan-Meier curves were constructed to visualize overall survival and recurrence-free survival. Separate curves were constructed for microadenomas and macroadenomas, thereby including tumor size as stratification factor, and separate curves were constructed stratified by surgeons' intraoperative findings. As sensitivity analyses, time-to-event curves were constructed comparing the early and late years of all endoscopic operations to assess a potential learning curve, and separate curves were constructed comparing microscopic operations after CT or X-ray imaging versus after MRI to check whether preoperative imaging quality influenced surgical success.

Cox proportional hazard regression analyses were performed to provide hazard ratios and to adjust for potential confounders (results presented in text and Table 3). To take into account different follow-up times, we performed recurrence-free survival analyses also separately with inclusion of only the first 5 years of follow-up. We considered the following variables as potential confounders based on literature and known biological pathways: age at diagnosis, sex, hypertension at diagnosis, diabetes mellitus at diagnosis, dyslipidemia at diagnosis, CSI score, tumor size, and prior medical treatment. After comparison of these potential confounders, we included age at diagnosis and tumor size as confounders in all our Cox analyses, unless otherwise specified.

Statistical analyses were performed with IBM SPSS Statistics 23.0 (IBM Corp, Armonk, NY, USA) and with Stata 14.2 (Stata Corp., College Station, TX, USA) for the two-sample test of proportions (command: prtesti) to calculate the difference between two proportions with 95% confidence interval (CI), as this was not provided by SPSS. All patients gave informed consent to use their data for scientific research and permission from the medical ethical committee in the LUMC was granted. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were used for reporting (25).

Results

Study population (Figure 1, and Table 1)

In total, 137 patients were included, of whom 87 (79.3% female, mean age 38.9 [range 12-80] years) underwent microscopic and 50 (68% female, mean age 44.4 [range 10-73] years) endoscopic surgery. Macroadenomas were relatively more common in the endoscopic group (32.0% versus 20.7% in the microscopy group), and preoperative medical treatment was more common in patients with endoscopic surgery (94.0% versus 65.5% in the microscopy group), in line with the changing treatment strategies over time. Fifteen patients were lost to follow-up (all in the

microscopic surgery group after an average of 101 months, range: 3-261 months). Histopathology confirmed a corticotroph adenoma in nineteen patients with negative imaging, and was inconclusive in one patient. See Table 2 for a detailed description of diagnostic strategy and results.

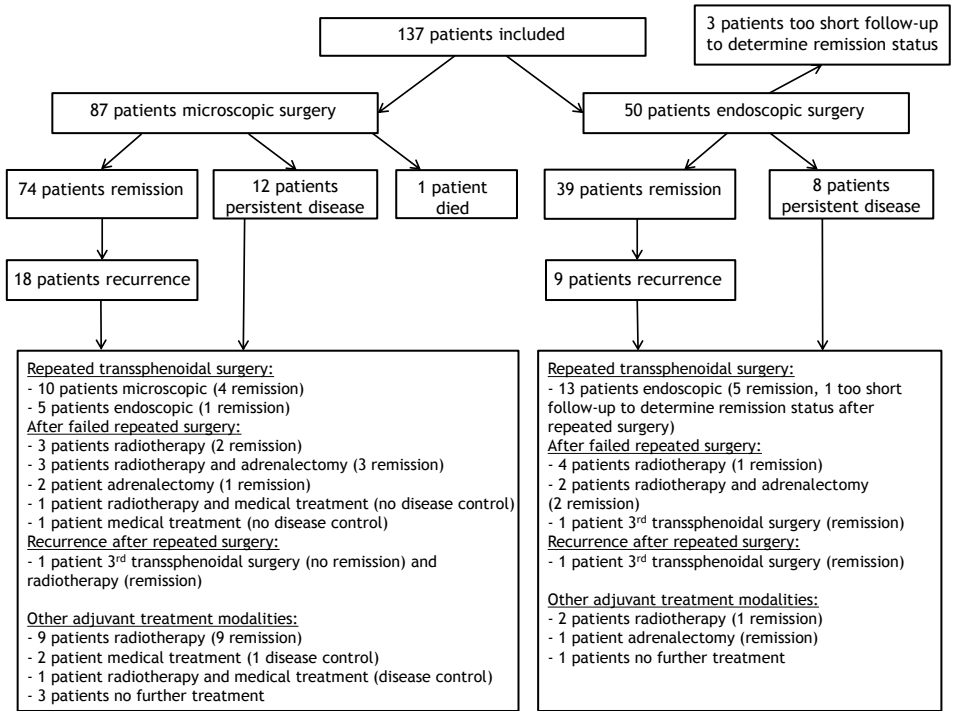

Figure 1: Flow chart of treatment and remission status.

Surgical outcome and recurrence-free survival

Three months after microscopic surgery, 74 of 86 patients (86.0%) were in remission, of whom 67 patients (77.9%) were hydrocortisone dependent. One patient died three days after microscopic surgery (cause not identified). The five-year recurrence-free survival rate for the patients in remission after surgery was 89% (95% CI: 82%-96%), and the ten-year recurrence free survival rate was 84% (95% CI: 75%-93%).

Three months after endoscopic surgery, 39 of 47 patients (83.0%) were in remission, of whom 33 patients (70.2%) were hydrocortisone dependent. Both the five-year and ten-year recurrence-free survival rates were 71% (95% CI: 55%-87%) for the patients in remission after surgery. All patients with negative pathology were in remission at the end of follow-up, except for one patient. Patients with negative pathology achieved remission after (repeat) transsphenoidal surgery or pituitary radiotherapy, and in three cases after adjuvant bilateral adrenalectomy.

Recurrence occurred less often in patients with microscopic surgery (hazard ratio 0.47, 95% CI: 0.19-1.14), see Figure 2. The hazard ratio was 0.38 (95% CI: 0.15-0.99) if only the first 5 years of follow-up were considered. After adjustment, the hazard ratio was 0.40 (95% CI: 0.16-1.01). As percentage macroadenomas changed over time (Figure 3), separate analyses for microadenomas and macroadenomas were performed. For microadenomas the risk of recurrence was similar for the two techniques: hazard ratio 0.99 (95% CI: 0.26-3.74), or hazard ratio 0.79 (95% CI: 0.20-3.16) for the first 5 years of follow-up, which was similar after adjustment for age at diagnosis. If only macroadenomas were considered, the hazard ratio was 0.18 (95% CI: 0.04-0.91, p=0.038), which was exactly the same for the first 5 years of follow-up, and 0.14 (95% CI: 0.03-0.77; p=0.023) after adjustment for age at diagnosis, see Figure 4. When patients with cavernous sinus invasion were excluded from analysis, the hazard ratio for macroadenomas remained similar, also after adjustment for age at diagnosis.

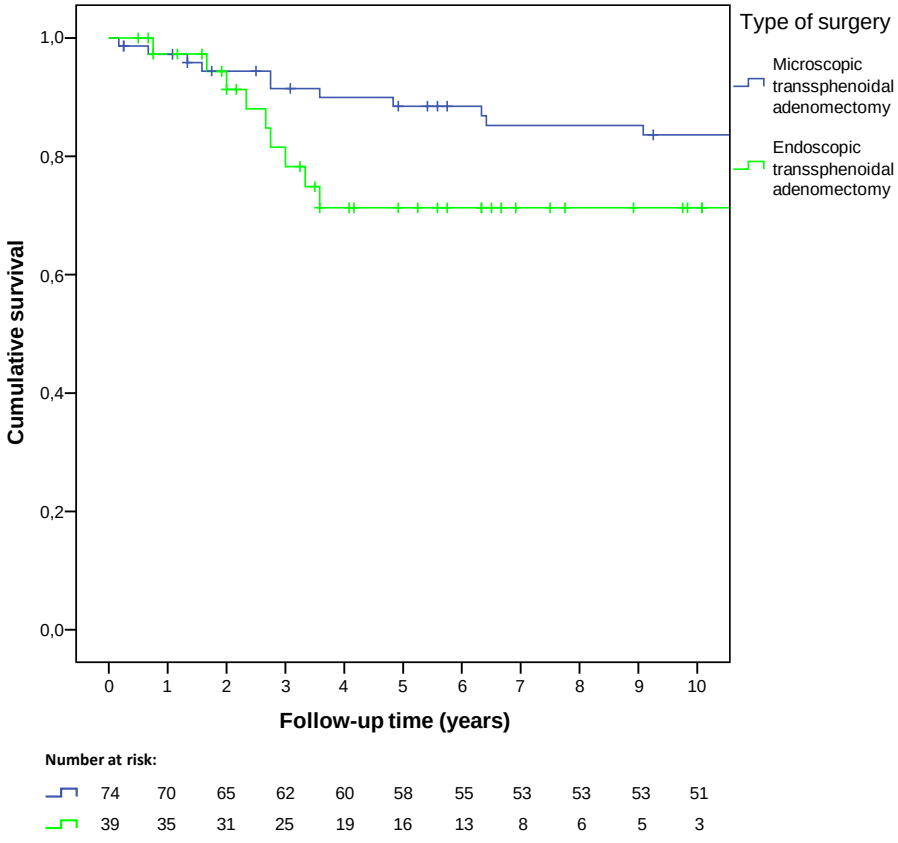

Figure 2: Recurrence-free survival after microscopic versus endoscopic transsphenoidal surgery.

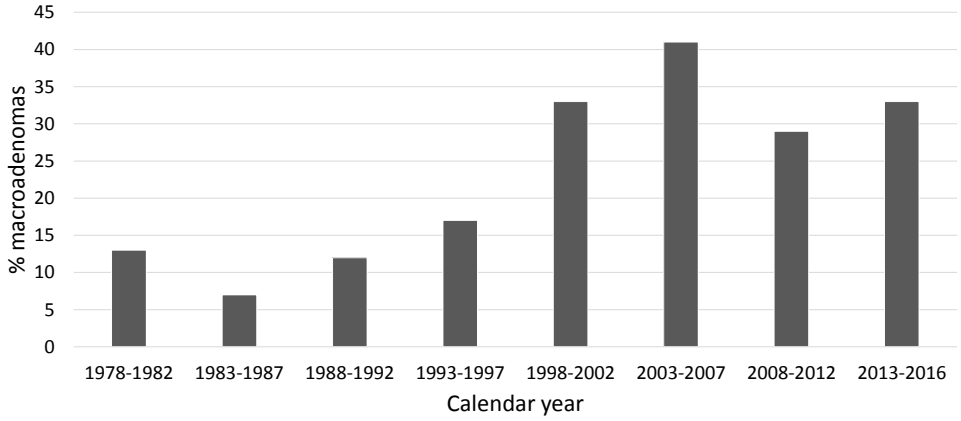

Figure 3: Percentage of macroadenomas operated during the study period.

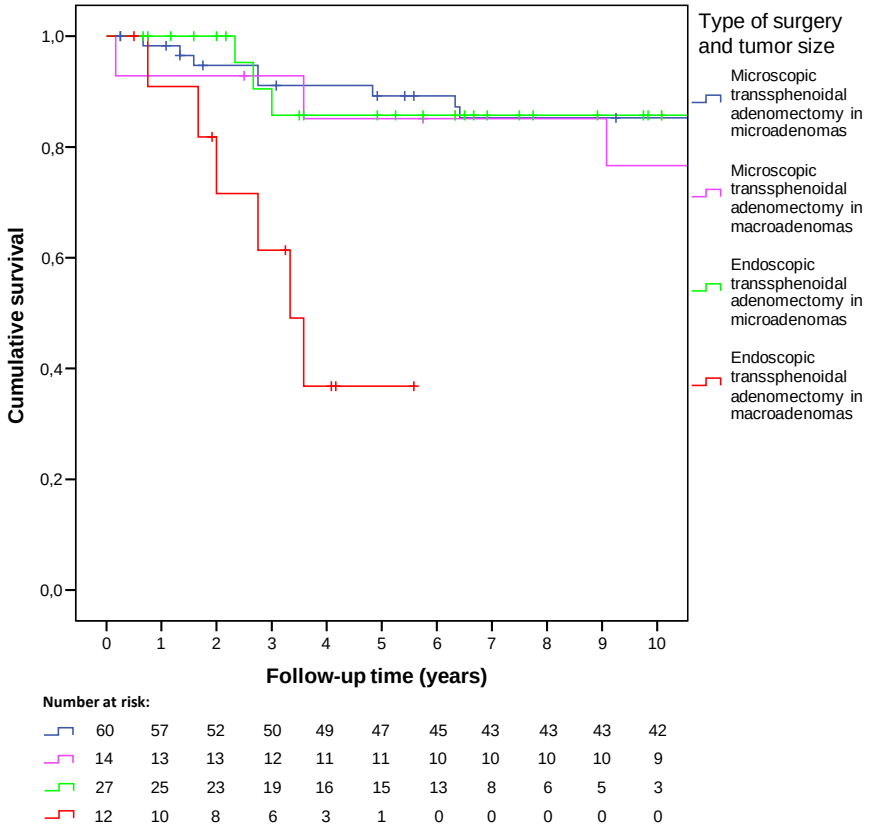

Figure 4: Recurrence-free survival per tumor size after microscopic versus endoscopic transsphenoidal surgery.

Surgeons' intraoperative findings were not clearly related to recurrence-free survival (see Figure 5). Hazard ratios compared to the reference group of complete adenomectomy were: 1.32 (95% CI: 0.57-3.06) for (at least) partial adenomectomy (1.21, 95% CI: 0.43-3.39, for the first 5 years of follow-up), and 3.90 (95% CI: 0.86-17.63) for residual tumor (4.49, 95% CI: 0.96-21.01, for the first 5 years of follow-up). Hazard ratios after adjustment for age at diagnosis, type of adenomectomy (microscopy or endoscopy), and tumor size (microadenoma or macroadenoma) were similar. To assess a potential learning curve within the endoscopic surgery group, patients that underwent endoscopic surgery until December 31st 2011 (N=21) were compared with patients that underwent endoscopic surgery after January 1st 2012 (N=18). To determine whether preoperative imaging quality influenced surgical success in microscopic surgery, patients with CT/X-ray imaging were compared with patients with MRI scans. We found no difference between early and late years of endoscopic surgery (hazard ratio 1.72, 95% CI: 0.46-6.52, which was exactly the same if only the first 5 years of follow-up were included; hazard ratio 1.06, 95% CI: 0.25-4.53 after adjustment), or between preoperative imaging techniques for microscopic surgery (hazard ratio 0.99, 95% CI: 0.36-2.73, which was 0.65, 95% CI: 0.16-2.72, if only the first 5 years of follow-up were included; similar after adjustment), see Figure 6.

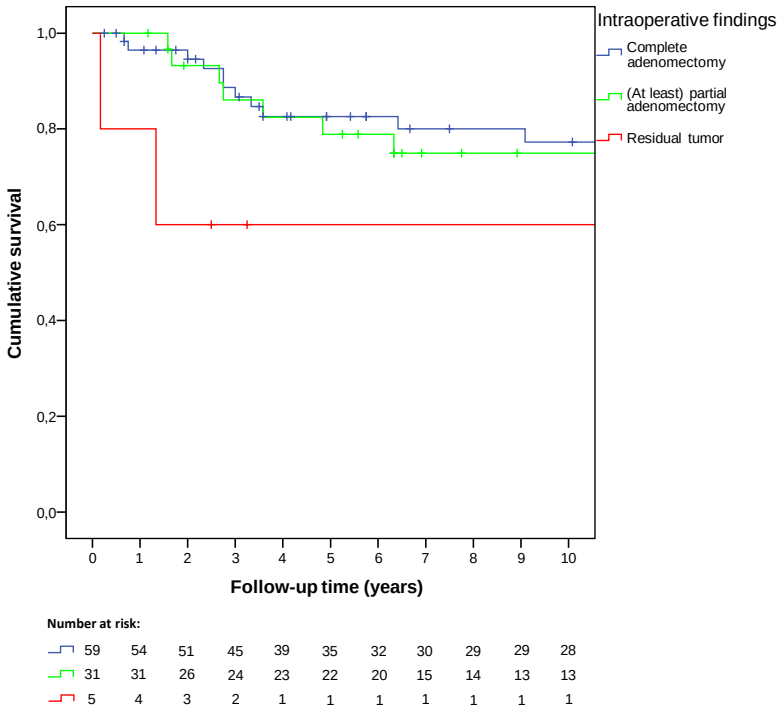

Figure 5: Recurrence-free survival according to surgeons' intraoperative findings.

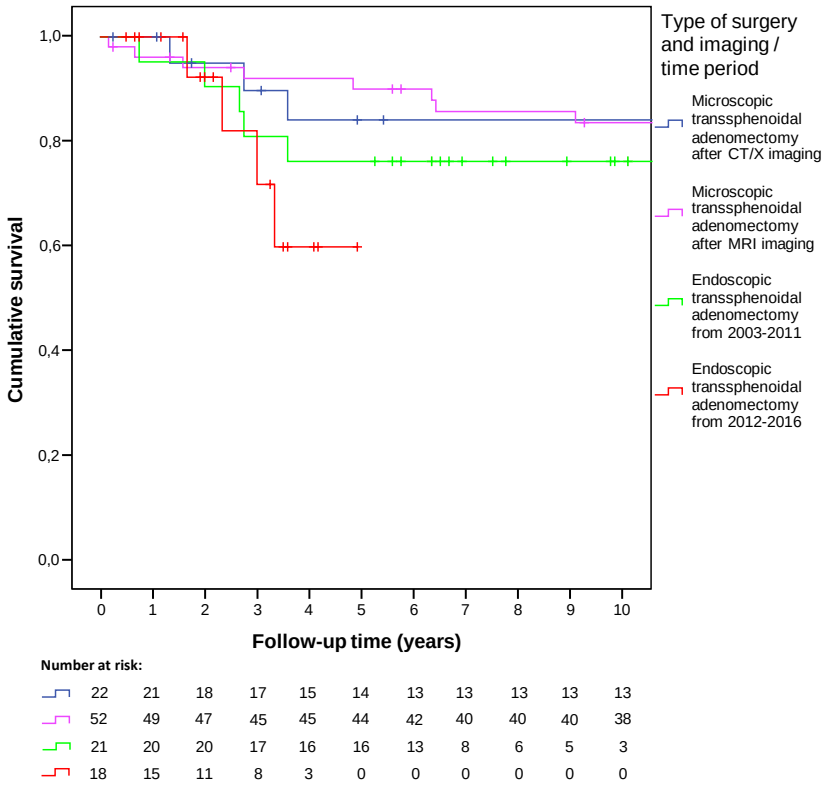

Figure 6: Recurrence-free survival according to imaging and time of surgery.

Overall survival

Within ten years after surgery, ten patients died: nine in the microscopic surgery group and one in the endoscopic surgery group (all-cause mortality). One patient died three days after microscopic surgery of unidentified cause, although a cardiogenic cause was most likely, as left ventricular hypertrophy, coronary artery disease with over 50% stenosis, and pulmonary edema were found at autopsy. Ten-year overall survival rate was 89% (95% CI: 82%-96%) for microscopic surgery and 94% (95% CI: 83%-100%) for endoscopic surgery. The hazard ratio for mortality was 2.79 (95% CI: 0.35-22.51, similar after adjustment) for microscopic versus endoscopic surgery, see Figure 7.

Short- and long-term morbidity

In the microscopic group, less patients experienced a corticosteroid withdrawal syndrome compared to the endoscopic group (hazard ratio after adjustment: 0.38, 95% CI 0.20 to 0.72). Patients with microscopic surgery also had less long-term neuropsychiatric morbidity after surgery than patients with endoscopic surgery. In

Table 3, a detailed overview is presented of hazard ratios for short- and long-term morbidity for endoscopic versus microscopic surgery.

Four patients were treated with antibiotics for meningitis, all after microscopic surgery (two of whom had positive CSF cultures, with pseudomonas and klebsiella species, respectively). Three patients had a severe bleeding (two in the microscopy group and one in the endoscopy group), which was discovered directly after surgery based on clinical findings/deterioration and confirmed on postoperative imaging: of these patients one patient had a subdural hematoma (microscopy), one patient had a intracerebral hematoma (microscopy) and a subarachnoid bleeding (both patients were treated surgically), and one patient had severe epistaxis and hematemesis (treated conservatively, but described as severe in the patient file, with at least 800 ml of blood loss; endoscopy). The other, non-severe bleedings were intraoperative venous bleedings (predominantly from the cavernous sinus), that were excessive compared to the expected amount of blood loss during surgery, and another episode of mild epistaxis which was treated conservatively.

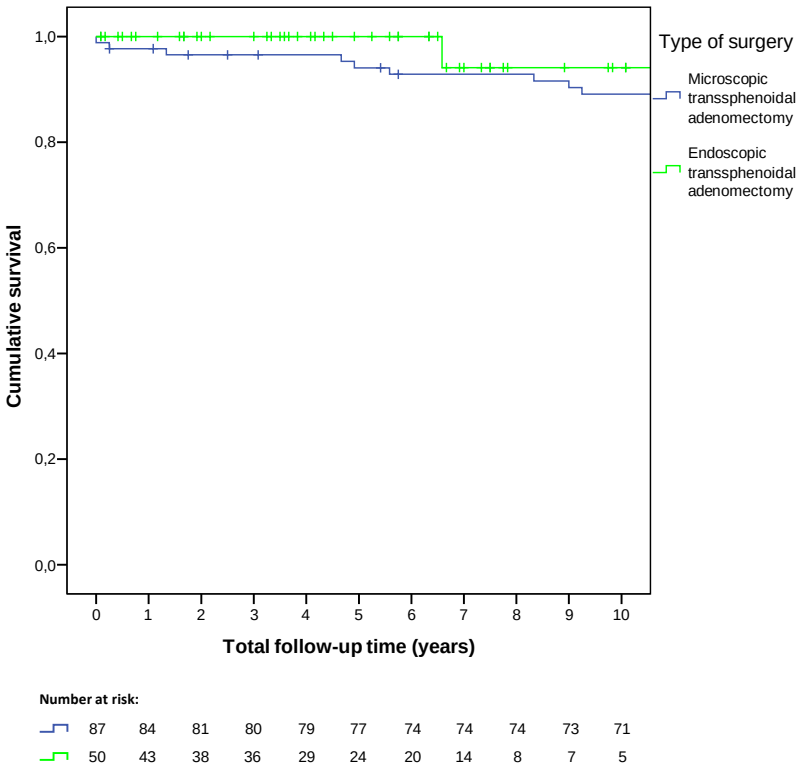

Figure 7: Overall survival after microscopic versus endoscopic transsphenoidal surgery.

Repeated transsphenoidal surgery

Fifteen patients with initial microscopic surgery underwent a second transsphenoidal surgery after a mean of 111 months (range: 7-242), of which ten were microscopic and five endoscopic. Remission was obtained in five of these patients (33.3%) (all hydrocortisone dependent [absolute deficiency], as evaluated three months after repeated surgery), one of whom had undergone endoscopic and four repeated microscopic surgery. In the endoscopic group, thirteen patients underwent repeated transsphenoidal surgery after a mean of 21 months (range: 1-43), all of which were endoscopic. Remission was obtained in five patients (41.7%) (four hydrocortisone dependent [absolute deficiency] three months after repeated surgery), and duration of follow-up was insufficient in one patient to determine remission status after repeated surgery. A third transsphenoidal surgical procedure was performed in three patients, resulting in remission in two patients (one hydrocortisone dependent [absolute deficiency]). For more detailed information on adjuvant treatment and remission status, see Figure 1.

Discussion

In this study, we compared remission rates, mortality risk, and short- and long-term complications between patients treated at a European reference center for Cushing's disease with microscopic versus endoscopic transsphenoidal pituitary surgery. Both techniques yielded similar results regarding remission rate and percentage of patients with hydrocortisone dependency. Patients with microscopic surgery had longer recurrence-free survival, but a higher mortality rate, than patients with endoscopic surgery. When analysed separately, patients with a macroadenoma had a longer recurrence-free survival after microscopic surgery than after endoscopic surgery. Our results could not prove indications of the presence of a learning curve for endoscopic surgery, nor did we find that the type of imaging technique influenced our results for microscopic surgery. No clear relation was found between the surgeons' intraoperative findings and recurrence-free survival, although patients with a certain residual tumor after surgery seemed to perform worse than patients with complete adenomectomy. After endoscopic surgery, more patients had symptoms consistent with corticosteroid withdrawal, and patients showed a higher hazard ratio for long-term neuropsychiatric morbidity.

While there have been previous cohort studies, a major strength of this study is that this study includes the largest cohort to date, thereby increasing reliability of results stratified by tumor size. This is also the first cohort study investigating long-term mortality in Cushing's disease after endoscopic surgery and comparing this to long-term mortality after microscopic surgery. The remission rates and surgical morbidity

obtained from this study are in line with previously published small cohort studies in Cushing's disease directly comparing both techniques (9-12). Our recently published systematic review comparing both surgical techniques in Cushing's disease showed an advantage of performing endoscopic surgery for macroadenomas based on remission and recurrence rates (26). This could not be confirmed in the current cohort study.

Table 3: Short- and long-term morbidity.

	Hazard ratio (95% CI; p-value)[#], unadjusted	**Hazard ratio (95% CI; p-value)[#], adjusted for age at diagnosis and tumor size (microadenoma or macroadenoma)**
Surgical outcome		
Persistent disease	0.82 (0.34 to 2.01; p=0.663)	0.88 (0.35 to 2.21; p=0.789)
Recurrent disease	0.47 (0.19 to 1.14; p=0.093)	0.40 (0.16 to 1.01; p=0.052)
Hydrocortisone dependency	1.11 (0.73 to 1.68; p=0.625)	1.04 (0.68 to 1.60; p=0.845)
- *Absolute deficiency*	1.06 (0.69 to 1.62; p=0.793)	0.97 (0.63 to 1.50; p=0.888)
- *Normal cortisol response**	°	°
- *Pragmatic replacement*[$]	°	°
Repeat surgery	0.30 (0.13 to 0.70; p=0.005)	0.27 (0.11 to 0.64; p=0.003)
Remission after first repeat surgery	0.21 (0.04 to 1.13; p=0.069)	0.17 (0.02 to 1.16; p=0.070)
Short term morbidity: ≤3 month after first surgery		
Cerebrospinal fluid leakage	°	°
Meningitis	°	°
Bleeding, all	0.56 (0.22 to 1.41; p=0.221)	0.56 (0.22 to 1.44; p=0.227)
Bleeding, severe	°	°
Syndrome of inappropriate antidiuretic hormone release	0.32 (0.09 to 1.09; p=0.069)	0.29 (0.08 to 1.02; p=0.054)[a]
Diabetes insipidus	1.04 (0.54 to 1.99; p=0.904)	0.96 (0.49 to 1.86; p=0.901)
Anterior pituitary deficiency	0.76 (0.37 to 1.54; p=0.444)	1.06 (0.51 to 2.22; p=0.870)
- *One axis*	0.85 (0.37 to 1.96; p=0.704)	1.18 (0.50 to 2.79; p=0.708)
- *Two axes*	0.41 (0.09 to 1.83; p=0.243)	0.52 (0.11 to 2.45; p=0.409)
- *Three axes*	°	°
Corticosteroid withdrawal syndrome	0.39 (0.21 to 0.72; p=0.003)	0.38 (0.20 to 0.72; p=0.003)
Cardiovascular morbidity	°	°
Long term morbidity: >3 months after first surgery		
Anterior pituitary deficiency after 1 year	0.79 (0.42 to 1.52; p=0.488)	1.03 (0.52 to 2.01; p=0.056)
- *One axis*	0.83 (0.38 to 1.83; p=0.642)	1.10 (0.48 to 2.48; p=0.827)
- *Two axes*	0.65 (0.17 to 2.41; p=0.517)	0.74 (0.19 to 2.89; p=0.663)
- *Three axes*	°	°
Hydrocortisone dependency >3 years	1.05 (0.56 to 1.96; p=0.878)	1.11 (0.59 to 2.10; p=0.742)
Diabetes insipidus >3 months	1.71 (0.68 to 4.29; p=0.251)	1.84 (0.72 to 4.69; p=0.204)
Cardiovascular morbidity	0.37 (0.12 to 1.16; p=0.088)	0.43 (0.13 to 1.38; p=0.155)
Hypertension	0.85 (0.51 to 1.41; p=0.529)	0.92 (0.53 to 1.58; p=0.749)
Diabetes mellitus	0.53 (0.22 to 1.29; p=0.160)	0.56 (0.23 to 1.36; p=0.198)
Neuropsychiatric morbidity	0.14 (0.03 to 0.68; p=0.015)	0.15 (0.03 to 0.76; p=0.022)

CI=confidence interval

[#]Reference group for hazard ratios was endoscopic surgery.

[a]Adjusted for age at diagnosis only.

*Hydrocortisone for symptoms despite normal cortisol response to CRH stimulation. °Insufficient data for analysis.

[$]Pragmatic hydrocortisone replacement (without stimulation test).

There are several study limitations that need to be taken into account when interpreting the study results. Patients treated microscopically were included in a different time period than patients treated by endoscopic surgery, which might have resulted in substantial differences between both study groups. The prevalence of certain comorbidities has changed over time, as well as the available treatment modalities for these comorbidities. However, patient characteristics were compared between the study groups, and only follow-up duration and the use of medical treatment prior to surgery differed largely. It is impossible to say in which direction mortality might have changed due to the differences in prevalence and treatment of comorbidities over time, as one might expect a worse survival with a higher prevalence of comorbidities, but a better survival due to improved treatment modalities. It is unlikely that recurrence-free survival is influenced by prevalence or treatment of comorbidities. Although we adjusted for confounders, residual confounding due to unmeasured or incorrectly measured confounders remains a potential issue. To exclude a potential chronological bias completely, a new study should be performed comparing both surgical techniques in the same study center and in the same time period.

Selective loss to follow-up could have led to selection bias, as all patients lost to follow-up were in the microscopic surgery group. As patients could have become lost to follow-up due to various reasons (including both excellent health as well as very poor health status), the direction in which the results may have been biased cannot be determined. This effect was minimized as our analyses were confined to the first ten years after surgery and loss to follow-up was on average 8.3 years after surgery. Selection due to selective referral for endoscopic surgery to our center of mainly complex cases (i.e. macroadenomas and/or those with inconclusive imaging features suggesting lateral invasion of the cavernous sinus), may have led to worse surgical outcomes and higher complication rates after endoscopic surgery than expected. This is exemplified by the higher percentage of adenomas with cavernous sinus invasion in the endoscopically treated group.

Over time, diagnostic strategies and treatment for Cushing’s disease have changed. Diagnostic imaging has changed from CT to MRI resulting in better visualization of suspect lesions, increasing the certainty of which target to address during surgery. There have been several treatment changes at our center, e.g. the surgical technique has changed from microscopic surgery to endoscopic surgery, and after 1990, preoperative medical treatment to control cortisol secretion became common practice at our center. Patients were not randomized to a certain treatment but were treated by the method available at that time. Therefore, effects of time, imaging technique, and use of medical therapy prior to surgery, cannot be separated from the effect of the surgical technique. To evaluate the effect of preoperative

medical treatment on outcome *per se*, a randomized trial is needed which compares patients with the same surgical technique with and without medical pretreatment, which has not yet been performed to our knowledge.

According to the STROBE statement, generalizability of the study needs to be discussed (25). Theoretically, this study is generalizable to all patients with Cushing's disease with a primary surgical treatment of transsphenoidal adenomectomy. However, generalizability is reduced due to specific effects produced by the setting (hospital, neurosurgeons) and time period of inclusion of patients per study group.

In the endoscopic group, a higher rate of corticosteroid withdrawal syndrome was seen. This is especially remarkable, as many patients received preoperative medical treatment to lower cortisol concentrations before endoscopic surgery. However, this result is in agreement with our clinical experience, and might be explained by increased awareness for the corticosteroid withdrawal syndrome during recent years, leading to better recognition and improved hydrocortisone adjustments, when needed. Patients treated endoscopically showed a higher hazard ratio for long-term neuropsychiatric morbidity. This is, at least in part, due to the increased awareness of persistent neuropsychiatric comorbidity in patients with (previous) Cushing's disease in recent years.

Better results were anticipated for endoscopic surgery compared to microscopic surgery regarding recurrence-free survival and complication rates, based on the better visualization of tumors, certainly for the laterally invasive or large tumors. Regarding recurrence-free survival, we found an advantage of microscopic surgery for macroadenomas only. Microadenomas are most likely completely within the field of vision regardless of the surgical technique, explaining the lack of difference between both surgical techniques. As our recently published review showed an advantage of endoscopic surgery for macroadenomas (26), the advantage of microscopic surgery for macroadenomas shown in this study regarding recurrence-free survival might be explained by the surgeons' attempt to perform a complete tumor resection with limited visibility of the entire tumor, although extent of anterior pituitary deficiency after surgery was similar for both techniques. Another explanation is that more difficult and invasive macroadenomas were referred to our center for endoscopic surgery (six endoscopic versus four microscopic treated patients were referred by other neurosurgeons or university medical centers), resulting in eight endoscopic (50.0%) versus five microscopic (27.8%) macroadenomas with cavernous sinus invasion. However, excluding patients with macroadenomas and cavernous sinus invasion did not change the hazard ratio for recurrence. Differences between both surgical techniques may also have occurred due to the small number of patients per study group after stratification by tumor size. Differences in outcomes between the

current cohort study and our recently published systematic review and meta-analysis on the same subject (26) may have resulted from differences in included patients in both studies, e.g. as a result of selective referral of more difficult and invasive macroadenomas to our center, or as a result of differences in experience in and use of both techniques for Cushing's disease or also other pituitary adenomas between our center and other centers included in the systematic review. Differences may also have resulted from variations in used definitions for remission, recurrence, and complications between study centers. The difference in outcome between the current cohort study and our recently published systematic review emphasizes the importance of combining data from multiple centers, as will be done for patient care purposes in the newly established European Reference Network on Rare Endocrine Conditions (Endo-ERN).

Conclusions

We found no clear advantage for either microscopic or endoscopic transsphenoidal surgery in Cushing's disease, based on surgical outcome or complications. In addition, no learning curve was found for endoscopic surgery. The transition from microscopic surgery to endoscopic surgery for Cushing's disease in our center was made without temporarily deterioration of outcomes, which may be reassuring for surgeons who consider changing to endoscopic surgery.

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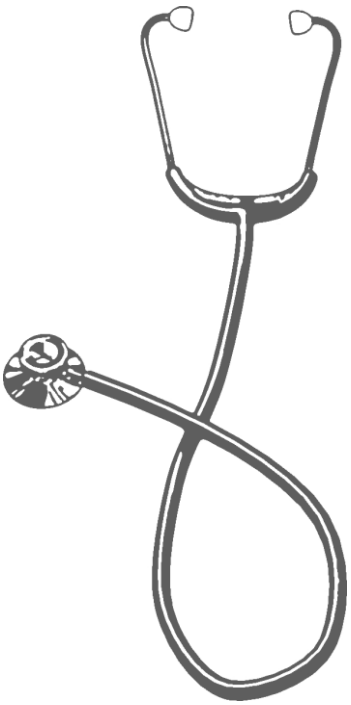
Chapter 5

Endoscopic versus microscopic transsphenoidal surgery for Cushing's disease: a systematic review and meta-analysis



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Pituitary. 2018 Oct;21(5):524-534.

Abstract

Purpose
Systematic review and meta-analysis comparing endoscopic and microscopic transsphenoidal surgery for Cushing's disease regarding surgical outcomes (remission, recurrence, and mortality) and complication rates. To stratify the results by tumor size.

Methods
Nine electronic databases were searched in February 2017 to identify potentially relevant articles. Cohort studies assessing surgical outcomes or complication rates after endoscopic or microscopic transsphenoidal surgery for Cushing's disease were eligible. Pooled proportions were reported including 95% confidence intervals.

Results
We included 97 articles with 6,695 patients in total (5,711 microscopically and 984 endoscopically operated). Overall, remission was achieved in 5,177 patients (80%), with no clear difference between both techniques. Recurrence was around 10% and short-term mortality <0.5% for both techniques. Cerebrospinal fluid leak occurred more often in endoscopic surgery (12.9% versus 4.0%), whereas transient diabetes insipidus occurred less often (11.3% versus 21.7%). For microadenomas, results were comparable between both techniques. For macroadenomas, the percentage of patients in remission was higher after endoscopic surgery (76.3% versus 59.9%), and the percentage recurrence lower after endoscopic surgery (1.5% versus 17.0%).

Conclusions
Endoscopic surgery for patients with Cushing's disease reaches comparable results for microadenomas, and probably better results for macroadenomas than microscopic surgery. This is present despite the presumed learning curve of the newer endoscopic technique, although confounding cannot be excluded. Based on this study, endoscopic surgery may thus be considered the current standard of care. Microscopic surgery can be used based on neurosurgeon's preference. Endocrinologists and neurosurgeons in pituitary centers performing the microscopic technique should at least consider referring patients with Cushing's disease with a macroadenoma.

Introduction

Cushing's disease is caused by an adrenocorticotropic hormone (ACTH)-secreting pituitary adenoma, with an estimated incidence of 1.2–1.7 per million each year (1). The resulting excess of glucocorticoids induces insulin resistance, dyslipidemia, central obesity, hypercoagulability, and increases the risk of osteoporosis, hypertension, and neuropsychiatric disorders (2, 3). First-choice treatment for Cushing's disease is transsphenoidal pituitary surgery, with selective adenoma removal (4). Despite biochemical cure, mortality risk in patients with Cushing's disease remains increased (5).

Two main techniques have been used for transsphenoidal pituitary surgery: microscopic and endoscopic surgery. Furthermore, the microscopic and endoscopic techniques have been used in combination, in which the endoscope was used to visually confirm findings of the microscope. The microscopic technique was the established method to perform transsphenoidal surgery, until the first reports on endoscopic pituitary surgery were published, starting in 1992 (6). With the operating microscope, intraoperative differentiation of pathologic tissue from normal tissue is achieved by providing three-dimensional vision in a direct line to the pituitary (7, 8). Endoscopic pituitary surgery provides a broader field of vision using endoscopes with various angles in close proximity to the pituitary, however losing the three-dimensional vision and thus depth perception (6, 8). From the introduction of the endoscope in transsphenoidal surgery, most surgical centers have chosen for microscopy or endoscopy. Only few small cohort studies have compared the microscopic and endoscopic surgical techniques in Cushing's disease performed in the same center (9–12). No clear differences in remission rate or surgical morbidity between microscopic and endoscopic surgery could be shown. However, the studies had only limited statistical power (9).

Several systematic reviews have compared endoscopic and microscopic surgical techniques in a heterogeneous population of patients with various pituitary adenomas. These studies have found a reduced rate of some complications (postoperative diabetes insipidus, rhinological complications), but an increased rate of other complications (vascular complications, cerebrospinal fluid leak, anterior pituitary hormone deficiency) for the endoscopic technique (8, 13, 14). These differences in outcomes may partially be explained by the surgeon's attempt for a more radical tumor excision with the newer endoscopic technique with better vision, by the larger proportion of more challenging macroadenomas and re-operations reported in literature, and by improved rhinological care by an otolaryngologist after endoscopic surgery (8, 13, 14). Until now, no systematic review has been published comparing the microscopic to the endoscopic surgical technique in Cushing's disease.

Convincing evidence supporting the choice for one of both techniques in the treatment of Cushing's disease, either based on treatment results or complication rate, is thus lacking.

Study aims

The primary aims of this systematic review were to compare remission and recurrence rate, and mortality, after microscopic versus endoscopic transsphenoidal pituitary surgery for Cushing's disease. Secondary study aims were to compare complication rates, remission and recurrence rates stratified by tumor size, and percentage remission after a repeat transsphenoidal surgical procedure.

Methods

Eligibility criteria

Randomized controlled trials and cohort studies in Cushing's disease assessing outcomes after endoscopic or microscopic transsphenoidal surgery were eligible. Studies describing endoscope-assisted microscopic surgery were considered microscopic surgery. Single-arm studies as well as direct comparisons were considered, mainly because we did not expect many direct comparisons in a single cohort. Study outcomes of interest were remission rate, recurrence rate, short- and long-term mortality risk, and complications of surgery. Studies reporting outcomes after primary as well as after repeat transsphenoidal surgery were eligible. Studies reporting <10 patients with Cushing's disease per treatment group were excluded to minimize the risk of selection bias. Articles were also excluded if the study included children only, if the study did not clearly report which surgery type was performed, or if no distinction between surgery types was made in the analysis. Articles including patients with selective adenomectomy as well as partial or total hypophysectomy were included as long as total hypophysectomy did not exceed 5%. If described separately, patients with total hypophysectomy were excluded from analyses. If multiple articles described (partially) the same population, the article with the largest cohort was included per analysis. Articles irretrievable online were requested by contacting the authors. Articles still irretrievable, but with sufficient data mentioned in the abstract for reliable eligibility assessment and data extraction, were included. Only articles in English were considered.

Search strategy

To identify potentially relevant articles, PubMed, Embase, Web of Science, COCHRANE Library, CENTRAL, Emcare, LWW, ScienceDirect, and Wiley were systematically searched in February 2017 in cooperation with a specialized librarian (see Supplemental Data 1 for the complete search strategy). References of included

articles were searched and the search strategy was manually extended in PubMed with the search term 'pituitary adenoma' to find more potentially eligible studies.

Data extraction
All identified articles were imported in endnote 8 (Thomson Reuters, Philadelphia, PA, USA). Studies were screened by title and abstract and potentially relevant articles were reviewed in detail to assess eligibility. Potentially relevant articles were screened and reviewed by two reviewers independently and disagreement was solved by consensus. The meta-analysis of observational studies in epidemiology (MOOSE) guidelines were used for reporting (15).

Risk of bias assessment
For risk of bias analysis we used a component approach. Risk of bias was assessed by two independent reviewers for all included studies using the following components, which could potentially bias a reported association between surgical technique and outcome:

1. Inclusion of patients (consecutive inclusion or a random sample is considered low risk of bias)
2. Loss to follow-up (<5% is considered low risk of bias)
3. Criteria for diagnosis of Cushing's disease (see below)
4. Clear reporting of criteria for main study outcome. For most studies the main outcome is remission of Cushing's disease. If remission is not a study outcome, studies will be checked for reporting criteria for their primary study outcome, most often one or more complications of treatment.

As criteria for diagnosis of Cushing's disease vary widely over time and per study center, and study outcomes also vary per included article, mentioning the criteria for diagnosis and study outcome is considered a low risk of bias. Classification of interventions was not considered in this risk of bias analysis, because the interventions of interest are one-time procedures and therefore unlikely to be misclassified.

Risk of bias analysis was used to explore potential heterogeneity. As most studies did not compare the two surgical techniques directly, confounding was not judged at the study level, but was assessed by comparing baseline characteristics between microscopically and endoscopically treated patients. Variables influencing the choice of treatment as well as co-interventions that could affect treatment outcome were reported.

Study endpoints

The main outcomes of this study were the percentage of patients reaching remission, the recurrence rate, and the short-term mortality risk after microscopic and endoscopic transsphenoidal pituitary surgery for Cushing's disease. Secondary outcomes were complication rates, rates of remission and recurrence stratified by tumor size, and the percentage of patients to reach remission after a repeat transsphenoidal surgical procedure. Because of the low number of studies with direct comparisons, percentages were reported per surgical technique.

Remission was considered direct postoperatively (until 6 months post-surgery). Hydrocortisone dependency was calculated as a percentage of the total patient population to maintain comparability with remission rate. Disease recurrence was estimated as percentage of the patients with initial remission. Mortality risk was analyzed for short-term mortality (<3 months after surgery). Long-term mortality risk (≥3 months after surgery) was not analyzed, as time since surgery was often unclear. Articles reporting mortality without mentioning time since surgery were excluded from mortality analyses.

The following complications were assessed: cerebrospinal fluid leak, meningitis, syndrome of inappropriate antidiuretic hormone secretion (SIADH), anterior pituitary hormone deficiency, thromboembolism, bleeding, transient diabetes insipidus, permanent diabetes insipidus, and psychopathology. If an article described diabetes insipidus without specifying the duration, it was excluded from diabetes insipidus analyses.

Statistical analysis

Percentages were pooled in a random-effects logistic regression model if there were ≥5 articles per analysis. A fixed-effects model was used for analyses with <5 studies. The Freeman-Tukey arcsine transformation was used to stabilize variances, in order to prevent exclusion of studies with 0% or 100% as outcome. All analyses were performed using Stata 11.2 (Stata Corp., College Station, TX, USA). Sensitivity analyses were performed to assess the potential effect of high risk of selection bias studies by excluding articles in which inclusion of patients was not consecutive or a random sample and/or loss to follow-up was ≥5%. Of note, articles not mentioning method of inclusion or loss to follow-up were not excluded in these sensitivity analyses, as this would not leave sufficient articles for analysis (13 for microscopic surgery and one for endoscopic surgery only). Sensitivity analyses were also performed for studies with a study period starting from the year 2000 or later, to assess the potential cohort effect of calendar year of surgery. Finally, sensitivity analyses were performed for studies reporting specific criteria for diagnosis of Cushing's disease (pituitary imaging or petrosal sinus sampling, and at least one of

the following laboratory measurements or tests: increased morning serum cortisol, increased 24-h urinary free cortisol, increased midnight salivary cortisol, no suppression of cortisol after a low dose dexamethasone test combined with a non-suppressed ACTH) to increase reliability of including only patients with Cushing's disease, as well as for studies using at least a low dose dexamethasone test in the determination of remission status to increase test homogeneity, and for studies assessing remission status 3–6 months postoperatively, as this is a more reliable timeframe to correctly assess remission status than direct postoperatively (16).

Results

Study selection

The initial search identified 932 articles. Searching through references of included articles and manually extending the search in PubMed with the search term 'pituitary adenoma' identified another 16 articles, thereby yielding a total of 948 articles. After screening the articles by title and abstract, 685 articles were excluded, leaving 263 articles for detailed review. Reasons for exclusion are summarized in Figure 1. There were 97 articles included in this review, two of which based on abstract only (17, 18).

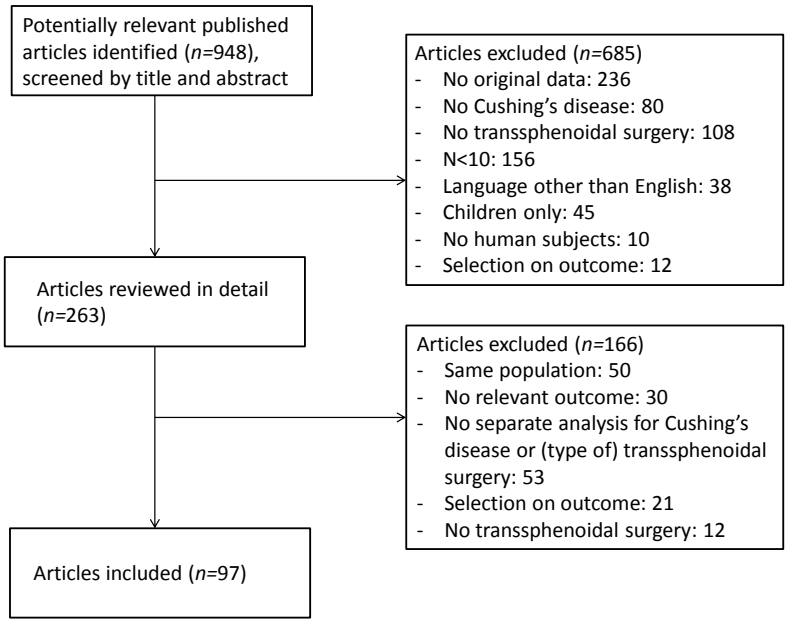

Figure 1: Flow-chart of inclusion of articles in this systematic review.

Study characteristics (Supplemental Data 2)

No randomized controlled trials were performed comparing microscopic to endoscopic surgery. There were 71 studies reporting on microscopic surgery only (4, 7, 17, 19–86), 22 studies reporting on endoscopic surgery only (18, 87–107), and four studies from four different centers reporting on both microscopic and endoscopic surgery in the same center (9–12). Studies reporting on both techniques were entered twice in the tables and analyses, separately for each of the techniques. Articles were published between 1978 and 2017 for microscopic surgery and from 2001 to 2017 for endoscopic surgery. Two included articles reported only results for patients after repeat transsphenoidal surgery (65, 82). A total of 5,711 patients were included for the microscopic technique, and 984 patients for the endoscopic technique.

Risk of bias assessment

Detailed risk of bias assessment per included article is shown in Supplemental Data 3. Reported loss to follow-up (reported in 35 studies [36%]) ranged from 0% to 26.9%. Inclusion of consecutive patients or a random sample of patients was explicitly stated in 73 articles (75%). There were 80 articles (82%) that reported the criteria for Cushing's disease diagnosis, or that referred to the article in which the exact criteria were published. Criteria for main study outcome were reported in 88 articles (91%). Remission of Cushing's disease was the main study outcome in 83 of these 88 articles (94%).

Differences in baseline characteristics (confounding) are likely as treatment assignment was dependent on calendar year of surgery and center. There were only three articles describing both techniques in the same center and in the same calendar period (see Supplemental Data 2). Furthermore, there was a slight difference in average age at treatment (microscopy 21.5–50 years; endoscopy 31.9–55.7 years) and in percentage female (microscopy 67%–93%; endoscopy 57%–95%). Co-interventions that could influence treatment outcome were reported per article in Supplemental Data 3. Nine included articles (9%) explicitly reported that no co-interventions were used, 20 articles (21%) reported use of co-interventions before or shortly after treatment in part of their included patients. The remaining 68 articles (70%) did not report on co-interventions.

Study outcomes

For a total of 91 study groups (87 articles), remission was the primary outcome of interest. Overall, remission was obtained in 80% (5,177 out of 6,484) of patients. There were 48 articles reporting a (short- and/or long-term) mortality rate, and 60 articles reported the rate of, at least one, complication. For details of study outcomes at the individual study level, see Supplemental Data 4.

Pooled proportions of surgical outcomes: remission, recurrence, mortality, and remission after repeat surgery (Figure 2; Table 1)

The percentage remission was similar for microscopically and endoscopically treated patients, both reaching around 80% remission. Hydrocortisone dependency was seen in 39.3% (95% confidence interval [CI]: 23.5%–56.4%) of patients after microscopic surgery and in 33.5% (95% CI: 13.3%–57.3%) after endoscopic surgery. Recurrence of disease occurred in around 10% of patients after both types of surgery. Average follow-up duration for studies reporting on disease recurrence was 1.0–15.4 years for microscopy and 1.4–5.9 years for endoscopy. Recurrence occurred after an average of 6–76 months in studies using the microscopic technique, and after an average of 24–54 months in studies using the endoscopic technique. Short-term mortality was 0.0% (95% CI: 0.0%–0.2%) for microscopic surgery and 0.4% (95% CI: 0.0%–2.2%) for endoscopic surgery. The percentage of patients that obtained remission after a repeated transsphenoidal surgical procedure was 55.7% (95% CI: 43.3%–67.8%) for microscopic surgery, and 42.6% (95% CI: 18.4%–68.4%) for endoscopic surgery. Measurements of treatment effect were consistent across individual studies, and spread of measurements is reflected by the 95% confidence interval of the outcomes of the analyses.

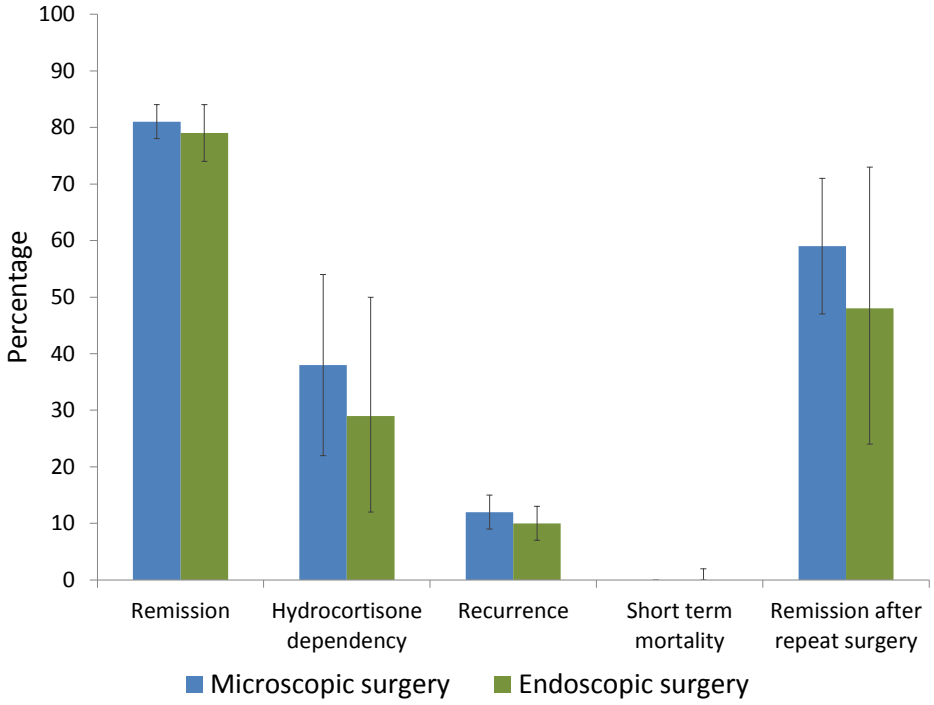

Figure 2: Analysis of surgical outcomes of transsphenoidal surgery for Cushing's disease. Bars: 95% confidence interval.

Pooled proportions of complications after transsphenoidal pituitary surgery for Cushing's disease (Figure 3; Table 1)

Cerebrospinal fluid leak was reported less often in patients after microscopic surgery (4.0% [95% CI: 2.3%–6.1%]), than after endoscopic surgery (12.9% [95% CI: 5.8%–22.1%]). Furthermore, SIADH, bleeding and permanent diabetes insipidus were seen slightly less often in patients after microscopic surgery, than in patients after endoscopic surgery. Transient diabetes insipidus was reported more often in patients after microscopic surgery (21.7% [95% CI: 15.0%–29.3%]), than in patients after endoscopic surgery (11.3% [95% CI: 6.6%–17.1%]). Meningitis (around 0.4%), anterior pituitary deficiency (around 10.5%), and thromboembolism (little over 1%), were seen in about equal percentages of patients, regardless of surgical technique. Psychopathology was reported in 0.7% (95% CI: 0.0%–3.1%) of patients after microscopic surgery. There were no articles on endoscopic surgery reporting on psychopathology.

Table 1: Results of meta-analyses comparing microscopic and endoscopic surgery for Cushing's disease.

	Microscopic surgery		**Endoscopic surgery**	
	Estimated percentage	**95% confidence interval**	**Estimated percentage**	**95% confidence interval**
Meta-analysis of surgical outcomes				
Remission	80.5	77.6-83.3	79.2	74.3-83.8
Hydrocortisone dependency	39.3	23.5-56.4	33.5	13.3-57.3
Recurrence	11.5	9.0-14.3	9.6	6.9-12.7
Short term mortality	0.0	0.0-0.2	0.4	0.0-2.2
Remission after repeat surgery	55.7	43.3-67.8	42.6	18.4-68.4
Meta-analysis of complications				
Cerebrospinal fluid leak	4.0	2.3-6.1	12.9	5.8-22.1
Meningitis	0.6	0.1-1.3	0.1	0.0-1.0
Syndrome of inappropriate antidiuretic hormone secretion	3.5	1.3-6.6	5.2	2.9-8.0
Anterior pituitary hormone deficiency	9.4	5.1-14.8	11.5	5.7-18.8
Thromboembolism	1.2	0.4-2.3	1.5	0.4-3.0
Bleeding	1.9	0.7-3.5	3.7	0.8-8.3
Transient diabetes insipidus	21.7	15.0-29.3	11.3	6.6-17.1
Permanent diabetes insipidus	2.4	1.1-4.1	4.0	2.2-6.3
Psychopathology	0.7	0.0-3.1	-	-
Meta-analysis of surgical outcomes according to tumor size				
Remission for microadenoma	85.5	81.2-89.3	83.9	76.8-90.0
Recurrence for microadenoma	9.8	6.8-13.2	8.1	4.3-12.8
Remission for macroadenoma	59.9	52.0-67.6	76.3	64.3-86.7
Recurrence for macroadenoma	17.0	5.6-31.5	1.5	0.0-6.4

Pooled proportions of remission and disease recurrence according to tumor size (Figure 4; Table 1)

For microadenomas, the percentage of patients that achieved remission was 85.5% (95% CI: 81.2%–89.3%) after microscopic surgery versus 83.9% (95% CI: 76.8%–90.0%) after endoscopic surgery. Recurrence of disease occurred in 9.8% (95% CI: 6.8%–13.2%) of patients after microscopic surgery versus 8.1% (95% CI: 4.3%–12.8%) after endoscopic surgery.

For macroadenomas, the percentage of patients that achieved remission was 59.9% (95% CI: 52.0%–67.6%) after microscopic surgery versus 76.3% (95% CI: 64.3%–86.7%) after endoscopic surgery. Disease recurrence occurred in 17.0% (95% CI: 5.6%–31.5%) after microscopic surgery versus 1.5% (95% CI: 0.0%–6.4%) after endoscopic surgery.

Sensitivity analysis

Generally, results from sensitivity analyses were similar to those found in the main analyses. Detailed results for sensitivity analyses and the number of studies per analysis can be found in Supplemental Data 5.

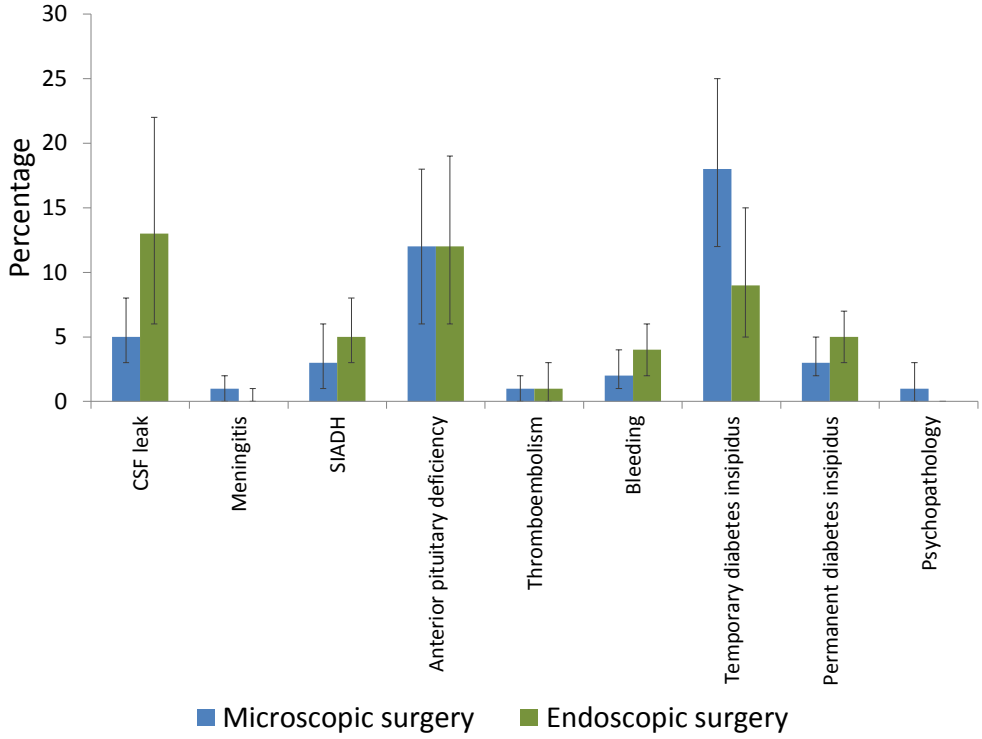

Figure 3: Analysis of complication rates after transsphenoidal surgery for Cushing's disease. Bars: 95% confidence interval.

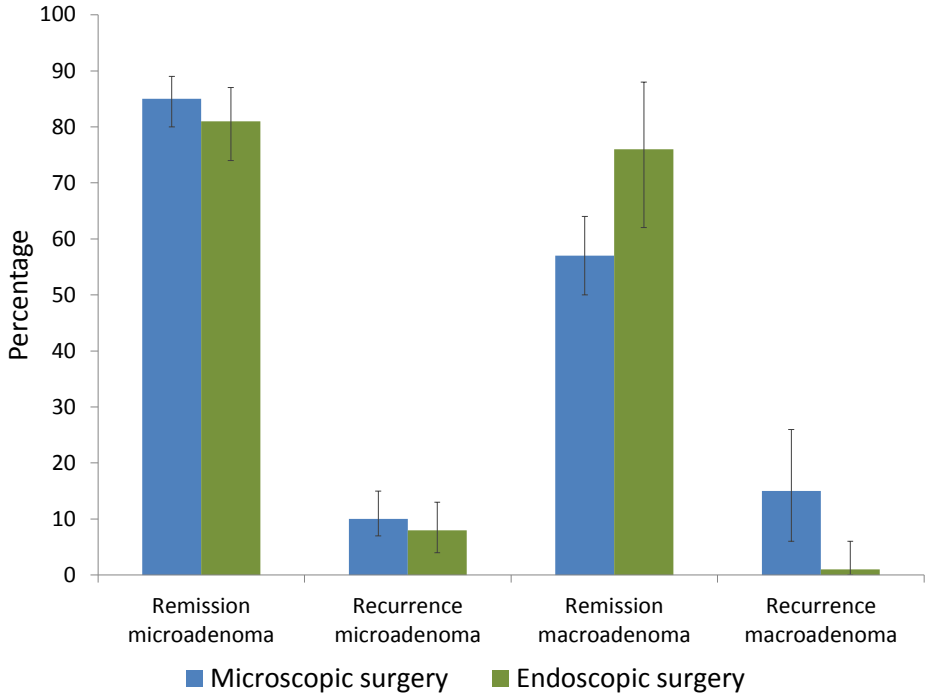

Figure 4: Analysis of surgical outcomes of transsphenoidal surgery for Cushing's disease, stratified by tumor size. Bars: 95% confidence interval.

Discussion

We performed a systematic review to compare surgical outcomes after microscopic versus endoscopic transsphenoidal pituitary surgery for Cushing's disease. Regardless of surgical technique, remission rates were around 80% and recurrence rates around 10% after transsphenoidal surgery. There were no clear differences between surgical techniques regarding mortality or remission rates after repeat transsphenoidal surgery. Complication rates ranged from 0.1% (for meningitis) to 21.7% (for transient diabetes insipidus), with minor differences between surgical techniques. Remission and recurrence rates for microadenomas were similar for both surgical techniques. However, remission rate was higher for macroadenomas (76.3% versus 59.9%), with a lower recurrence rate (1.5% versus 17.0%) after endoscopic surgery than after microscopic surgery. Thus, for macroadenomas only there seems to be an advantage of the endoscopic over the microscopic surgical technique for transsphenoidal treatment of Cushing's disease.

This is the first systematic review comparing microscopic and endoscopic transsphenoidal pituitary surgery specifically for Cushing's disease. We found comparable remission and mortality rates for both surgical techniques, which is in line with results of meta-analyses of heterogeneous populations of various pituitary adenomas, and some small cohort studies comparing both techniques directly for Cushing's disease (8–14). Differences in complications rates found in meta-analyses of heterogeneous populations of various pituitary adenomas can partially be confirmed by our analysis (reduced rate of transient diabetes insipidus, and increased rate of vascular complications and cerebrospinal fluid leak for the endoscopic technique) (8, 13, 14). The increased rate of anterior pituitary hormone deficiency for endoscopic transsphenoidal surgery was not found in the present study (14). The difference in remission rate between the surgical techniques for macroadenomas, but not microadenomas, is in line with the results from a cohort study on multiple pituitary adenomas described separately, that reported an advantage of the endoscopic technique for macroadenomas, but not for microadenomas. This difference was statistically significant for the population as a whole, but was supported by differences in the same direction for all included types of pituitary adenoma, including Cushing's disease (11).

In interpreting the results, the following study limitations need to be taken into account. Most included studies in this study were single-arm studies, limiting the possibilities of directly comparing microscopic to endoscopic transsphenoidal surgery. However, as treatment assignment in most studies was based on availability of a specific technique in the surgical center, for endoscopy often based on preference of the neurosurgeons after a test period, other baseline characteristics, such as age and sex distribution, are unlikely to have influenced treatment assignment largely. As microscopy was the established surgical technique until the introduction of the endoscope for transsphenoidal surgery for Cushing's disease, year of surgery varied widely for included studies (108). For endoscopic surgery, a learning curve has been described (97, 98). As most studies did not report patient level data, the effect of a potential learning curve per surgical center could not be analyzed in this study. However, to avoid measuring an effect of a collective learning curve, the earliest studies using endoscopic surgery, with study periods starting before the year 2000, were excluded in the previously mentioned sensitivity analysis.

Included studies showed heterogeneity in criteria used for diagnosing Cushing's disease, both in tests used to determine remission status, and in time period after surgery for assessment of remission status. Sensitivity analyses showed generally comparable results to the main analyses. Differences are likely to have occurred because of the small number of studies included in these sensitivity analyses compared to the number of studies in the corresponding main analyses. However, too

many studies did not clearly report loss to follow-up, method of inclusion of patients, or both, preventing us from performing a sensitivity analysis excluding both articles with unclear risk of selection bias as well as high risk of bias, as this restriction would have resulted in one low bias risk article only in endoscopy. Follow-up duration differed between publications, which could potentially lead to a bias in the analysis of recurrences, as this is the only truly long-term outcome. However, given that most recurrences occur early after initial surgery (with only one microscopic study reporting average time to recurrence longer than any average follow-up duration of an endoscopic study), and given that the average follow-up duration for studies reporting on disease recurrence is 1.0–15.4 years for microscopy and 1.4–5.9 years for endoscopy, the bias is probably not very large.

From a pathophysiological perspective, the similar results yielded for microscopic and endoscopic transsphenoidal surgery may be explained by the large percentage of microadenomas in the population of patients with Cushing's disease (109). For microadenomas, there may not be an advantage in increasing field of vision at the cost of losing three-dimensional vision and thereby depth perception (6, 8). Most likely, due to their small size, microadenomas are completely within the field of vision regardless of surgical technique. Our results concerning remission and recurrence for microadenomas indeed showed no clear advantage for either technique. For macroadenomas, we did show an advantage of the endoscopic surgical technique. As macroadenomas are larger and more often invasive, a broader field of vision in close proximity to the tumor may aid the neurosurgeon in achieving a complete tumor resection, causing higher remission and lower recurrence rates after endoscopic surgery. In microscopic surgery, these tumors are more often partially out of vision for the neurosurgeon. Unfortunately, due to lack of data, we were unable to perform separate analyses for invasive versus non-invasive macroadenomas, as well as for small versus larger microadenomas. The increased rate of cerebrospinal fluid leak after endoscopic transsphenoidal surgery may partially be explained by the neurosurgeon's attempt to achieve complete tumor resection also in more difficult cases with the newer endoscopic technique, whereas the reduced rate of transient diabetes insipidus may originate from the more precise tumor excision due to improved vision close to the tumor, causing less damage to the posterior lobe of the pituitary. Publication bias has been suggested as partial explanation for the increased rate of cerebrospinal fluid leak after endoscopic surgery, as more often challenging macroadenomas have been described (14).

For most patients with Cushing's disease, this study shows no clear advantage of either microscopic or endoscopic transsphenoidal surgery regarding surgical outcomes and complication rates. For macroadenomas, the endoscopic technique yields better results regarding remission and recurrence rate. These results are present despite the

presumed learning curve of the newer endoscopic technique within the study period, although confounding by indication and improved radiological investigations with time cannot be excluded. As most patients with Cushing's disease have microadenomas (109), there is no reason that all neurosurgical centers treating patients with Cushing's disease should change to the endoscopic technique. However, there is also no particular reason to keep using the microscopic technique for patients with Cushing's disease, other than neurosurgeon's preference. Based on this study, centers that choose to use the microscopic technique should consider referral of patients with Cushing's disease and a macroadenoma to another surgical center that performs endoscopic surgery.

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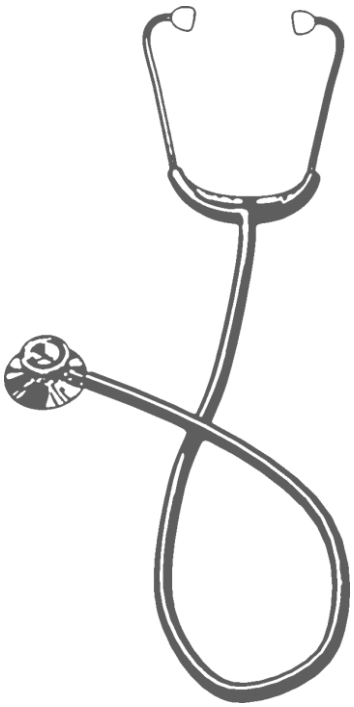
Chapter 6

Effectiveness of medical treatment for Cushing's syndrome: a systematic review and meta-analysis



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Abstract

Purpose
To systematically review the effectiveness of medical treatment for Cushing's syndrome in clinical practice, regarding cortisol secretion, clinical symptom improvement, and quality of life. To assess the occurrence of side effects of these medical therapies.

Methods
Eight electronic databases were searched in March 2017 to identify potentially relevant articles. Randomized controlled trials and cohort studies assessing the effectiveness of medical treatment in patients with Cushing's syndrome, were eligible. Pooled proportions were reported including 95% confidence intervals.

Results
We included 35 articles with in total 1,520 patients in this meta-analysis. Most included patients had Cushing's disease. Pooled reported percentage of patients with normalization of cortisol ranged from 35.7% for cabergoline to 81.8% for mitotane in Cushing's disease. Patients using medication monotherapy showed a lower percentage of cortisol normalization compared to use of multiple medical agents (49.4% versus 65.7%); this was even higher for patients with concurrent or previous radiotherapy (83.6%). Mild side effects were reported in 39.9%, and severe side effects were seen in 15.2% of patients after medical treatment. No meta-analyses were performed for clinical symptom improvement or quality of life due to lack of sufficient data.

Conclusions
This meta-analysis shows that medication induces cortisol normalization effectively in a large percentage of patients. Medical treatment for patients with Cushing's disease is thus a reasonable option in case of a contraindication for surgery, a recurrence, or in patients choosing not to have surgery. When experiencing side effects or no treatment effect, an alternate medical therapy or combination therapy can be considered.

Introduction

Cushing's syndrome due to endogenous glucocorticoid excess is either adrenocorticotropic hormone (ACTH)-dependent or ACTH-independent, both with a variety of underlying causes (1). Cushing's disease results from an ACTH-secreting pituitary adenoma and has a reported incidence of approximately 1.2–1.7 patients per million each year (2). Ectopic Cushing's syndrome is a rare condition resulting from a non-pituitary ACTH-producing source. ACTH-independent Cushing's syndrome is caused by a cortisol-producing adrenal adenoma or carcinoma (1). Excess of glucocorticoids alters body composition and metabolic profile, inducing fat maldistribution, muscle wasting, insulin resistance, dyslipidemia, hypercoagulability, and increasing the risk of osteoporosis, hypertension, and neuropsychiatric disorders (3, 4).

Transsphenoidal pituitary adenomectomy is a well-established and effective treatment for Cushing's disease (5). Cushing's syndrome is generally approached by removing the ACTH-producing tumor in ectopic Cushing's syndrome and by adrenalectomy in ACTH-independent Cushing's syndrome (6). However, there is increasing experience with first line medical treatment, both for patients with contraindications for surgery and for patients with recurrent disease (7). Furthermore, drugs can be used to control cortisol secretion preoperatively and to bridge the time period until control of hypercortisolism is achieved by radiotherapy (7). Drugs used in medical practice vary per country and underlying cause of Cushing's syndrome and include ketoconazole, metyrapone, mitotane, cabergoline, pasireotide, and mifepristone (7, 8). A recent review described the percentage of patients achieving cortisol normalization after monotherapy with the steroidogenesis inhibitors ketoconazole and metyrapone (9). However, until now no systematic review and meta-analysis has been performed to summarize the effectiveness of all medical agents used in clinical practice (ketoconazole, metyrapone, mitotane, cabergoline, pasireotide, and mifepristone).

Study aims

The primary aim of the present systematic review and meta-analysis was to evaluate the effectiveness of medical treatment for Cushing's syndrome in clinical practice. Effectiveness of medical treatment was evaluated regarding cortisol secretion, clinical symptom improvement, and quality of life. The secondary study aim was to compare these medical therapies according to occurrence of side effects.

Methods

Eligibility criteria

Randomized controlled trials and cohort studies assessing the effectiveness of FDA/EMA approved medical treatment for treatment of Cushing's syndrome, either *de novo* or with persistent or recurrent disease, were eligible for inclusion, as well as cabergoline, which has been used for Cushing's syndrome in multiple investigator initiated clinical trials. Medical agents considered were ketoconazole, metyrapone, mitotane, cabergoline, pasireotide, and mifepristone. Articles were excluded if reporting broad inclusion categories without separating the subgroup of Cushing's patients, or if the study included children (age <18 years) only. For eligibility, at least ten patients had to be included per treatment group to minimize risk of selection bias. For multiple articles describing (partially) overlapping populations, the article with the largest study population was included in the analysis. Articles irretrievable online were requested by contacting the authors. Only articles in the English language were considered.

Search strategy

To identify potentially relevant articles, PubMed, Embase, Web of Science, COCHRANE Library, CENTRAL, Emcare, LWW, and ScienceDirect were systematically searched in March 2017 in cooperation with a specialized librarian (see Supplemental Data 1 for the complete search strategy). The search was repeated in PubMed in May 2017. Furthermore, references of included articles were searched to increase the number of potentially eligible articles.

Data extraction

All identified articles were imported in EndNote 8 (Thomson Reuters, Philadelphia, PA, USA). Studies were screened by title and abstract and two independent reviewers reviewed potentially relevant articles in detail. The Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines were used for reporting (10).

From included articles we extracted the following data: number and type of patients with Cushing's syndrome included, type of medical agent used, treatment dose, treatment duration, duration of follow-up, number of patients pre-treated with medication before surgery, number of patients with normalization of cortisol, clinical improvement, well-being, quality of life, and side effects. Where available, separate outcomes were extracted for patients with primary treatment (before any other treatment for Cushing's syndrome) and patients with secondary treatment (after recurrence or failure of surgery and/or radiotherapy). For clinical improvement, all reported symptoms as well as general statements (e.g. "Clinical signs regressed in full responders", without specifying which clinical signs) were extracted. However,

only hypertension and diabetes mellitus were considered for analysis, because these symptoms were expected to be reported homogeneously by multiple articles. For quality of life, all general and Cushing's disease specific questionnaires were considered. All reported side effects were extracted.

Risk of bias assessment

We used a component approach to assess risk of bias for all included studies. The following components were included, which could potentially bias a reported association between medical treatment for Cushing's syndrome and outcome:

1. Inclusion of patients (consecutive inclusion from all patients eligible or a random sample is considered low risk of bias)
2. Loss to follow-up (<5% is considered low risk of bias)
3. Criteria for diagnosis of Cushing's syndrome adequately reported (see below)
4. Outcome measurement for cortisol normalization: urinary free cortisol, midnight salivary cortisol or a low dose dexamethasone test is considered low risk of bias
5. Reporting of outcome definition (see below)
6. Description of protocol for laboratory measurements (see below)
7. Description of dose and duration of intervention (see below)

As criteria for diagnosis of Cushing's syndrome vary widely over time and even by study center, and per underlying etiology, adequately reporting the criteria used for diagnosis is considered a low risk of bias. Reporting of outcome definition is considered adequate if the article at least mentioned which outcome was studied, which test was used to determine the outcome, and if applicable, which cortisol level had to be measured. Description of protocol for laboratory measurements is considered adequate if the assay used for measuring cortisol is reported, or the assay for the main outcome if this was not cortisol. Description of dose and duration of intervention is considered adequate if dose (per day or per week) and duration of medical treatment are reported (average and range, or exact dose and duration if this is equal for all patients). Also considered adequate is reporting of exact treatment protocol for trials with dose increase based on cortisol levels. Referring to another published article in which the information is reported is also considered adequate.

Risk of bias assessment was conducted to explore potential heterogeneity. As there were no studies that compared two different medical agents directly, confounding was not judged at the study level, but was assessed by comparing baseline characteristics for all included studies.

Study endpoints

Primary outcome of this study was the effectiveness of medical treatment for Cushing's syndrome, represented by the pooled percentage of patients reaching normalization of cortisol secretion (definition according to the authors) after medical treatment, patients showing symptom improvement, improved well-being and improved quality of life. Main analyses were performed in studies reporting on pituitary Cushing patients. Separate analyses were performed for publications reporting on (1) mixed etiologies other than adrenocortical carcinomas, (2) mixed etiologies including adrenocortical carcinomas, and (3) ectopic Cushing only, if sufficient data were available. Studies were categorized according to type of medical agent (ketoconazole, metyrapone, mitotane, cabergoline, pasireotide, and mifepristone). Studies using more than one of the above mentioned medical agents at the same time or consecutively were assessed separately.

For normalization of cortisol, all measurements of cortisol were considered. However, measurement of urinary free cortisol, midnight salivary cortisol or morning cortisol after a low dose dexamethasone test was considered low risk of bias (see above). Data on reduction of cortisol as a percentage of baseline were not considered for analysis.

Secondary outcomes were the pooled percentages of patients with mild or severe side effects stratified by medical agent. Severe side effects were considered those that required therapy adjustment or withdrawal, as well as all side effects categorized as severe by the authors. Mild side effects were all not categorized as severe. For articles reporting only specific side effects, the side effect that affected the most patients was included in the analysis.

Subgroup analyses were performed according to indication (primary therapy, including pre-treatment before surgery, and therapy for recurrence) if described separately. As few studies provided separate data for primary/secondary analysis, a separate subgroup analysis was performed, in which studies were categorized as low (≤20%) or high (≥80%) percentage of patients using medical agents as pre-treatment before surgery. A separate analysis was performed for normalization of cortisol according to the presence of multiple medical treatments and concurrent or previous radiotherapy.

Statistical analysis

A random-effects logistic regression model was used to pool percentages for analyses including ≥5 articles, whereas a fixed-effects logistic regression model was used for analyses including <5 articles. All pooled percentages are accompanied by 95% confidence intervals (CI). The Freeman-Tukey arcsine transformation was used to

stabilize variances, in order to prevent exclusion of studies with 0% or 100% as outcome. All analyses were performed using Stata 11.2 (Stata Corp., College Station, TX, USA).

Sensitivity analyses were performed for normalization of cortisol for low risk of bias studies, and for the combination of low and intermediate risk of bias studies. Articles were considered low risk of bias if they adhered to at least six (out of seven) of the above-mentioned criteria for risk of bias. Only one article adhered to all seven criteria (11). Articles were considered intermediate risk of bias if they adhered to five of the above-mentioned criteria for risk of bias.

Results

Study selection

The initial search identified 960 potentially relevant articles. Searching through references of included articles identified one additional article, thereby yielding a total of 961 articles. By screening these articles by title and abstract, 874 articles were excluded. The remaining 87 articles were reviewed in detail. Reasons for exclusion are summarized in Figure 1. In total, 35 articles were included, reporting on six different drugs.

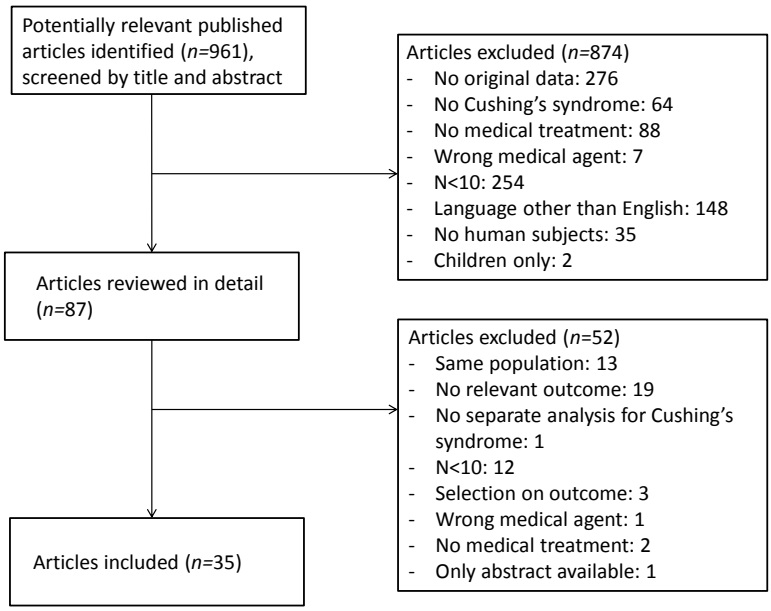

Figure 1: Flow-chart of inclusion of articles in this systematic review.

Study characteristics (Supplemental Data 2)
We included five studies on mitotane (12–16), two on pasireotide (17, 18), three on cabergoline (19–21), eight on ketoconazole (22–29), five on metyrapone (30–34), two on mifepristone (35, 36), and ten on multiple medical agents (11, 37–45). Studies were published between 1971 and 2017. There were eleven single-arm trials, two randomized trials with two treatment arms (pasireotide 600 µg versus pasireotide 900 µg; first cabergoline, then add ketoconazole versus first ketoconazole, then add cabergoline), and 22 cohort studies. We included eighteen studies on Cushing's disease only, two on ectopic Cushing's syndrome only, and fifteen on patients with Cushing's syndrome due to various underlying etiologies. In total, 1,520 patients were included. There were 28 articles measuring normalization of cortisol by at least urinary free cortisol, midnight salivary cortisol or a low dose dexamethasone test (88% of 32 articles reporting normalization of cortisol as an outcome). There were 25 studies reporting on clinical improvement, and three studies reporting on quality of life.

Baseline characteristics of included studies show clear differences between studies. Reported average age varied between 32.2 and 60.0 years. Percentage female varied between 21.7% and 95.0%. Average duration of follow-up was 2 weeks to 11.5 years. Seven articles reported that at least part of their study population received radiotherapy in addition to medical treatment (two with mitotane, one with ketoconazole, and four with metyrapone). Pasireotide and cabergoline were used only for Cushing's disease, whereas all other medical agents were used for various etiologies of Cushing's syndrome.

Risk of bias assessment (Supplemental Data 3)
Loss to follow-up (reported in twelve studies [34%]) ranged from 0% to 60%. Inclusion of consecutive patients or a random sample was explicitly stated in seventeen articles (49%). Criteria for diagnosis of Cushing's syndrome were adequately reported in 31 articles (89%). Reporting of outcome definition was adequate in 25 articles (71%). Description of protocol for laboratory measurements was adequate in 23 articles (66%). Description of dose and duration of intervention was adequate in 23 articles (66%). A total of nine articles were with low risk of bias (adherent to at least six out of seven criteria) and another nine articles with intermediate risk of bias (adherent to five out of seven criteria).

Study outcomes
For 26 articles (1,000 patients) normalization of cortisol was reported as outcome measure. There were 25 articles reporting on clinical improvement. One article reported no improvement in any of the measured clinical symptoms (weight, blood pressure, glucose, and HbA1c) (19). All other articles reported improvement in one or

more clinical symptoms. Well-being was not reported by any of the included articles. Three articles (228 patients) reported on quality of life. These reported an improvement in CushingQoL score, SF-36 score, and emotional reaction on the Nottingham Health Profile (NHP), and on the other hand more pain measured by the RAND-36 (18, 36, 44). There were 30 articles (86%) reporting at least one side effect. Two of these articles, using mifepristone, described an increase in cortisol levels during the study period in a total of 47 out of 70 included patients (67%) (35, 36). No meta-analysis was performed for clinical improvement, as results were considered too heterogeneous. Hypertension and diabetes mellitus were described by heterogeneous articles (type of medical agent, etiology of Cushing's syndrome) and the type of outcome was heterogeneous (difference in blood pressure and glucose versus number of patients with improved values, only patients with disturbed values at start of the study versus all patients analyzed). Detailed study outcomes at the individual study level are reported in Supplemental Data 4.

Meta-analyses of normalization of cortisol (Table 1; Figure 2: normalization of cortisol per medical agent in pituitary Cushing)

For Cushing's disease, pooled reported treatment effect ranged from 32.3% if a large percentage of patients used medication as pre-treatment before surgery, to 83.6% if medication was combined with radiotherapy. When comparing medical agents, a relatively high percentage of patients using mitotane showed normalization of cortisol (81.8%), whereas treatment with cabergoline and pasireotide less often normalized cortisol secretion (35.7% and 41.1%). For detailed results, including data on different etiologies (mixed etiologies other than adrenocortical carcinomas, mixed etiologies including adrenocortical carcinomas, and ectopic Cushing), see Table 1.

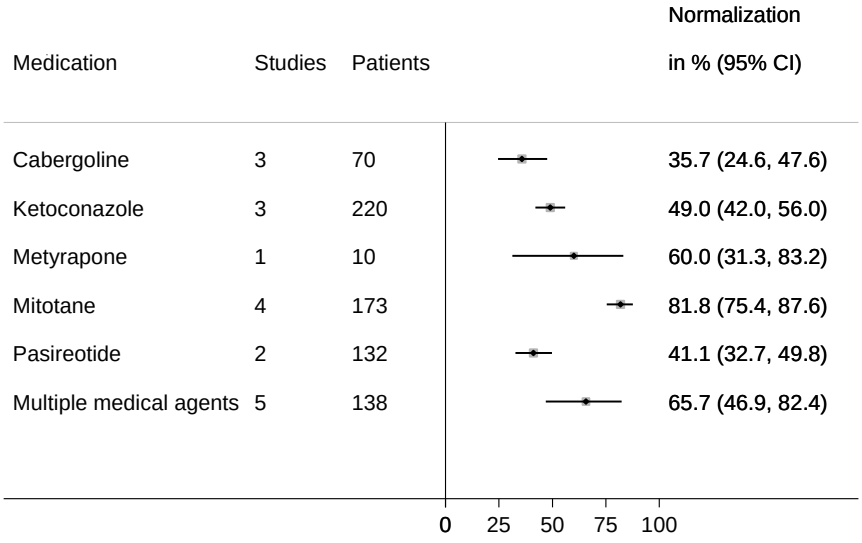

Figure 2: Meta-analysis of normalization of cortisol after medical treatment in Cushing disease.

Table 1: Results of meta-analyses according to etiology of Cushing's syndrome.

	Cushing's disease		All etiologies (pituitary, adrenal, ectopic) other than adrenal carcinoma		All etiologies (pituitary, adrenal, ectopic) including adrenal carcinoma		Ectopic Cushing's syndrome	
	%	95% CI (N)	%	95% CI (N)	%	95% CI (N)	%	95% CI (N)
Normalization of cortisol	59.5	48.4-70.1 (18)	61.6	51.8-71.0 (22)	64.7	55.6-73.3 (26)	83.3	64.9-96.8 (3)
Per medical agent								
- *Mitotane*	81.8	75.4-87.6 (4)	81.8	75.4-87.6 (4)	79.8	73.3-85.7 (4)	-	-
- *Pasireotide*	41.1	32.7-49.8 (2)	41.1	32.7-49.8 (2)	41.1	32.7-49.8 (2)	-	-
- *Cabergoline*	35.7	24.6-47.6 (3)	35.7	24.6-47.6 (3)	35.7	24.6-47.6 (3)	-	-
- *Ketoconazole*	49.0	42.0-56.0 (3)	49.3	42.6-56.0 (4)	71.1	51.6-87.5 (7)	-	-
- *Metyrapone*	60.0[a]	31.3-83.2[a]	75.9	57.5-90.9 (2)	75.9	57.5-90.9 (2)	-	-
- *Mifepristone*	-	-	-	-	-	-	-	-
- *Multiple medical agents*	65.7	46.9-82.4 (5)	67.8	51.9-81.9 (7)	67.6	53.6-80.3 (8)	-	-
Primary treatment	58.1	49.7-66.2 (4)	49.4	41.3-57.5 (4)	49.4	41.3-57.5 (4)	-	-
Secondary treatment	57.8	41.3-73.6 (5)	48.6	41.2-56.1 (4)	48.6	41.2-56.1 (4)	-	-
Per percentage pretreatment								
- *≤20%*	59.7	49.4-69.6 (8)	59.7	49.4-69.6 (8)	59.7	49.4-69.6 (8)	-	-
- *≥80%*	32.3	20.0-45.8 (2)	42.6	33.5-51.9 (3)	53.6	45.0-62.0 (4)	-	-
Adjuvant treatment								
- *No other treatment*	49.4	36.0-62.9 (10)	52.7	40.1-65.1 (12)	57.2	44.4-69.6 (14)	-	-
- *Multiple medical agents*	65.7	46.9-82.4 (5)	67.8	51.9-81.9 (7)	67.6	53.6-80.3 (8)	-	-
- *Radiotherapy*	83.6	75.5-90.4 (3)	83.6	75.5-90.4 (3)	84.8	78.0-90.6 (4)	-	-
Sensitivity analysis (low risk of bias)								
- *Mitotane*	71.6[a]	59.9-81.0[a]	71.6[a]	59.9-81.0[a]	71.6[a]	59.9-81.0[a]	-	-
- *Pasireotide*	17.2[a]	7.6-34.5[a]	17.2[a]	7.6-34.5[a]	17.2[a]	7.6-34.5[a]	-	-
- *Cabergoline*	35.0	20.6-50.9 (2)	35.0	20.6-50.9 (2)	35.0	20.6-50.9 (2)	-	-
- *Ketoconazole*	-	-	-	-	-	-	-	-
- *Metyrapone*	-	-	-	-	-	-	-	-
- *Mifepristone*	-	-	-	-	-	-	-	-
- *Multiple medical agents*	50.6	40.9-60.2 (3)	50.6	40.9-60.2 (3)	50.6	40.9-60.2 (3)	-	-

Table 1: Results of meta-analyses according to etiology of Cushing's syndrome (continued).

	Cushing's disease		All etiologies (pituitary, adrenal, ectopic) other than adrenal carcinoma		All etiologies (pituitary, adrenal, ectopic) including adrenal carcinoma		Ectopic Cushing's syndrome	
	%	95% CI (N)	%	95% CI (N)	%	95% CI (N)	%	95% CI (N)
Sensitivity analysis (low and intermediate risk of bias)								
- *Mitotane*	71.6[a]	59.9-81.0[a]	71.6[a]	59.9-81.0[a]	71.6[a]	59.9-81.0[a]	-	-
- *Pasireotide*	17.2[a]	7.6-34.5[a]	17.2[a]	7.6-34.5[a]	17.2[a]	7.6-34.5[a]	-	-
- *Cabergoline*	35.7	24.6-47.6 (3)	35.7	24.6-47.6 (3)	35.7	24.6-47.6 (3)	-	-
- *Ketoconazole*	48.5[a]	41.7-55.4[a]	48.3	41.5-55.2 (2)	59.8	54.1-65.4 (4)	-	-
- *Metyrapone*	-	-	83.3[a]	60.8-94.2[a]	83.3[a]	60.8-94.2[a]	-	-
- *Mifepristone*	-	-	-	-	-	-	-	-
- *Multiple medical agents*	53.1	43.8-62.3 (4)	67.5	46.5-85.7 (5)	67.4	50.0-82.8 (6)	-	-
Mild side effects	39.9	25.0-55.8 (13)	40.2	27.4-53.8 (15)	35.3	25.6-45.7 (23)	-	-
Per medical agent								
- *Mitotane*	68.5	59.1-77.2 (3)	68.5	59.1-77.2 (3)	69.1	60.0-77.6 (3)	-	-
- *Pasireotide*	58.3	51.4-65.1 (2)	58.3	51.4-65.1 (2)	58.3	51.4-65.1 (2)	-	-
- *Cabergoline*	24.0	14.4-35.1 (3)	24.0	14.4-35.1 (3)	24.0	14.4-35.1 (3)	-	-
- *Ketoconazole*	-	-	-	-	22.6	15.1-31.0 (3)	-	-
- *Metyrapone*	30.8[a]	12.7-57.6[a]	32.2	16.3-50.3 (2)	19.7	12.2-28.2 (3)	-	-
- *Mifepristone*	-	-	-	-	35.6	24.5-47.4 (2)	-	-
- *Multiple medical agents*	18.0	10.6-26.7 (4)	25.5	7.6-48.5 (5)	26.7	12.8-43.2 (7)	-	-
Severe side effects	15.2	9.1-22.4 (12)	16.2	10.1-23.3 (14)	15.3	10.1-21.3 (21)	-	-
Per medical agent								
- *Mitotane*	28.4[a]	19.0-40.1[a]	28.4[a]	19.0-40.1[a]	28.4[a]	19.0-40.1[a]	-	-
- *Pasireotide*	15.7	10.9-21.2 (2)	15.7	10.9-21.2 (2)	15.7	10.9-21.2 (2)	-	-
- *Cabergoline*	4.8	0.5-11.9 (3)	4.8	0.5-11.9 (3)	4.8	0.5-11.9 (3)	-	-
- *Ketoconazole*	20.5[a]	15.5-26.6[a]	18.8	13.5-24.6 (2)	14.2	10.3-18.7 (4)	-	-
- *Metyrapone*	7.7[a]	1.4-33.3[a]	27.1	12.2-44.7 (2)	16.2	9.4-24.3 (3)	-	-
- *Mifepristone*	-	-	-	-	42.0	30.4-54.0 (2)	-	-
- *Multiple medical agents*	20.9	13.6-29.2 (4)	20.9	13.6-29.2 (4)	14.6	4.2-28.8 (6)	-	-
Result of severe side effect								
- *Adjust therapy*	23.9	15.9-32.8 (4)	23.6	10.0-40.4 (6)	20.4	8.6-35.2 (9)	-	-
- *Stop therapy*	8.5	2.8-16.3 (9)	8.5	2.8-16.3 (9)	8.5	4.6-13.4 (15)	-	-

[a]No meta-analysis was performed, as the total number of studies equaled 1 (results only shown for analyses per medical agent if other medical agents were reported in multiple articles). CI=confidence interval, N=number of articles included in meta-analysis.

Seven studies reported data separately for medication as primary (n=4) and/or secondary therapy (n=5). For patients with Cushing's disease, medication as primary therapy normalized cortisol in 58.1% (95% CI: 49.7%–66.2%), similar to the effect of medication as secondary therapy, 57.8% (95% CI: 41.3%–73.6%). Articles in which ≤20% of patients were medically pre-treated before surgery showed normalization of cortisol in 59.7% of patients (95% CI: 49.4%–69.6%). Articles in which ≥80% of patients were pre-treated with medication before surgery showed a preoperative normalization of cortisol in 32.3% (95% CI: 20.0%–45.8%) for patients with Cushing's disease. Patients with medical monotherapy showed a relatively low percentage of cortisol normalization. This percentage was higher for patients using multiple agents, and highest for patients with concurrent or previous radiotherapy.

The sensitivity analyses, both excluding articles with high risk of bias (n=18 included), as well as excluding articles with high and intermediate risk of bias (n=9 included), showed similar results as the main analysis. The most remarkable difference is that lower percentages of patients with normalization of cortisol were seen for multiple medical agents in both sensitivity analyses than for multiple medical agents in the main analysis.

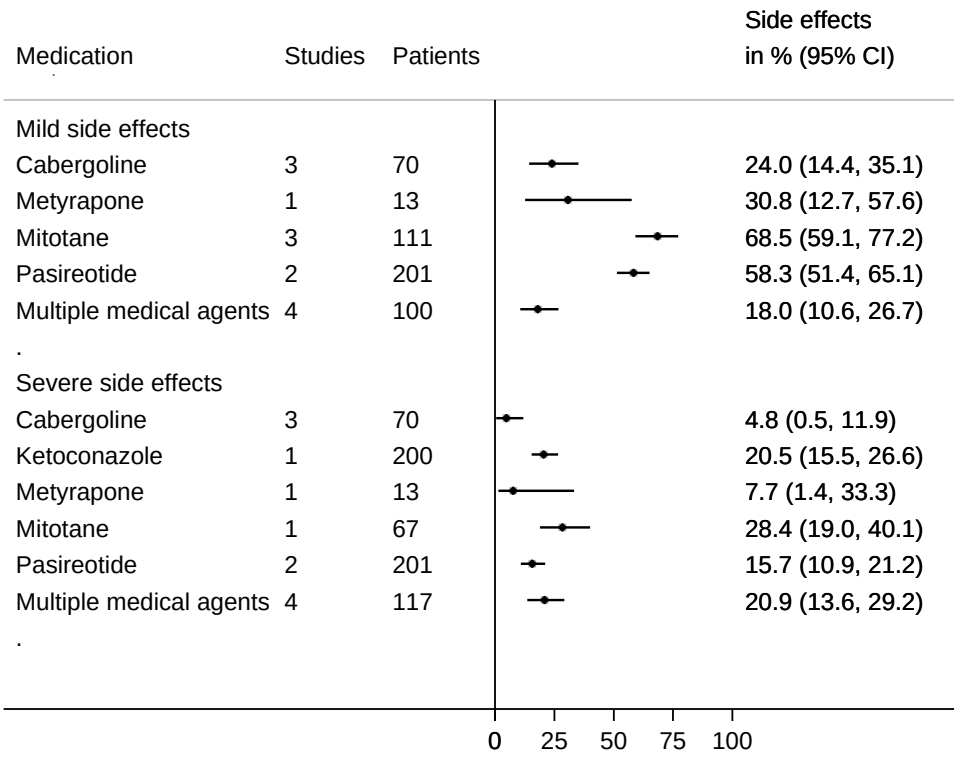

Figure 3: Meta-analysis of side effects after medical treatment in Cushing disease.

Meta-analyses of side effects (Table 1; Figure 3: side effects per medical agent in pituitary Cushing)
For Cushing's disease, mild side effects were reported in 39.3% (95% CI: 25.0%–55.8%) of patients after medical treatment. Patients using mitotane and pasireotide more often showed mild side effects compared to the total population (68.5% and 58.3%).

For Cushing's disease, severe side effects were seen in 15.2% (95% CI: 9.1%–22.4%). In the group of mixed etiologies including adrenocortical carcinoma, patients using mifepristone showed severe side effects relatively often (42.0%; 95% CI: 30.4%–54.0%). Due to the severe side effects, 23.9% (95% CI: 15.9%–32.8%) of patients adjusted their medical therapy, and 8.5% (95% CI: 2.8%–16.3%) of patients stopped their medical therapy.

Discussion

We performed a systematic review and meta-analysis to evaluate effectiveness of medical treatment in routine clinical practice in Cushing's syndrome. Medical treatment was effective in normalizing cortisol levels in Cushing's syndrome in 35.7% (cabergoline) to 81.8% (mitotane) of patients. Furthermore, the combined use of medical agents at the same time or consecutively increased the percentage of patients with normalized cortisol secretion (65.7%). Importantly, medical agents for hypercortisolism can cause severe side effects, leading to therapy adjustment or withdrawal in 4.8% (cabergoline) to 28.4% (mitotane) of patients. These results suggest that medical therapy can be considered a reasonable treatment alternative to the first-choice surgical treatment when regarding treatment effectiveness and side effects.

This study is the first systematic review and meta-analysis of all medical agents currently used in clinical practice for Cushing's syndrome. Only one previous study performed a systematic review and meta-analysis of two medical agents in Cushing's syndrome. Daniel *et al.* studied normalization of cortisol after monotherapy with ketoconazole or metyrapone, and found that urinary free cortisol normalized in 60% (ketoconazole) and that normalization of hypercortisolism as defined by the authors occurred in 75% of patients using metyrapone (9). This is in line with the results obtained in the current study (49.0%–71.1% for ketoconazole and 60.0%–75.9% for metyrapone depending on patient categories). Since our last search in May 2017, one more research article on medical treatment in Cushing's syndrome was published. Lacroix *et al.* described a 12-month clinical trial using pasireotide in various dosages in 150 patients. Urinary free cortisol normalized in 41.3% of patients. This is in line with our own results for cortisol excretion normalization for patients using

pasireotide (41.1%). Adverse events grade 1–2 were described in up to 48% of patients, and adverse events grade 3–4 in up to 16% of patients. This is in line with our own results for mild side effects (58.3%) and severe side effects (15.7%) for patients using pasireotide (46).

In interpreting the results, the following study limitations need to be taken into account. There was a large amount of heterogeneity in included studies, regarding medical agent, etiology of Cushing's syndrome, indication for use of medical therapy for Cushing (presurgical cortisol control, contra-indication for surgery, post-surgical therapy failure or recurrence), outcome measurement (definition of cortisol normalization), and concurrent or previous use of radiotherapy. Heterogeneity concerning medical agent and etiology was handled by performing separate analyses per medical agent and per etiology, although this substantially reduced the number of articles in some categories. Due to heterogeneity regarding outcome measurement for clinical symptoms (difference in blood pressure and glucose versus number of patients with improved values, only patients with disturbed values at start of the study versus all patients analyzed), we were unable to perform a quantified analysis of clinical improvement after medical therapy. Included articles showed various levels of risk of bias. However, sensitivity analyses excluding articles with high risk of bias or with high and intermediate risk of bias showed similar results to the main analysis.

For patients with Cushing's disease, first line transsphenoidal surgery yields better results than medical therapy (80% remission). However, remission after a repeat surgery was shown to be only 42.6%–55.7% in a recent meta-analysis. Various complications occurred in up to 18.5% of patients (47). This underlines that medical treatment is a reasonable alternative to repeat surgical procedure.

Differences in effectiveness and side effects between the various etiological groups were small. In ectopic Cushing's syndrome only, a higher percentage of patients reached normalization of cortisol levels than in the other etiological categories. As there were only three articles with separate data on patients with ectopic Cushing's syndrome (of which one article only reported on one patient with ectopic Cushing's syndrome, besides reporting on Cushing patients with other etiologies), no further subanalyses were possible, and reliability of this result is uncertain. However, we found no explanation for this high percentage of cortisol normalization in ectopic Cushing's syndrome when considering risk of bias, type of medical agent, or additional treatment.

When comparing different medical agents, it seems that a high percentage of patients with cortisol normalization corresponds to a high percentage of patients with

side effects and vice versa. However, all dosages used and studied were within the boundaries advised by the European Medicines Agency (EMA) and the Food and Drug Administration (FDA). It might mean that advised and commonly used dosages are in fact not the optimal dosages for treatment of Cushing's syndrome considering the balance between treatment effect and side effects. Furthermore, medical agents with a high percentage of patients with normalized cortisol secretion (mitotane and metyrapone) are relatively often combined with radiotherapy, which may lead to overestimating the effect and side effects of the drug *per se*. The use of combined medical therapy, in combination or consecutively, increases the likelihood of successful treatment, i.e. in normalizing cortisol levels. This suggests that sensitivity for different medical agents may vary per patient.

No difference was shown in normalization of cortisol between patients using medical agents as primary treatment versus secondary treatment, suggesting that effectiveness of medical agents is independent of other treatment modalities. A higher percentage of patients reached normalization of cortisol in studies where a small part of included patients received medication preoperatively (including patients with contraindications to surgery and patients after previous surgery) than in studies where a large part of included patients received medication before elective surgery. The most likely explanation is that in patients with planned surgery, medication is given to control cortisol excess, and surgery is performed before complete normalization of cortisol occurs. Unfortunately, we do not know the average time until cortisol normalization, as this was not reported by most articles. Total follow-up time was not different for studies with a high percentage of medical pre-treatment before surgery (average 0.1–11.5 years) than for all included studies. It would be interesting to know if the effectiveness of surgery is dependent upon the normalization of cortisol with medical treatment before surgery compared to presurgical medical treatment without cortisol normalization and compared to no presurgical medical treatment at all. Especially in Cushing's disease, the percentage of patients with preoperative medical treatment that reaches cortisol normalization is low. This might be due to the higher expectations of surgery in Cushing's disease compared to other etiologies, which may be why surgery is performed before patients reach normalization of cortisol levels. However, these results should be interpreted with caution, as a small number of articles were included in these analyses, and in the articles with a mixed etiologies population, a large proportion of included patients had Cushing's disease (23, 43).

Based on the current study, medication can be regarded a valuable alternative to pituitary surgery for patients with Cushing's disease with contraindications to surgery, patients with a recurrence considering repeat surgery, and patients that choose not to undergo surgery. For all other patients with Cushing's disease, pituitary

surgery remains the first-choice treatment. For other etiologies of Cushing's syndrome, at present, there is insufficient evidence to recommend when to use medical treatment to lower cortisol levels. However, from the total group of patients, it suggests that medical agents have similar effectiveness in normalizing cortisol levels for all etiologies of Cushing's syndrome. For a higher chance of treatment success, a different medical agent could be tried if there is no treatment effect or if the patient experiences side effects. There is no evidence for which drug should be used first. Based on the current study, mitotane or metyrapone seem to be most effective in normalizing cortisol levels, but also cause the highest percentage of patients with side effects.

In conclusion, we consider medical treatment for Cushing's disease a reasonable option in patients with contraindication to surgery, with a recurrence, or that choose not to have surgery. Patients that experience side effects or no treatment effect should be advised to start treatment with a different medical agent to increase the chance of treatment success.

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Part III

Clinical outcome in Cushing's syndrome

Chapter 7

Sex differences in presentation but not in outcome for ACTH-dependent Cushing's syndrome

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Submitted

Abstract

Background
Sex differences in the clinical picture of adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome are controversial, except for the known higher prevalence in females. We compared a broad range of potential differences to enable a more accurate understanding of the clinical picture of sex-specific ACTH-dependent Cushing's syndrome.

Methods
Cohort study including consecutive patients with ACTH-dependent Cushing's syndrome from Leiden and Berlin diagnosed between 2000-2016, comparing clinical presentation, biochemical parameters, diagnostic tests, surgical outcome, and comorbidities between men and women.

Results
We included 130 patients: 37 males and 93 females. With similar cortisol concentrations, ACTH concentrations were higher in males than females at time of diagnosis (median: 116 ng/L versus 57 ng/L). The prevalence of osteoporosis was higher in males than in females (48.6% versus 25.0%), persisting after surgery, with more vertebral fractures (16.2% versus 5.4%) before surgery. Males showed more anemia (75.9% versus 36.8%) after surgery. There were no differences in etiology, pituitary tumor size, diagnostic and therapeutic strategy, or surgical outcome between sexes.

Conclusions
Based on this study, males and females with ACTH-dependent Cushing's syndrome present different clinical patterns. However, these differences do not justify different diagnostic strategies or treatment based on sex, considering the similar surgical outcome. Clinicians should be alert to diagnose accompanying osteoporosis (with fractures) in male patients with ACTH-dependent Cushing's syndrome.

Introduction

Cushing's syndrome is characterized by endogenous glucocorticoid excess, either adrenocorticotropic hormone (ACTH)-dependent or ACTH-independent, both with a variety of underlying causes (1). The vast majority of patients has Cushing's disease caused by an ACTH-secreting pituitary adenoma, with an estimated incidence of 1.2-1.7 per million each year (2). First-choice treatment for Cushing's disease is transsphenoidal pituitary surgery, selectively removing the corticotroph adenoma (3). Ectopic Cushing's syndrome is a rare condition resulting from a non-pituitary ACTH-producing source, and is generally approached by removing the ACTH-producing tumor, if identified and resectable. Excess of glucocorticoids causes osteoporosis, central obesity, insulin resistance, dyslipidemia, hypertension, hypercoagulability and neuropsychiatric disorders (4, 5). Despite biochemical cure, mortality risk remains increased in patients with Cushing's disease (6).

Sex distribution differs markedly between different etiologies. There is consensus that Cushing's disease occurs up to five times more often in females, and that male patients with ACTH-dependent Cushing's syndrome have a relatively higher risk of an ectopic ACTH-secreting tumor. However, exact figures are lacking and a pathophysiological explanation for the sex distribution is absent (7, 8). Interestingly, the female preponderance is not yet present in prepubertal cases, suggesting that males are diagnosed with Cushing's syndrome at a younger age (9, 10). This is confirmed by some studies in adults with Cushing's syndrome (11, 12), but rejected by others (13, 14). Some cohort studies have reported males to have more severe clinical presentation (higher body mass index and waist circumference, reduced libido and sexual dysfunction, more striae, myopathy, and hypokalemia), biochemical parameters (higher ACTH, serum cortisol, and urinary free cortisol [UFC] concentrations), complications at diagnosis (higher HbA1c concentrations, more often hypertension, anemia, spine osteoporosis with vertebral fractures, rib fractures, and hypercoagulable state), and worse outcome after surgery (more often anemia, lower cortisol normalization rate, and higher recurrence rate), than females (8, 9, 11-16). Pituitary tumors were less easily visualized by pituitary magnetic resonance imaging (MRI) in males (12, 13). One study reported more macroadenomas, with higher invasion and apoplexy rate (11), whereas another study reported equal percentages of macroadenomas in males and females (12). No differences were found in quality of life between males and females on multiple validated questionnaires (CushingQoL, EQ-VAS, and SCL-90-R) (8, 11, 17). Table 1 summarizes the reported literature regarding sex differences (also reported as gender differences) in Cushing's syndrome (8-18).

Table 1: Studies on sex differences in Cushing's syndrome.[#]

Author	Year	Study center, country	Number of patients (male/ female)	Type of patients	Mean age (years)	Study outcome: sex differences
Studies with sex differences as primary study aim:						
Ambrogio	2014	Milan, Italy	80 (17/63)	Cushing's disease	39.1 (range: 15-62)	Male: lower mean hemoglobin and RBC values than healthy subjects, with increased MCV. RBC and hemoglobin correlated to testosterone levels. After surgery more anemic, slower normalization of hemoglobin. Both sexes: hemoglobin reduced 1.5-2x after surgery, regardless of surgical outcome. MCV decreased 3 months after surgery.
Huan	2014	Ji'nan, China	87 (23/64)	Cushing's disease	43.33 (range: 23-65)	Male: younger age at diagnosis, larger adenoma diameter, higher invasion rate and apoplexy rate, more osteoporosis, hypokalemia, sexual dysfunction, and hypertension, higher preoperative and postoperative (six months after surgery) cortisol levels, and a higher recurrence rate (30.4% vs. 7.8%). Both sexes: no differences in CushingQoL scores. Cortisol values 3 days postoperatively lower than before and 6 months after surgery.
Libuit	2015	Bethesda, USA	102 (54/48)	Cushing's disease, only pediatric patients	12.9 (SD: 3.0)	Male: more likely to present with higher BMI Z-score, lower height Z-score, higher plasma ACTH. Female: no specific signs or symptoms more often at presentation, increased risk of metabolic syndrome based on LDL at presentation. Both sexes: no difference in cure rate, in all patients decrease in prevalence of metabolic syndrome after surgery, equal tumor size.
Liu	2015	Shanghai, China	73 (13/60)	Cushing's disease	Male: 30.0 (range: 14-64) Female: 33.5 (range: 16-62)	Male: significantly higher ACTH (not explained by tumor size), BMI, HbA1c, ALT, AST, GGT, systolic blood pressure, and hemoglobin, more frequently purple striae, more fatty liver assessed by ultrasound. Both sexes: no differences in plasma cortisol, no difference in age.
Milian	2014	Tübingen, Germany	72 (11/61)	Cushing's disease, only patients biochemically cured	45.9 (range: 22-76)	Male: prolonged time to diagnosis strong predictive factor for worse psychopathological status (multiple dimensions, SCL-90-R). Both sexes: no significant difference in the frequency of psychopathology (in any of the dimensions). Presence of hypocortisolism was associated with phobic anxiety in male and psychoticism in female patients. Hypopituitarism was correlated with somatization in male and psychoticism in female patients.
Pecori Giraldi	2003	Milan, Italy	280 (47/233)	Cushing's disease	Male: 30.5 (SEM: 1.93) Female: 37.0 (SEM: 0.86)	Male: presentation at younger age, higher UFC and ACTH, lower sensitivity of high dose dexamethasone test, more symptoms indicative of hypercatabolic state (osteoporosis, muscle wasting, striae, nephrolithiasis), more often negative pituitary imaging, and immediate and late surgical outcome less favorable (lower surgical success rate and more frequent recurrence). Both sexes: similar time interval between appearance of first symptoms of hypercortisolism and diagnosis. Same percentage macroadenoma.

Table 1: Studies on sex differences in Cushing's syndrome (continued).[#]

Author	Year	Study center, country	Number of patients (male/ female)	Type of patients	Mean age (years)	Study outcome: sex differences
Rockall	2003	London, UK	31 (7/24)	Cushing's disease (n=20), adrenal Cushing's syndrome (n=5), ectopic Cushing's syndrome (n=3), unknown etiology (n=3)	45 (range: 17-79)	Male: significant increase in the V:S (visceral fat:subcutaneous fat) ratio compared with non-cushingoid controls (control data from literature). Female: significant increase in the V:S ratio compared with non-cushingoid controls. Both sexes: There was no difference in the V:S ratio between male and female patients.
Storr	2004	London, UK	50 (21/29)	Cushing's disease, only patients ≤30 years	18.0 (range: 6.4-30.0)	In patients 18 years of age or younger, there was no difference in the severity of hypercortisolemia or ACTH at diagnosis between males and females.
Studies with sex differences as secondary study aim:						
Zilio	2014	Padova, Italy	84 (17/67)	Cushing's disease	42.2 (range: 15-70)	Male: higher UFC and ACTH values, lower ACTH response to DDAVP stimulation. Pituitary tumor less easily visualized by pituitary MRI. More frequent or more severe complications, in particular hypokalemia, hypercoagulable state, and osteoporosis at lumbar spine, with consequent higher risk of vertebral fractures. Male sex was an independent risk factor for dyslipidemia, severity of hypertension, lumbar osteoporosis and fractures. Both sexes: No differences in age at diagnosis, disease duration and BMI. The prevalence of hypogonadism did not significantly differ.
Patil	2007	Stanford, USA	3525 (649/2871)	Cushing's disease	Reported only in categories: <18: 147 18-44: 2246 45-64: 966 >64: 161	Women were less likely than men to have an adverse outcome (OR 0.3).
Valassi	2011	Multicenter (Europe)	481 (91/391)	Cushing's disease (n=317), adrenal Cushing's syndrome (n=130), ectopic Cushing's syndrome (n=24), other etiology (n=10)	44.2 (range: 15-84)	Male: significantly higher proportion ectopic Cushing's syndrome than other etiologies. Reduced libido more prevalent than in women. Higher prevalence of spine osteoporosis, and more vertebral and rib fractures. Mean waist significantly higher. Hypertension (83%), myopathy (71%), and reduced libido (69%) more common. Female: weight gain more common. Both sexes: no difference in the specialists consulted (before correct diagnosis) (other than gynecologists). Mean CushingQoL and EQ-VAS score not different.

[#]All mentioned studies were cohort studies.

ACTH=adrenocorticotropic hormone, ALT=alanine aminotransferase, AST=aspartate aminotransferase, BMI=body mass index, CushingQoL=Cushing Quality of Life questionnaire, EQ-VAS=EuroQoL-visual analogue scale, GGT=gamma-glutamyltransferase, HbA1c=glycated hemoglobin, LDL=low-density lipoprotein, MCV=mean corpuscular volume, OR=odds ratio, RBC=red blood cell, SCL-90-R: Symptom Checklist-revised, SD=standard deviation, SEM=standard error of the mean, UFC=urinary free cortisol

Potential differences between males and females could for example be based on: 1) Different concentrations of corticosteroid binding globulin (CBG) (19), 2) Different interaction between corticosteroids and the gonadotroph axis, and 3) Different disease entity of Cushing's syndrome. A large cohort study focusing on a broad range of potential differences between both sexes is needed to confirm the sometimes conflicting results from previous small studies focusing on a limited number of aspects, to enable a more accurate understanding of the clinical picture of sex-specific ACTH-dependent Cushing's syndrome.

Study aims

To compare the phenotype of male and female patients with ACTH-dependent Cushing's syndrome regarding: 1) Clinical presentation, 2) Biochemical parameters and diagnostic test results, 3) Surgical outcome (i.e. percentage remission, hydrocortisone dependency, recurrence, and mortality), and comorbidities. Based on previous literature, we hypothesized that males show more symptoms related to hypercortisolism at diagnosis, with higher concentrations of ACTH and cortisol, and more comorbidity at diagnosis, specifically hypertension, anemia, and osteoporosis. We also hypothesized that males show worse outcome after surgery regarding remission and recurrence rate.

Methods

Study population

Consecutive patients with ACTH-dependent Cushing's syndrome from the Leiden University Medical Center and the Charité Universitätsmedizin Berlin were included. Only patients with a diagnosis from January 1st 2000 onwards were included, as this guaranteed data collection from equal time periods for both centers. There were no restrictions regarding treatment (transsphenoidal surgery, adrenalectomy, radiotherapy, medical treatment, and ectopic tumor resection).

The process to diagnose ACTH-dependent Cushing's syndrome was published previously (20). Cut-off levels for the used diagnostic tests varied between study center and time period, due to use of different assays. All patients had pituitary imaging by MRI, except for three patients who had computed tomography (CT) only: one Cushing's disease patient due to a contraindication for MRI, and two ectopic Cushing patients who already had CT for other reasons, revealing tumors with a high suspicion of ectopic ACTH secretion.

First choice treatment for Cushing's disease was transsphenoidal adenomectomy (TSA). One patient underwent bilateral adrenalectomy (ACTH-dependent Cushing's

syndrome without clear pituitary adenoma, but also no ectopic source), two did not have surgery yet within the study period, and two were being treated long-term medically (one with cabergoline and one with levoketoconazole in a controlled trial setting). For ectopic Cushing's syndrome, first choice treatment was removal of the ectopic ACTH-producing tumor. In 2001, one patient had TSA first, as a pituitary adenoma was identified on MRI, before the diagnosis of ectopic Cushing's syndrome was established. Two patients underwent adrenalectomy (one unilateral because the ectopic tumor was located in the adrenal, and one bilateral because this could be combined with a nephrectomy for a renal cell carcinoma), and two patients died before treatment was instituted. The method of postoperative evaluation and definitions of surgical outcomes (remission, recurrence, and persistent disease) were presented previously (20).

Outcomes and follow-up

Outcomes of interest were clinical presentation of ACTH-dependent Cushing's syndrome, surgical outcome, and short- and long-term morbidity. Surgical outcome included percentage remission, hydrocortisone dependency, recurrence, and mortality. Hydrocortisone dependency was measured three months after surgery and was divided in three categories: a) Absolute deficiency if insufficient cortisol response to a stimulation test [corticotropin-releasing hormone (CRH)-test, ACTH-test or insulin tolerance test], b) Hydrocortisone for symptoms despite normal cortisol response to stimulation, and c) Pragmatic hydrocortisone replacement (without stimulation test).

Short-term morbidity (≤3 months after first surgery) included 1) Anemia (defined as hemoglobin concentrations of <8.5 mmol/L for males and <7.5 mmol/L for females, measured within two weeks after surgery), 2) Anterior pituitary deficiency other than ACTH requiring medication (number of deficient axes, with gonadal axis deficiency also described separately), 3) Severe bleeding (requiring surgical intervention or bleeding described as severe in patient file), and 4) Cardiovascular event (thrombosis, pulmonary embolism, cerebrovascular accident, transient ischemic attack, and myocardial ischemia).

Long-term morbidity (>3 months after first surgery) included 1) Anterior pituitary deficiency (one year after surgery), 2) Hypertension (de novo as well as persisting after surgery), 3) Diabetes mellitus (de novo as well as persisting after surgery), 4) Neuropsychiatric morbidity (complaints as well as consultation of psychologist or psychiatrist), 5) Osteoporosis (defined as a bone mineral density of -2.5 standard deviation [SD]), and 6) Fractures (symptomatic as well as radiologically diagnosed asymptomatic fractures were included, clinical vertebral and femoral fractures

described separately). Anterior pituitary deficiency was described only for patients after a transsphenoidal adenomectomy.

We followed patients from date of diagnosis until death, loss to follow-up, or 31 December 2016, whichever came first. The following patient information was collected at time of diagnosis: age, comorbidities (cardiovascular event, hypertension, diabetes mellitus, dyslipidemia, neuropsychiatric morbidity, anemia, osteoporosis, fractures in patient history), and all eight items of the Cushing's syndrome Severity Index score (CSI score) (21).

Ectopic Cushing's syndrome was classified according to the following underlying disorders: neuroendocrine tumor of the gastrointestinal tract, lung tumor, and other source of ACTH production. Pituitary tumor size was divided into microadenomas (≤10 mm) and macroadenomas (>10 mm).

Risk of bias

This study included all eligible patients to prevent selection bias. However, selective loss to follow-up could have led to selection bias, if more patients from one sex were lost to follow-up than from the other sex caused by e.g. presence of comorbidities. This could alter the percentages of patients with long-term comorbidity after treatment in our study, leading to biased results. Confounding was not assessed as a potential source of bias, as study groups were formed based on sex, and no factor of interest was thought to influence sex. Factors associated with sex could have influenced our results due to selection bias, e.g. by differences in age, and these factors were compared between both sexes, as described in the next paragraph.

Statistical analysis

The following contingency tables were prepared, comparing male to female patients with ACTH-dependent Cushing's syndrome: 1) Demographic characteristics, phenotype of Cushing's syndrome, and medical history (above mentioned patient information collected at time of diagnosis, as well as duration of follow-up), and 2) Surgical outcome, and short- and long-term morbidity. Furthermore, diagnostic strategy and results (biochemical parameters at diagnosis, type and result of radiologic imaging, simultaneous bilateral inferior petrosal sinus sampling, etiology of Cushing's syndrome, tumor size for pituitary adenomas, medical treatment prior to surgery, histology results, and immunohistochemistry results) were compared between male and female patients. The unpaired T-test was used to compare outcomes for continuous variables, and for categorical variables the two-sample test of proportions was used. To correct for multiple testing, the Bonferroni method was used and tests were considered significant if $p<0.001$, although this was probably too conservative for this study due to correlations between the analyses (e.g.

osteoporosis and fractures). All performed analyses were reported in this article. In the tables, percentages were reported according to the total number of patients with a valid value for the specific parameter. If per parameter, data were missing for ≥5% of patients, this was marked in the tables. If variables with ≥5% missing data showed a clear difference between sexes, we also calculated percentages according to total number of patients, thereby assuming that patients with a missing value were rightfully unmeasured, and reported this in the results section only.

Kaplan-Meier curves were constructed for overall survival since time of diagnosis, and for recurrence-free survival since time of surgery between male and female patients. For recurrence-free survival, only patients at risk for recurrence were included in the analysis. Cox proportional hazard regression analyses were performed to provide hazard ratios with 95% confidence intervals.

IBM SPSS Statistics 23.0 (IBM Corp, Armonk, NY, USA) was used to perform all statistical analyses, except the two-sample test of proportions (command: prtesti), which was performed using Stata 14.2 (Stata Corp., College Station, TX, USA), to calculate the difference between two proportions with 95% confidence interval, as this was not provided by SPSS. Patients gave informed consent for use of their data for scientific research. Permission from the ethical committees in the LUMC and Charité Universitätsmedizin was granted. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were used for reporting (22).

Results

Study population (Table 2)

In total, 130 patients were included (n=85 from Leiden, n=45 from Berlin), of whom 37 are males (28.5% of total, mean age: 45.3 years, range: 14-74 years) and 93 females (mean age: 44.8 years, range: 10-80 years). With similar serum cortisol concentrations (median: 670 nmol/L versus 680 nmol/L) and UFC (median: 4.5 times upper limit of normal versus 3.4 times upper limit of normal), males had higher ACTH concentrations at time of diagnosis when compared to females (median: 116 ng/L versus 57 ng/L). There were no differences regarding etiology of Cushing's syndrome (males 5.4% ectopic, females 4.3% ectopic), and tumor size of pituitary adenoma (males 40.0% macroadenomas, females 32.2% macroadenomas). Radiologic findings (pituitary adenoma, ectopic ACTH-secreting tumor, no tumor, or inconclusive), and percentage of patients with inferior petrosal sinus sampling were comparable for both sexes. Osteoporosis was more prevalent at time of diagnosis in males (48.6% versus 25.0%) than in females. Male patients also reported more fractures in their

patient history (27.0% versus 19.6%), mainly vertebral (16.2% versus 5.4%). None of these differences was statistically significant after Bonferroni correction. Males less often reported sex-related disturbances than females (positive item on Cushing's syndrome Severity Index score: 22.8% versus 64.0%).

Table 2: Demographic characteristics and phenotype of Cushing's syndrome.

	Male		**Female**		**Tested difference (95% CI; p-value*)**
	N	**%**	**N**	**%**	
Total number of patients	37	100.0	93	100.0	
Age at diagnosis, years[#]	45.3	16.4	44.8	15.1	0.5 (-5.4 to 6.5; p=0.87)
Duration of follow-up (years)^	5.9	2.2-9.8	5.6	1.9-12.2	0.6 (-1.2 to 2.5; p=0.50)
Comorbidities at diagnosis					
Cardiovascular event	6	16.2	10	10.9	5.3% (-8.2% to 18.8%; p=0.41)
Hypertension	28	75.7	64	69.6	6.1% (-10.6% to 22.8%; p=0.49)
Diabetes mellitus	12	32.4	28	30.4	2.0% (-15.8% to 19.8%; p=0.82)
Dyslipidemia	7	18.9	18	19.6	0.7% (-14.3% to 15.7%; p=0.93)
Neuropsychiatric morbidity	14	37.8	37	39.8	2.0% (-16.5% to 20.5%; p=0.83)
Anemia°	3	9.1	7	8.4	0.7% (-10.8% to 12.2%; p=0.90)
- *Hemoglobin (mmol/L)*[#°]	9.4	1.0	8.8	1.0	
Osteoporosis	18	48.6	23	25.0	23.6% (5.2% to 42.0%; p=0.009)
Fractures in patient history	10	27.0	18	19.6	7.4% (-9.0% to 23.8%; p=0.36)
- *Vertebral fracture*	6	16.2	5	5.4	
- *Femoral fracture*	1	2.7	1	1.1	
Cushing's syndrome Severity Index score[#]	6.2	3.1	6.9	2.6	-0.7 (-1.8 to 0.4; p=0.20)
Fat distribution	31	88.6	86	96.6	8.0% (-3.2% to 19.2%; p=0.083)
- *Mild*	16	45.7	28	31.4	
- *Severe*	15	42.9	58	65.2	
Skin lesions	27	77.1	64	71.9	5.2% (-11.6% to 22.0%; p=0.56)
- *Mild*	20	57.1	40	44.9	
- *Severe*	7	20.0	24	27.0	
Muscle weakness	21	60.0	51	57.3	2.7% (-16.5% to 21.9%; p=0.78)
- *Mild*	8	22.9	19	21.3	
- *Severe*	13	37.1	32	36.0	
Mood disorder	14	40.0	42	47.2	7.2% (-12.1% to 26.5%; p=0.47)
- *Mild*	9	25.7	24	27.0	
- *Severe*	5	14.3	18	20.2	
Hypertension	28	80.0	63	70.8	9.2% (-7.1% to 25.5%; p=0.30)
- *Mild*	24	68.6	51	57.3	
- *Severe*	4	11.4	12	13.5	
Diabetes mellitus	11	31.4	32	36.0	4.6% (-13.7% to 22.9%; p=0.63)
- *Mild*	3	8.6	16	18.0	
- *Severe*	8	22.8	16	18.0	
Hypokalemia	11	31.4	18	20.2	11.2% (-6.3% to 28.7%; p=0.19)
- *Mild*	3	8.6	5	5.6	
- *Severe*	8	22.8	13	14.6	
Sex-related disturbances	8	22.8	57	64.0	41.2% (24.1% to 58.3%; p=0.000)
- *Mild*	2	5.7	29	32.6	
- *Severe*	6	17.1	28	31.4	

CI=confidence interval

*Due to the Bonferroni correction, tests were considered significant if p<0.001, [#]mean + standard deviation, ^median + IQR, °data were missing for ≥5% of patients

Males and females were treated for Cushing's syndrome similarly: percentage of patients with cortisol-lowering medication preoperatively (males 68.6%, females 65.9%) was comparable. After surgery, histology results (tumor identified, no tumor identified, or inconclusive) and immunohistochemistry results (ACTH-positive or negative) were also similar for both sexes.

Twenty patients were lost to follow-up (four males [10.8%] and sixteen females [17.2%], seventeen patients from Berlin, three patients from Leiden) after an average follow-up time of 71 months. For a detailed description of demographic characteristics, see Table 2, and Figures 1A-C. As there were only six patients with ectopic Cushing's syndrome, no analyses were performed stratified by etiology. The ectopic ACTH-secreting tumors were: pancreatic neuroendocrine tumor (n=1), lung tumors (carcinoid [n=1] and carcinoma [n=1]), thymoma (n=1), thymic carcinoma (n=1), and pheochromocytoma (n=1).

Surgical outcome

There were 34 male patients with at least three months follow-up after surgery, of whom 28 were in remission (82.4%), and 22 were hydrocortisone dependent (64.7%). Of the 28 males in remission after surgery, recurrence occurred in eight patients (28.6%), after a mean of 55 months (range: 9-130 months). Five of these eight patients were hydrocortisone dependent three months after first surgery (62.5%). There were 85 female patients with at least three months follow-up after surgery, of whom 68 were in remission (80.0%), and of whom 55 were hydrocortisone dependent (64.7%). Of the 68 females in remission after surgery, recurrence occurred in fifteen patients (22.1%), after a mean of 30 months (range: 0-109 months). Eight of these fifteen patients were hydrocortisone dependent three months after first surgery (53.3%). The hazard ratio for recurrence was 1.22 (95% confidence interval: 0.52-2.89) for males compared to females (Figure 2). Three months after surgery, ACTH concentrations were higher in males than in females (mean: 30 ng/L versus 11 ng/L). More detailed surgical outcome results can be found in Table 3.

Overall survival

Within the study period twelve patients died, five males and seven females. The hazard ratio for mortality was 2.35 (95% confidence interval: 0.73-7.51) for males compared to females. The Kaplan-Meier curves of overall survival are shown in Figure 3.

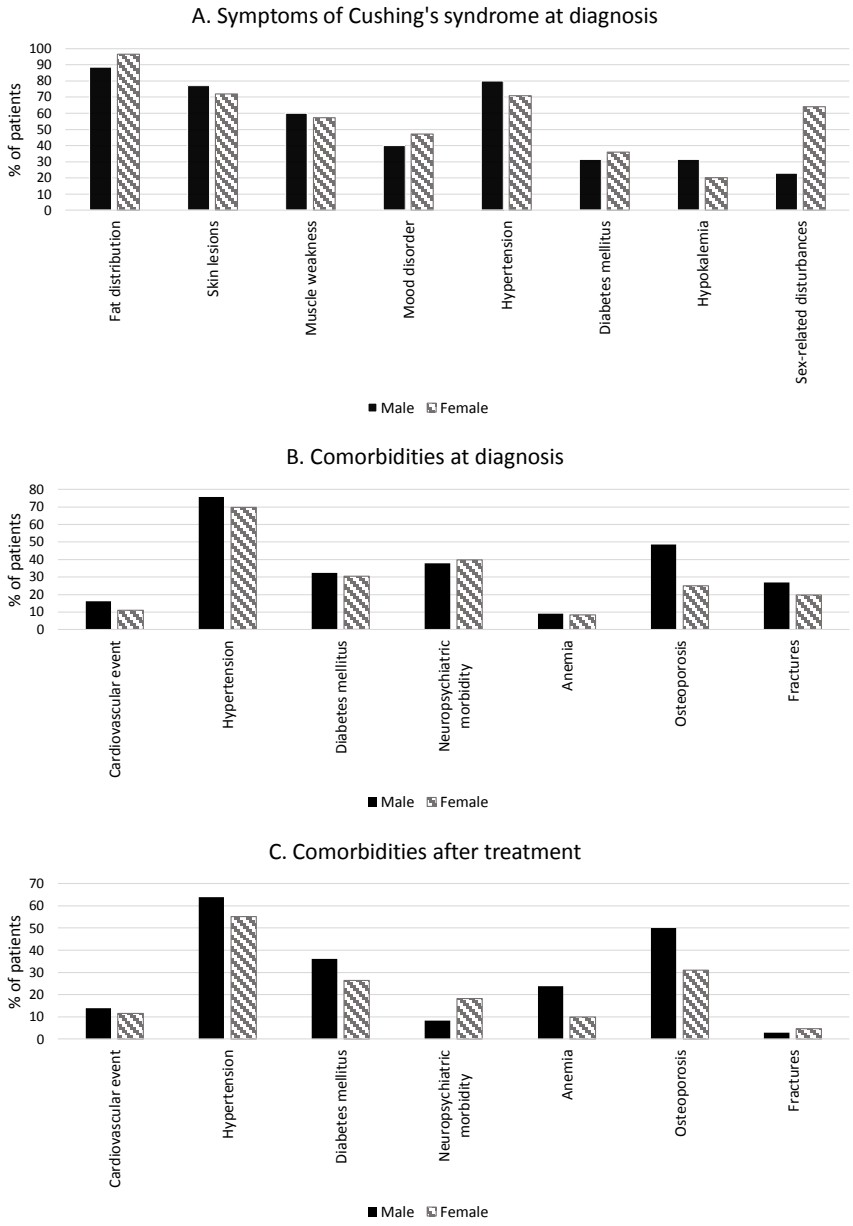

Figure 1: Comorbidities and symptoms of ACTH-dependent Cushing's syndrome by sex. A: Symptoms of Cushing's syndrome at diagnosis. B: Comorbidities at diagnosis. C: Comorbidities after treatment.

Table 3: Surgical outcome and short and long-term morbidity.

	Male		Female		Tested difference (95% CI; p-value*)
	N	%	N	%	
Surgical outcome					
Cortisol direct postoperatively (nmol/L)^°	220	50-510	60	30-360	100 (-20 to 220; p=0.096)
Cortisol 3 to 6 months postoperatively (nmol/L)^°	180	80-280	130	40-320	30 (-70 to 120; p=0.56)
ACTH 3 to 6 months postoperatively (ng/L)^°	30	8-72	11	5-33	50 (-13 to 113; p=0.12)
Hydrocortisone dependency	22	66.7	55	67.1	0.4% (-18.6% to 19.4%; p=0.97)
- *Absolute deficiency*	19	57.6	51	62.2	
- *Normal cortisol response*[#]	0	0.0	1	1.2	
- *Pragmatic replacement*[§]	3	9.1	3	3.7	
Persistent disease	6	17.1	17	19.5	2.4% (-12.6% to 17.4%; p=0.76)
Recurrent disease	8	22.9	15	17.2	5.7% (-10.3% to 21.7%; p=0.47)
Adjuvant treatment	15	41.7	32	36.4	5.3% (-13.7% to 24.3%; p=0.58)
- *Radiotherapy*	12	33.3	13	14.8	
- *TSA*	8	22.2	22	25.0	
- *Adrenalectomy*	2	5.6	6	6.8	
- *Ectopic tumor resection*	0	0.0	1	1.1	
- *Medical treatment*	5	13.9	10	11.4	
Short term morbidity: ≤3 month after first surgery					
Anemia°	22	75.9	25	36.8	39.1% (19.8% to 58.4%; p=0.000)
- *Hemoglobin (mmol/L)*¯°	7.7	1.3	7.7	1.0	
Anterior pituitary deficiency°⁺	10	34.5	17	22.1	12.4% (-7.2% to 32.0%; p=0.19)
- *One axis*	6	20.7	13	16.9	
- *Two axes*	2	6.9	3	3.9	
- *Three axes*	2	6.9	1	1.3	
- *Gonadal axis deficiency*	7	24.1	5	6.5	
Bleeding, severe	2	6.1	1	1.2	4.9% (-3.6% to 13.4%; p=0.13)
Cardiovascular event	3	9.1	4	4.8	4.3% (-6.5% to 15.1%; p=0.38)
Long term morbidity: >3 months after first surgery					
Anterior pituitary deficiency after 1 year°⁺	9	32.1	18	25.3	6.8% (-13.2% to 26.8%; p=0.49)
- *One axis*	4	14.3	14	19.7	
- *Two axes*	3	10.7	2	2.8	
- *Three axes*	2	7.1	2	2.8	
- *Gonadal axis deficiency*	6	21.4	4	5.6	
Hypertension	23	63.9	48	55.2	8.7% (-10.2% to 27.6%; p=0.37)
Diabetes mellitus	13	36.1	23	26.4	9.7% (-8.5% to 27.9%; p=0.28)
Neuropsychiatric morbidity	3	8.3	16	18.2	9.9% (-2.2% to 22.0%; p=0.17)
Osteoporosis	18	50.0	27	31.0	19.0% (0.0% to 38.0%; p=0.047)
Fractures	1	2.8	4	4.6	1.8% (-5.2% to 8.8%; p=0.65)
- *Clinical vertebral fracture*	1	2.8	2	2.3	
- *Femoral fracture*	0	0.0	0	0.0	

ACTH=adrenocorticotropic hormone, CI=confidence interval, CSF=cerebrospinal fluid, SIADH=syndrome of inappropriate antidiuretic hormone release, TSA=transsphenoidal adenomectomy

*Due to the Bonferroni correction, tests were considered significant if p<0.001, ¯mean + standard deviation, ^median + IQR, °data were missing for ≥5% of patients, ⁺only for patients with TSA

[#]Hydrocortisone for symptoms despite normal cortisol response to CRH stimulation.

[§]Pragmatic hydrocortisone replacement (without stimulation test).

Short- and long-term morbidity

Postoperatively, anemia was more prevalent in males than in females (75.9% versus 36.8% for patients with a valid value only). If all patients were included in this analysis, assuming that patients without a valid value were rightfully unmeasured, this difference was even larger (78.4% versus 26.9%). After surgery, males continued to have osteoporosis more often than females (50.0% versus 31.0%). Overall, anterior pituitary deficiency was more prevalent in males than females (34.5% versus 22.1) three months after surgery. Specifically, gonadal axis deficiency needing replacement therapy was more prevalent in males (24.1% versus 6.5%). This difference remained one year after surgery. More detailed morbidity results can be found in Table 3, and in Figure 1B-C.

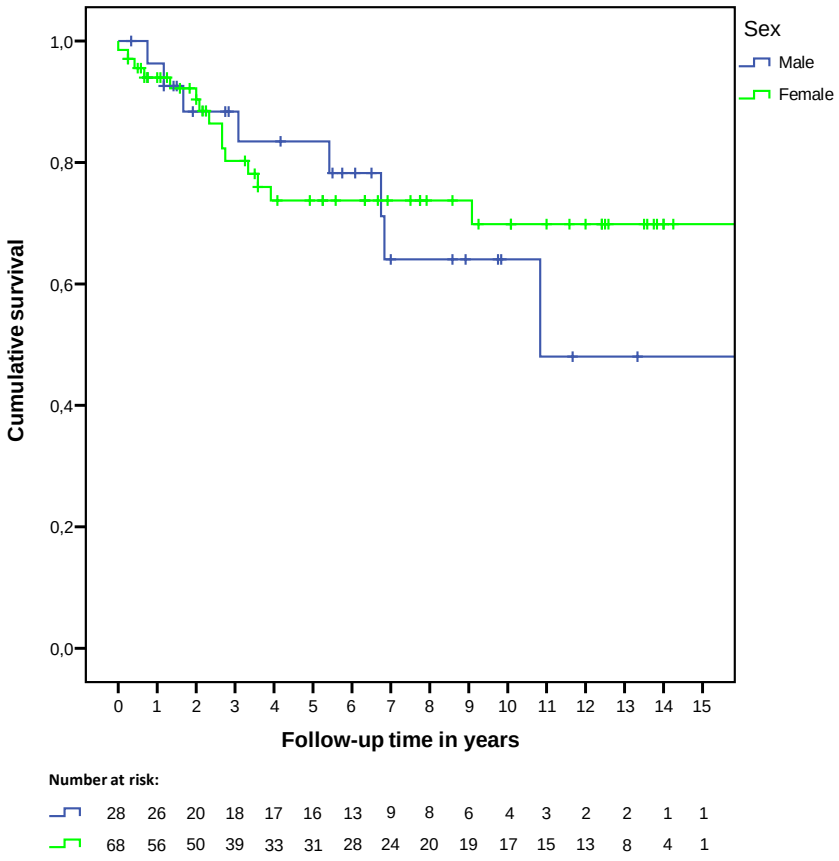

Figure 2: Recurrence-free survival by sex.

Discussion

In this cohort study, we compared clinical presentation, biochemical parameters, diagnostic test results, surgical outcome, and morbidity in male versus female patients with ACTH-dependent Cushing's syndrome. With similar serum cortisol concentrations and UFC, males had higher ACTH concentrations at time of diagnosis than females, with no difference in etiology of Cushing's syndrome and pituitary tumor size between the sexes. Cushing's disease was the most common cause of ACTH-dependent Cushing's syndrome in both male and female patients. At diagnosis of Cushing's syndrome, we found a higher prevalence of osteoporosis with (vertebral) fractures in males than females. After surgery, the higher prevalence of osteoporosis with (vertebral) fractures persisted, and a higher prevalence of anemia in males than in females was found. It is important to note that there were no differences in surgical outcome, recurrence, or mortality between sexes. Thus, male patients with ACTH-dependent Cushing's syndrome seem to constitute a different clinical pattern regarding symptoms and biochemistry, which does, however, not affect the further diagnostic strategy, therapy, or surgical outcome in this cohort study, although comorbidities did differ between sexes. Therefore, no different diagnostic or therapeutic strategy is indicated based on sex. However, based on the differences in comorbidities, extra attention should be given to male patients for diagnosing and treating osteoporosis (with fractures).

A major strength of this study is that it includes a large cohort from two centers and focuses on a broad spectrum of potential differences between sexes, thereby allowing a more accurate description and clear understanding of the clinical picture of ACTH-dependent Cushing's syndrome based on sex, than previous cohort studies. The differences in biochemistry and morbidity found in this study are largely in line with previous studies: higher plasma ACTH (9, 12-14), more often anemia (16) and osteoporosis (with fractures) (8, 11-13). The lack of difference in etiology and pituitary tumor size was in agreement with some studies (9, 12), but not with others (8, 11). Similarly, the lack of difference in surgical outcome was in agreement with one study (9), but not with two others (11, 12). Thus, this study adds evidence to the existence of a sex difference with respect to ACTH, anemia, and osteoporosis (with fractures), and to the lack of a sex difference in etiology and pituitary tumor size. The existence of a sex difference regarding surgical outcome is doubtful, as now two studies show a difference, whereas two others do not, indicating that more research is needed into the existence of a sex difference in surgical outcome.

When interpreting the results, the following study limitations should be taken into account. As this study compared a broad spectrum of potential differences between sexes, the Bonferroni method was used to control for multiple testing, leading to

tests considered significant only if p<0.001. Consequently, the cohort size was insufficient to detect any differences that were smaller than 40% between the study groups. In order to find a 20% difference between the study groups with a significance level of p<0.001, we would have needed at least 450 patients in total, given the sex distribution in this study, which would have taken many more study centers or multiple extra decades to assemble. However, none of the previously published studies on potential sex differences controlled for multiple testing, without exception using a significance level of p<0.05 despite using many statistical tests per study. This should be taken into account when comparing our study results to the literature. Furthermore, as there were only six patients with ectopic Cushing's syndrome, no analyses could be performed for patients with ectopic Cushing's syndrome separately.

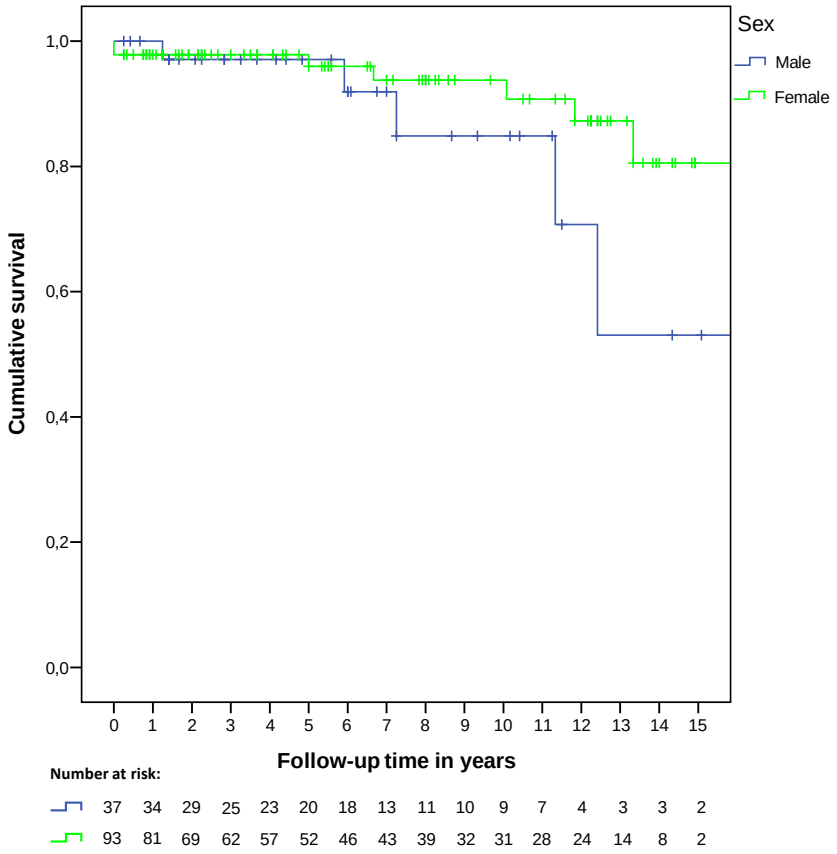

Figure 3: Overall survival by sex.

Loss to follow-up could have led to selection bias, since more female than male patients were lost to follow-up. However, as no patient was lost to follow-up within six months postoperatively, and most study endpoints were measured within six months postoperatively, selective loss to follow-up is unlikely to have influenced study results.

As this study was performed retrospectively, using information from patient files, information could not always be collected from similar time points for each patient, leading to missing data in some patients (e.g. anemia at diagnosis), and information bias is likely to have occurred due to selective questioning of patients (e.g. sex-related disturbances in the Cushing's syndrome Severity Index score). This presumably led to the improbable low percentage of males reporting sex-related disturbances, whereas other studies found males to have sex-related disturbances more often than females (8, 11). Thus, it is important to inquire patients at disease presentation thoroughly about this subject and ideally also interview the patient's partner in this regard.

As should be discussed according to the STROBE guideline (22), this study is theoretically generalizable to all patients with ACTH-dependent Cushing's syndrome. However, the small number of patients with ectopic Cushing's syndrome precludes generalizability for this etiological ultrarare subgroup.

ACTH has been reported to be higher in males than in females with ACTH-dependent Cushing's syndrome consistently across multiple studies (9, 12-14). Some studies, that also reported higher concentrations of UFC in males than in females, suggested that ACTH-dependent Cushing's syndrome is a more aggressive disease in males (12, 13). The increased ACTH concentrations could not be related to larger tumor size of the pituitary adenoma (14). Likewise, as the percentage of patients with ectopic Cushing's syndrome was similar for both sexes, etiological differences are no satisfying explanation for the observed variation in ACTH concentrations. Until now, no study has found a convincing pathophysiological explanation why ACTH is higher in males than in females. Interestingly, higher concentrations of ACTH in males do not consistently seem to increase concentrations of cortisol (serum cortisol as well as UFC), reducing the probability that ACTH-dependent Cushing's syndrome in males actually is a more aggressive disease than in females. Future research with the aim to discover why ACTH is higher in males than in females could focus first on which factors other than pituitary tumor size or etiology might be related to a higher ACTH per sex.

Osteoporosis (with fractures) and anemia were more prevalent in males than in females, which may be explained, at least in part, by patient delay during the

diagnostic process, as males may have less pronounced symptoms than females in the earlier stages of the disease (i.e. menstrual disturbances), leading to a greater delay in diagnosis and therefore more, and more severe complications at time of diagnosis in males. Furthermore, it has been suggested that interaction between corticosteroids and the gonadotropic axis leads to hypogonadism in males more often than in females, which may markedly influence bone damage in Cushing's syndrome (8, 13). This is in accordance with our study results, which showed postoperative hypogonadism more often in males than females. This hypogonadism may also cause an endocrine anemia due to the insufficient drive of testosterone on erythropoiesis (23). However, a meta-analysis of prior corticosteroid use and (osteoporotic) fracture risk found no difference between men and women (24).

In conclusion, male patients with ACTH-dependent Cushing's syndrome seem to show a different clinical picture than females. However, no different diagnostic strategy or treatment is indicated based on sex, in view of the similar surgical outcome. Clinicians should pay more attention to male patients with ACTH-dependent Cushing's syndrome in particular regarding diagnosis and treatment of accompanying osteoporosis (with fractures).

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Chapter 8

Adrenal crisis in treated Cushing's disease and Cushing's syndrome patients

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Abstract

Background
Adrenal crisis, the most feared complication of adrenal insufficiency, is a potentially life-threatening state of acute glucocorticoid deficiency. After successful surgery for Cushing's syndrome, many patients develop (transient) adrenal insufficiency. The incidence of adrenal crisis in patients treated for hypercortisolism is unknown.

Methods
Cohort study including consecutive patients with Cushing's syndrome with adrenal insufficiency after surgery from Leiden and Berlin from 2000-2015. We summarized incidence of adrenal crisis, compared patients with and without adrenal crisis regarding potential risk factors for its occurrence, and assessed the effect of better education in time on incidence of adrenal crisis.

Results
We included 106 patients, of whom 19 patients had a total of 41 adrenal crises. There were 9.0 crises per 100 patient-years at risk (95% confidence interval [CI]: 6.7-12.0). All crises occurred while on hydrocortisone replacement. The risk ratio for a recurrent crisis was 2.3 (95% CI: 1.2-4.6). No clear change in incidence of adrenal crisis due to better education in time was observed. There was no difference in recurrence rate between patients with, and without any crisis, but patients with adrenal crisis had more often pituitary deficiencies.

Conclusions
The incidence of adrenal crises after treatment for Cushing's syndrome is substantial, and patients who suffered from an adrenal crisis have higher risk for recurrent crisis. However, further risk factor analysis is needed to identify risks for a first crisis. Effective education methods to prevent adrenal crises should be identified and implemented, including stress instructions by trained nursing staff before hospital discharge.

Introduction

An adrenal crisis is a potentially life-threatening situation of an acute state of glucocorticoid deficiency, which can develop after any situation of increased demand for stress hormones, such as intercurrent illness or psychological stress, and is the most feared complication of adrenal insufficiency (1). The incidence of adrenal crisis in the heterogeneous population of patients with adrenal insufficiency is estimated to be 4.1 to 9.3 per 100 patient-years (2-9). There is no consensus on the definition of (imminent) adrenal insufficiency. However, the proposed definition by Allolio *et al.* is useful for clinical practice: a combination of 1) Major impairment of general health with at least two of the following signs/symptoms: hypotension, nausea or vomiting, severe fatigue, fever, somnolence, hyponatremia or hyperkalemia, hypoglycemia, and 2) Parenteral glucocorticoid administration followed by clinical improvement (10). A recent Dutch guideline developed by a multi-disciplinary group, that also included patient representatives, provides clear guidance on when to act to prevent adrenal crisis in daily life, e.g. in case of elective surgical procedures or acute illness with intensive care admission, and how to treat acute adrenal crisis (11).

Cushing's syndrome is the result of a prolonged state of endogenous hypercortisolism. This can be adrenocorticotropic hormone (ACTH)-dependent (e.g. Cushing's disease) or ACTH-independent (e.g. an adrenal cortisol-producing adenoma) (12). Excess of glucocorticoids causes osteoporosis, central obesity, insulin resistance, dyslipidemia, hypertension, hypercoagulability, cognitive dysfunction, and neuropsychiatric disorders (13, 14). First-choice treatment is transsphenoidal selective adenomectomy for Cushing's disease (15), surgical removal of the tumor in case of ectopic ACTH-producing tumors, and adrenalectomy for an adrenal adenoma (16).

After successful surgery, patients with Cushing's disease develop (transient) severe adrenal insufficiency due to suppression of the physiological hypothalamic-pituitary-adrenal (HPA) axis by the pathological secretion of ACTH by the adenoma, for which hydrocortisone replacement therapy is needed. A significant number of these patients even need life-long replacement therapy, because of incomplete recovery of the HPA-axis either due to isolated downregulation of the adrenal glands or because of pituitary function loss, which is seen in 42% of patients after 5 years of follow-up (17). The incidence of adrenal crisis in the subset of patients with a history of hypercortisolism is unknown. Due to long-standing hypercortisolism, the activity of the HPA-axis may have altered the response to cortisol deficiency in patients treated for Cushing's syndrome (including Cushing's disease) compared to other adrenal insufficient patients. The history of hypercortisolism and potential occurrence of corticosteroid withdrawal syndrome and a phase of lowering replacement doses may complicate diagnosis and awareness in these patients, as both patients and doctors

may not recognize early symptoms of adrenal crisis in time. Corticosteroid withdrawal syndrome can occur in patients with previous hypercortisolism despite apparently acceptable cortisol concentrations, meaning that these patients need higher replacement doses of hydrocortisone than other adrenal insufficient patients. This may suggest that they are at higher risk of an adrenal crisis if they receive normal replacement doses that result in apparently acceptable cortisol concentrations (18).

For clinical practice, it is important to know how often adrenal crisis occurs and what the possible risk factors are in the population of patients in the aftermath of an episode of endogenous hypercortisolism.

Study aims

The primary study aim was to summarize the incidence of adrenal crisis, defined as an acute impairment of general health requiring hospital admission (excluding emergency room visit only) and administration of intravenous glucocorticoids, with subsequent resolution of symptoms, in a population of patients with Cushing's syndrome treated by transsphenoidal pituitary surgery or adrenalectomy during the time that they were adrenal insufficient. Secondary study aims were to find risk factors identifying patients with Cushing's syndrome who may be at higher risk for an adrenal crisis after surgical treatment, to describe the course and underlying cause of adrenal crisis, and to assess a potential change in incidence of adrenal crises due to better education in recent time.

Methods

Study population

Consecutive patients with Cushing's disease and adrenal Cushing's syndrome from the Leiden University Medical Center and the Charité Universitätsmedizin Berlin were included in this cohort study. Patients with an adrenal carcinoma were excluded. Patients were included between January 1st 2000 and December 31st 2015. Only patients with adrenal insufficiency and glucocorticoid dependency after transsphenoidal pituitary surgery or adrenalectomy were included, as this constitutes the population at risk for an adrenal crisis. There were no restrictions in prior or adjuvant treatment, such as reoperation, prior or adjuvant radiotherapy or pre-operative cortisol-lowering medication.

The diagnostic process for ACTH-dependent Cushing's syndrome was described previously (19). For ACTH-independent Cushing's syndrome, the same criteria were used, except that ACTH had to be suppressed, and that adrenal imaging was

performed by either magnetic resonance imaging (MRI) or computed tomography (CT). Due to the use of different assays, cut-off levels for the used tests varied between both study centers and over time.

Transsphenoidal adenomectomy (TSA) was the first-choice treatment for Cushing's disease. One patient underwent bilateral adrenalectomy, because no pituitary adenoma was visualized on MRI and no lateralization was present during inferior petrosal sinus sampling, but also no ectopic ACTH-producing source was found. For patients with adrenal Cushing's syndrome, adrenalectomy was the first-choice treatment. Details of postoperative evaluation as well as definitions of remission, recurrence and persistent disease were presented previously (19). We included the time period that a patient was hydrocortisone dependent, as explained more extensively under "follow-up". During disease recurrence, patients were excluded from our study, but they could re-enter the study if after further treatment, they were again hydrocortisone dependent. Starting from three months postoperatively, potential normalization of the HPA-axis was tested dynamically with synthetic ACTH, corticotropin-releasing hormone (CRH), or insulin tolerance test (ITT). The HPA-axis was considered normalized if the dynamic test showed a normal test result, and consequently hydrocortisone replacement was discontinued.

Study outcomes and follow-up

Primary outcome of interest was adrenal crisis. Further outcomes of interest were mortality, (surgical) outcome including recurrent disease, and complications. Unless otherwise specified, outcomes were reported in relation to the first surgery.

Adrenal crisis was defined as an acute impairment of general health requiring hospital admission (not only emergency room, as emergency room adrenal crises could not be scored reliably) and administration of intravenous glucocorticoids, after which a resolution of symptoms followed (10). Self-treatment for adrenal crisis at home was not included as endpoint, as the accuracy of this diagnosis cannot be guaranteed. Regarding adrenal crisis, the total number of crises and the total number of patient-years at risk were summarized, as well as the number of crises per patient with at least one crisis, the type of hospital admission (regular ward or intensive care), duration of hospital stay in days, hydrocortisone dose before hospital admission for the adrenal crisis in mg/day (in case of a crisis before first hospital release after surgery, the last dose given before start of symptoms was entered), and, at hospital admission for the adrenal crisis, the number of patients with a subphysiological dose of hydrocortisone replacement (<20 mg/day), the number of patients with an infection, and the number of patients with psychological stress.

Besides recurrent disease, the following variables were described: hydrocortisone release dose from the hospital after the first surgery in mg/day, number of patients with restoration of the HPA-axis within one year after first surgery, and adjuvant treatment (radiotherapy, transsphenoidal adenomectomy, adrenalectomy, and medical treatment).

Complications (measured ≤3 months after first surgery) included diabetes insipidus (requiring medication at least once) and anterior pituitary deficiency other than ACTH requiring medication. Complications were described only for patients that underwent transsphenoidal adenomectomy.

We followed patients from date of surgery for Cushing's syndrome until death, loss to follow-up, or December 31st 2016, whichever came first. Patients were followed only during the period that they were at risk of having an adrenal crisis, i.e. from the moment they had adrenal insufficiency and glucocorticoid dependency until normalizing adrenal function, evidenced by clinical stop of hydrocortisone replacement and at least one normal dynamic test (ACTH, CRH or ITT). Patients were potentially included again after a period of normal adrenal function if they once more experienced adrenal insufficiency and glucocorticoid dependency after a repeat surgical procedure or radiotherapy.

Presurgical information was collected regarding diagnosis, comorbidities (hypertension, diabetes mellitus, dyslipidemia), and cortisol-lowering medical treatment prior to surgery. Pituitary tumor size was divided into microadenomas (≤10 mm) and macroadenomas (>10 mm).

All eligible patients were included in this study to prevent selection bias. However, selective loss to follow-up could have led to selection bias. Confounding was assessed as a potential source of bias by comparing baseline characteristics between groups.

Statistical analysis

In the contingency tables, patients with at least one adrenal crisis were compared to patients without any adrenal crisis: 1) Demographic characteristics and medical history (age at diagnosis, sex, duration of follow-up, Cushing's syndrome Severity Index (CSI) score (20), type of surgical treatment, comorbidities, tumor size for pituitary adenomas, and cortisol-lowering medical treatment prior to surgery), and 2) Adrenal crisis details, (surgical) outcome, and complications. The difference between the two study groups was tested with an unpaired T-test for continuous outcomes and with the two-sample test of proportions for outcomes reported as proportions. These tables were also stratified by study center. If data were missing for ≥5% of patients, this was marked in the tables.

The Andersen-Gill adaptation of the Cox proportional hazard model was used to analyze the effect of the following variables on occurrence of an adrenal crisis: study center (Leiden versus Berlin) and etiology of Cushing's syndrome (21). Univariate and multivariate analyses were performed. Only hydrocortisone-dependent time was included in the model, because this was the patient-time at risk for an adrenal crisis. By using the Andersen-Gill model, we were able to include recurrent adrenal crisis and take into account time between crises. Hydrocortisone replacement dose before hospital admission for adrenal crisis was related to the time between last surgery and the occurrence of adrenal crisis. This was the only analysis in which time since last surgery before adrenal crisis was used, as only patients with at least one crisis were included in this analysis and we were specifically interested in time since last surgery for this outcome. Prior adrenal crisis was related to risk of recurrent adrenal crisis by calculating the risk ratio, which was performed by dividing risk of recurrent crisis for patients with a first adrenal crisis by the risk of a first adrenal crisis for all included patients. Number of adrenal crises per 100 patient-years at risk was stratified by time period (2000-2004, 2005-2009, 2010-2016), to assess a change in incidence due to better education in time.

Statistical analyses were performed with IBM SPSS Statistics 23.0 (IBM Corp, Armonk, NY, USA), and with Stata 14.2 (Stata Corp., College Station, TX, USA) for the Andersen-Gill model and the two-sample test of proportions (command: prtesti) to calculate the difference between two proportions with 95% confidence interval (CI), as this was not provided by SPSS. Patients gave informed consent to use their data for scientific research. Permission was granted from the ethical committees in the LUMC and Charité Universitätsmedizin. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were used for reporting (22).

Results

Study population

In total, 106 patients were included in this study (66 from Leiden and 40 from Berlin), of whom nineteen patients (17.9%) suffered from at least one adrenal crisis. There was no difference in sex or age between both groups. For the nineteen patients with adrenal crisis, mean age was 46.2 years (range: 16-72), and most (n=14, 73.7%) were female. Of the 87 patients without adrenal crisis, mean age was 42.9 years (range: 10-80), and 68 (78.2%) were female.

At diagnosis of Cushing's syndrome, patients with adrenal crises had more often diabetes mellitus (68.4% versus 24.1%), and more often macroadenomas (53.8% versus 24.6%) than patients without adrenal crises. There was no difference in Cushing's

syndrome Severity Index score between both groups. Twenty patients were lost to follow-up after an average of 81.2 months (range 3-165), of whom four with at least one crisis (21% out of 19 patients with at least one crisis) and sixteen without any crisis (18% out of 87 patients without any crisis). For a detailed description of demographic characteristics, see Table 1. Results per study center can be found in Supplemental Data 1.

In Leiden, patients received a lower hydrocortisone replacement dose at hospital discharge postoperatively than in Berlin (median of 20 mg/day [interquartile range (IQR): 20-40; mean 25 mg/day] versus 30 mg/day [IQR: 25-50; mean 35 mg/day]), in accordance with the institutional protocols. However, in Berlin, the hydrocortisone replacement dose at hospital discharge was higher in patients with an adrenal crisis (median 50 mg/day), which was not observed in patients from Leiden.

Table 1: Demographic characteristics.

	Adrenal crisis		**No adrenal crisis**		**Tested difference (95% CI)**
	N	**%**	**N**	**%**	
Total number of patients	19	100.0	87	100.0	
*Age at diagnosis, years**	46.2	12.5	42.9	14.8	3.3 (-4.0 to 10.5)
Sex (female)	14	73.7	68	78.2	4.5% (-17.1% to 26.1%)
*Duration of follow-up (years)^*	6.3	4.6-11.3	7.8	3.7-11.7	0.5 (-1.7 to 2.7)
*Cushing's syndrome Severity Index score**	6.7	2.5	6.7	2.4	0.1 (-1.2 to 1.3)
Surgical treatment: transsphenoidal pituitary surgery	14	73.7	64	73.6	0.1% (-21.8% to 22.0%)
Comorbidities at diagnosis					
Hypertension	13	68.4	66	75.9	7.5% (-15.3% to 30.3%)
Diabetes mellitus	13	68.4	21	24.1	44.3% (21.5% to 67.1%)
Dyslipidemia	6	31.6	11	12.6	19.0% (-3.0% to 41.0%)
Patients with pituitary microadenoma	6	46.2	49	75.4	29.2% (0.1% to 58.3%)
Prior medical treatment	8	44.4	50	58.1	13.7% (-11.5% to 38.9%)

CI=confidence interval
*mean + standard deviation; ^median + IQR

Adrenal crisis

There were 41 adrenal crises in nineteen patients during 457 patient-years at risk, measured in all 106 patients. This translates into 9.0 crises per 100 patient-years at risk (95% CI: 6.7-12.0). Patients had on average 2.2 crises with a range of 1 to 7 crises per patient. Time since last surgery until first crisis had a median of 2 months (IQR: 1-22 months), and time since last surgery until any crisis had a median of 20 months (IQR: 1-57 months). This means generally patients with adrenal crises had their first crisis early after their last surgery, after which they remained at risk for recurrent

crises for at least 5 years. Five patients had a crisis during the same hospital admission in which the surgery was performed, most within one week after surgery, and one patient two weeks after surgery. For most adrenal crises, patients were admitted to a regular ward only. One patient was first admitted to the intensive care and afterwards to a regular ward. Median hospital stay duration was 5 days. The average hydrocortisone replacement dose used at home (or at least advised) just before hospital admission for adrenal crisis was 30 mg/d (range 0-100 mg/d). In seven cases of adrenal crisis, the used dose was <20 mg/d. There were 29 cases of adrenal crisis with documented infection, and two with clear psychological stress. For detailed results, see Table 2.

Table 2: Adrenal crisis, (surgical) outcome, and complications.

	Adrenal crisis		**No adrenal crisis**		**Tested difference (95% CI)**
	N	**%**	**N**	**%**	
Adrenal crisis	41 crises in 457 patient-years at risk				
Number of crises per patient[#]	2.2	1-7	-	-	
Type of hospital admission					
- *Regular ward*	40	97.6	-	-	
- *Intensive care*	1	2.4	-	-	
Duration of hospital stay (days)^	5	3-9	-	-	
Hydrocortisone dose at hospital admission (mg/day)[#]	30	0-100	-	-	
Subphysiological dose of hydrocortisone replacement	7	17.1	-	-	
Infection	29	70.7	-	-	
Psychological stress	2	4.9	-	-	
Surgical outcome					
Hydrocortisone dose (mg/day) at discharge^	35	20-50	20	20-40	5 (-7.5 to 17.5)
Serum cortisol direct postoperatively (nmol/L)^°	30	20-600	40	20-100	170 (-150 to 480)
Serum cortisol 3 to 6 months postoperatively (nmol/L)^°	150	10-450	110	30-240	120 (-20 to 390)
Restoration of HPA-axis in the first year postoperatively°	1	7.1	10	14.5	7.4% (-8.4% to 23.2%)
Recurrent disease	2	10.5	12	13.8	3.3% (-12.3% to 18.9%)
Adjuvant treatment	5	26.3	18	20.7	5.6% (-15.9% to 27.1%)
Complications					
Diabetes insipidus°[+]	5	41.7	15	24.6	17.1% (-12.8% to 47.0%)
Anterior pituitary deficiency[+]	6	50.0	15	23.8	26.2% (-4.0% to 56.4%)
- *One axis*	3	25.0	11	17.4	
- *Two axes*	2	16.7	2	3.2	
- *Three axes*	1	8.3	2	3.2	

CI=confidence interval, HPA-axis=hypothalamus-pituitary-adrenal-axis
[#]mean + range, ^median + IQR, °data were missing for ≥5% of patients, [+]only for patients with transsphenoidal surgery

As there were nineteen patients with at least one adrenal crisis out of 106 patients, the risk of an adrenal crisis at baseline was 17.9%. Risk of recurrent crisis for patients with at least one adrenal crisis was eight out of nineteen (42.1%). The risk ratio of having a recurrent crisis for patients with at least one crisis was 2.3 (95% CI: 1.2-4.6) compared to having a first adrenal crisis.

Four patients experienced more than three adrenal crises (two patients with four crises, one with five crises, and one with seven crises). There is no clear pattern, which discriminates these patients from other patients in this study. All had Cushing's disease, and three were female. One had multiple adjuvant treatments (repeat transsphenoidal surgery, radiotherapy, and bilateral adrenalectomy), whereas the others only had one surgical procedure. After surgery, one patient had diabetes insipidus, one had anterior pituitary deficiency (only thyroid axis deficient), and one had both (also only thyroid axis deficient). Two patients had a cortisol stimulation test within two weeks after surgery, showing peak cortisol concentrations of 10 and 50 nmol/L. Their average hydrocortisone replacement dose before hospital admission for adrenal crisis was 30 mg/day, and in one case only, the patient received <20 mg/day (17.5 mg/day).

In Leiden, patients had 6.7 crises per 100 patient-years (95% CI: 4.5-10.0), which was 14.7 crises per 100 patient-years (95% CI: 9.7-22.3) in Berlin. In Leiden, patients were admitted to hospital for adrenal crises using lower hydrocortisone replacement doses than in Berlin (20 mg/day versus 40 mg/day), and there were more patients with a hydrocortisone replacement dose <20 mg/d at hospital admission for adrenal crisis (six cases versus one in Berlin). In five cases, hydrocortisone replacement <20 mg/d was given during a hydrocortisone withdrawal schedule, whereas in two cases, a dose <20 mg/d was already given within one month postoperatively (one in Leiden and one in Berlin), due to suspicion of persisting disease. For patients with Cushing's disease, there were 9.6 crises per 100 patient-years (95% CI: 7.0-13.3), versus 7.0 crises per 100 patient-years (95% CI: 3.6-13.6) for adrenal Cushing's syndrome. For more detailed results, see Supplemental Data 2.

The hazard ratio for patients with adrenal Cushing's syndrome compared to Cushing's disease was 0.73 (95% CI: 0.32-1.65) in a univariate Andersen-Gill model, meaning the risk for adrenal crisis was lower in patients with adrenal Cushing's syndrome. The hazard ratio for patients from Berlin compared to patients from Leiden was 2.00 (95% CI: 1.02-3.91). In a multivariate Andersen-Gill model using both etiology and research center, we found a hazard ratio for adrenal Cushing's syndrome compared to Cushing's disease of 0.59 (95% CI: 0.25-1.39) and a hazard ratio for patients from Berlin compared to patients from Leiden of 2.23 (95% CI: 1.13-4.42). Due to the low number of events, no further risk factor analyses were performed.

In Figure 1, the relation between hydrocortisone replacement dose before hospital admission for adrenal crisis and time from last surgery until adrenal crisis is shown. Patients with a short period between last surgery and adrenal crisis had higher replacement doses of hydrocortisone at the occurrence of adrenal crisis than patients with a long duration between last surgery and adrenal crisis. This means that, due to the corticosteroid withdrawal syndrome, shortly after surgery patients with Cushing's syndrome need higher replacement doses of hydrocortisone than longer after surgery to prevent adrenal crisis. Despite in general higher dosing for withdrawal complaints, patients are at risk for adrenal crisis, pointing at an increased need for hydrocortisone in these patients.

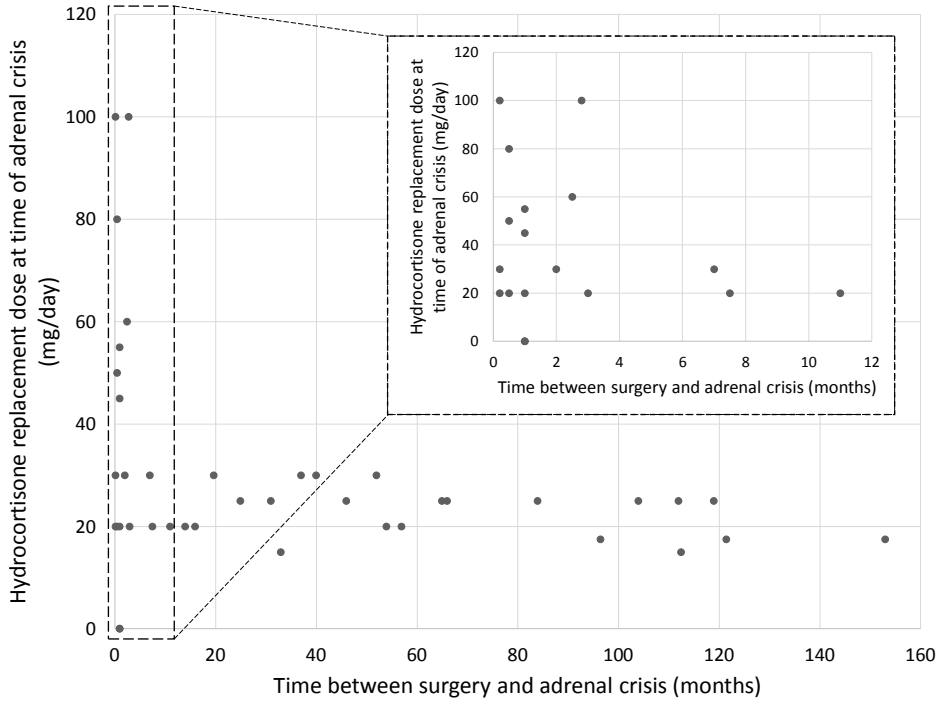

Figure 1: Relation between hydrocortisone replacement dose at time of adrenal crisis and time between last surgery and occurrence of adrenal crisis.

Stratified by time period, there were two crises in 44 patient-years at risk from 2000-2004 (5 crises per 100 patient-years, 95% CI: 1-18), fourteen crises in 142 patient-years at risk from 2005-2009 (10 crises per 100 patient-years, 95% CI: 6-16), and 25 crises in 272 patient-years at risk from 2010-2016 (9 crises per 100 patient-years, 95% CI: 6-13).

Surgical outcome and state of the HPA-axis
From the patients without adrenal crisis, twelve experienced a recurrence (14.8% out of 81 initially in remission). From the patients with at least one adrenal crisis, two experienced recurrent disease, for which they underwent repeat surgery (12.5% out of 16 initially in remission). Adrenal crises only occurred after the final treatment in each patient.

Postoperative serum cortisol concentrations were similar for patients with (median 30 nmol/L, IQR: 20-600) and without adrenal crisis (median 40 nmol/L, IQR: 20-100). There was one patient (7.1%) with restoration of the HPA-axis in the first year postoperatively who had an adrenal crisis, whereas the HPA-axis restored within a year in ten patients (14.5%) without adrenal crisis. A detailed overview of surgical outcome results can be found in Table 2, and results stratified by study center in Supplemental Data 2.

Overall survival
Five patients died in the study period, three without any adrenal crisis, and two patients with at least one adrenal crisis. Both deaths in the latter group were not related to adrenal crisis. No further mortality analyses were performed due to the small number of deaths.

Complications
Postoperatively, patients with adrenal crises had additional anterior pituitary deficiency more often than patients without any adrenal crisis (50.0% versus 23.8%), and they had a higher incidence of diabetes insipidus (41.7% versus 24.6%).

Discussion

In this exploratory study, we summarized the incidence of adrenal crisis in a population of patients with Cushing's syndrome after treatment during the postoperative period that they were adrenal insufficient. We compared patients with and without any adrenal crisis regarding potential risk factors for adrenal crisis and characteristics of Cushing's syndrome.

The incidence of adrenal crisis was 9.0 crises per 100 patient-years at risk. The risk of adrenal crisis was higher for patients from Berlin than for patients from Leiden, and higher for patients with Cushing's disease than for patients with adrenal Cushing's syndrome. First adrenal crisis occurred early after cure despite high replacement doses of hydrocortisone, and was a risk factor for recurrent crisis. No decline in incidence of adrenal crisis in time, due to better education and increased general awareness across Europe for necessity of stress instruction, was observed. Additional

pituitary deficits are more often present in patients with adrenal crisis, and may therefore be a risk factor for the occurrence of adrenal crises.

A strength of this study is the focus on adrenal crisis as primary outcome in a population of patients treated for Cushing's syndrome, including a detailed description of the underlying disease and course of the adrenal crisis. Our incidence of adrenal crisis is in line – on the high end – with the incidences reported in the literature on adrenal insufficient populations (4.1-9.3 per 100 patient-years) (2-9), and especially with one systematic review about patients with Cushing's syndrome after bilateral adrenalectomy (9.3 per 100 patient-years) (5). Our risk ratio for recurrent crisis is in line with the current literature (6).

The following study limitations need to be taken into account when interpreting our results. Due to the low absolute number of adrenal crises in our study population, the planned extensive risk factor analysis could not be performed. High percentages of loss to follow-up in both study groups, though equally divided, may have led to selection bias. As loss to follow-up could have resulted from various reasons, including both excellent health as well as very poor health status, the direction in which the results may have been biased cannot be determined. However, since our main results are in line with the literature, selection due to high loss to follow-up is unlikely to have biased our results extensively.

As should be discussed according to the STROBE guideline (22), this study is generalizable to all patients with Cushing's disease and adrenal Cushing's syndrome who are adrenal insufficient and glucocorticoid dependent after transsphenoidal pituitary surgery or adrenalectomy.

Our risk ratio for recurrent crisis indicates that certain patients are at larger risk than others to develop (multiple) adrenal crises. This may be due to decreased glucocorticoid sensitivity at the tissue level caused by polymorphisms in the glucocorticoid receptor gene (23). For these patients, the increase in hydrocortisone replacement dose during stress, or maybe even regular hydrocortisone replacement doses, may not be sufficient to prevent adrenal crisis. A further explanation may be an insufficient understanding in these patients of the instructions how to adequately anticipate with increasing the dose of hydrocortisone during stress, or insufficient compliance in increasing the hydrocortisone replacement dose. Severity of Cushing's syndrome (measured by CSI score) could not be related to occurrence of adrenal crisis. However, risk factors such as severity of Cushing's syndrome can be difficult to define, and should be studied in a large population to obtain reliable results.

The large difference in incidence of adrenal crisis between Leiden and Berlin may partially be explained by a different approach in hospital admission for patients presenting to the emergency room with signs and symptoms of early onset adrenal crisis. Patients in Leiden may preferably be treated in the emergency room, whereas patients in Berlin may rather be treated during hospital admission. On the other hand, the difference may partially be explained by different patient education strategies in the prevention of adrenal crises, possibly because in Berlin patients use higher hydrocortisone replacement doses, both at hospital discharge as well as prior to hospital admission for adrenal crisis. The difference in incidence of adrenal crisis between patients with Cushing's disease and adrenal Cushing's syndrome is small and may not represent a true difference. A difference might be explained by the different treatment methods for both etiologies. A higher risk of adrenal insufficiency and therefore adrenal crisis might be expected after adrenalectomy, which could have led to better patient education after adrenalectomy than after transsphenoidal surgery. Better awareness through education and thereby timely treatment of early symptoms of adrenal crisis could have resulted in the lower risk of adrenal crisis for patients with adrenal Cushing's syndrome.

The lack of difference in recurrence rates for Cushing's syndrome between patients with and without adrenal crisis is most likely due to selection of our patient population, as only patients with at least one period of adrenal insufficiency and glucocorticoid dependency were included in this study. Patients with adrenal crisis more often had additional pituitary hormone deficiencies than patients without any adrenal crisis, which may indicate which patients are more vulnerable, and therefore at higher risk of adrenal crisis.

The lack of improvement over time due to better education is supported by recent publications stating that patients with adrenal insufficiency are still threatened by the risk of adrenal crisis (24), that there is a clear mismatch between self-perceived understanding of adrenal insufficiency/adrenal crisis and objectively tested knowledge (9), and that definitive and timely diagnosis of adrenal crisis is often not possible before treatment needs to be initiated to prevent mortality in patients with adrenal crisis (25). Further research is needed to investigate the effects of various education methods in preventing adrenal crises and how often education should be repeated. Despite proper education, some patients may remain at higher risk of adrenal crisis due to e.g. altered glucocorticoid sensitivity (23). Furthermore, adrenal crisis occurring during hospital admission in which patients undergo surgery for Cushing's syndrome cannot be prevented merely by educating the patient. Other ways to prevent crises need to be explored, e.g. education of the endocrine nursing staff in order to recognize early stages of adrenal crisis timely. A low threshold for

contact between patient and physician could cause overtreatment, but could also result in less severe cases of adrenal crisis (26).

In conclusion, patients with a history of Cushing's disease are at risk for adrenal crisis. Adrenal crisis tends to present early after institution of cure. Those patients that have experienced adrenal crisis are at increased risk for recurrent crisis. Although some risk factors tend to point towards increased risk for adrenal crisis (e.g. additional pituitary deficiencies), we believe more outcome data are necessary to identify groups at increased risk. Further research is needed to find effective education methods for preventing adrenal crisis, and to find ways to prevent the first adrenal crisis already during the hospital stay directly after surgery.

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Chapter 9

Improvement but no normalization of quality of life and cognitive functioning after treatment for Cushing's syndrome: a systematic review and meta-analysis

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Abstract

Background
Cushing's syndrome is characterized by glucocorticoid excess, which induces physical and mental symptoms, and impairments in quality of life. Biochemical cure improves symptoms, but quality of life and cognitive function may remain impaired.

Objective
To perform a systematic review and meta-analysis evaluating changes in health-related quality of life and cognitive functioning in patients with Cushing's syndrome after treatment.

Methods
Eight electronic databases were searched in March 2017, and PubMed again in May 2018, to identify potentially relevant articles. Eligible studies were (randomized controlled) trials, cohort studies, and cross-sectional studies assessing quality of life or cognitive functioning in patients treated for Cushing's syndrome. Quality of life measures were standardized; differences were expressed as standardized mean difference, and reported with 95% confidence intervals. We compared patients before and after treatment (improvement), and patients after treatment and healthy controls (normalization).

Results
We included 47 articles with in total 2,643 patients. Most patients had Cushing's disease and were in remission after treatment. Both quality of life and cognitive functioning improved after treatment in all studied domains. Compared to a healthy control population, quality of life did not normalize. Cognitive functioning normalized in part, but not all, of the studied domains.

Conclusions
Treatment of Cushing's syndrome improves quality of life and cognitive functioning. As normalization was not achieved in quality of life and in some aspects of cognitive functioning, special and continuous attention should be given to these aspects for patients after treatment. Effective interventions for further improvement and possibly normalization are urgently needed.

Introduction

Cushing's syndrome due to endogenous glucocorticoid excess is a rare condition and is either adrenocorticotropic hormone (ACTH)-dependent or ACTH-independent, both with a variety of underlying causes (1). Glucocorticoid excess causes osteoporosis, central obesity, insulin resistance, dyslipidemia, proximal muscle weakness, hypertension, hypercoagulability, and neuropsychiatric disorders. Patients report fatigue, a variety of mental and physical symptoms, and impairment in quality of life (2-3). Mortality and morbidity are increased even after long-term correction of glucocorticoid excess, including cognitive functioning, indicating irreversible adverse effects of previous hypercortisolism (4-6).

Cushing's disease resulting from an ACTH-secreting pituitary adenoma accounts for approximately 70% of cases of endogenous Cushing's syndrome, and has a reported incidence of 1.2-1.7 patients per million each year (7). The other causes for endogenous Cushing's syndrome are ectopic Cushing's syndrome resulting from a non-pituitary ACTH-producing source (approximately 5% of cases), and ACTH-independent Cushing's syndrome that is caused by a cortisol-producing adrenal adenoma or carcinoma (approximately 25% of cases) (1, 8). First-choice treatment for Cushing's disease is transsphenoidal pituitary surgery, selectively removing the corticotroph adenoma (9). Cushing's syndrome is generally approached by removing the ACTH-producing tumor in ectopic Cushing's syndrome and by adrenalectomy in ACTH-independent Cushing's syndrome (10). If necessary to establish cure, repeat surgeries, radiotherapy, and pharmaceutical therapies are considered. After surgical treatment, many patients face a period of transient or permanent adrenal insufficiency and sometimes other hormone deficits (11). A particular issue after surgery, but sometimes also during medical therapy, is the steroid withdrawal syndrome with its severe musculoskeletal pains, fatigue, and emotional lability (12).

In 2012, a literature review summarized the effects of Cushing's disease on clinical symptoms, including health-related quality of life and cognitive functioning, stating that current treatment options may not completely reverse the effects of chronic hypercortisolism (13). In 2015, another systematic review summarized quality of life in patients with a pituitary adenoma, concluding that patients with Cushing's disease, along with patients with acromegaly, demonstrated the greatest impairment in quality of life, and the smallest improvement (14). For Cushing's disease, two disease-specific quality of life questionnaires have been developed: the Tuebingen Cushing's disease quality of life inventory (Tuebingen CD-25), and the Cushing Quality of Life questionnaire (CushingQoL) (15-17). Also, a pituitary patient-specific questionnaire, the Leiden Bother and Needs Questionnaire, was developed for use in Cushing's disease (18). It is now generally accepted that disease-specific

questionnaires should be combined with a generic questionnaire to assess health-related quality of life. In addition, structural and functional brain abnormalities were shown to be persistent after biochemical cure of Cushing's syndrome, which was related to both quality of life and cognitive functioning impairments in patients with Cushing's syndrome (5). Until now, no meta-analysis has been performed to evaluate health-related quality of life or cognitive functioning in patients with Cushing's syndrome before and after treatment.

Study aims

The aim of the present study is to evaluate improvement in, and normalization of, health-related quality of life and cognitive functioning in patients with Cushing's syndrome. Improvement in health-related quality of life and cognitive functioning will be evaluated by comparing patients before treatment to patients after treatment of Cushing's syndrome. Whether health-related quality of life and cognitive functioning can normalize will be evaluated by comparing patients with Cushing's syndrome after (multimodality) treatment to a healthy control population.

Methods

Eligibility criteria

(Randomized controlled) trials, cohort studies (measuring at different time points) and cross-sectional studies (measuring at one point in time), assessing quality of life or cognitive functioning in patients with Cushing's syndrome were eligible for inclusion. Comparative studies (before-after treatment comparisons, or patients with Cushing's syndrome compared to healthy controls) and non-comparative studies were considered for inclusion. Eligible quality of life questionnaires were validated generic, disease-specific (for Cushing's syndrome), and domain-specific questionnaires. Articles were excluded if no separate results for patients with Cushing's syndrome were described, if the study included children only, or if no quantitative data of quality of life questionnaires or cognitive functioning tests were presented (e.g. only figure without numbers). There were no restrictions regarding treatment for Cushing's syndrome. If multiple studies with (partially) overlapping populations described the same questionnaire or test, only the data from the largest cohort were included per analysis. To minimize risk of selection bias, at least ten patients had to be included per study group. Articles irretrievable online were requested by contacting the authors. Only articles written in English were considered.

Search strategy

PubMed, Embase, Web of Science, COCHRANE Library, CENTRAL, Emcare, LWW, and ScienceDirect were systematically searched in March 2017 in cooperation with a specialized librarian to identify potentially relevant articles (see Supplemental Data 1 for the complete search strategy). In May 2018, the search was repeated in PubMed. References of included articles were searched for relevant eligible articles.

Data extraction

The identified articles were all entered in EndNote 8 (Thomson Reuters, Philadelphia, PA, USA). First, the studies were screened by title and abstract. Two independent reviewers reviewed potentially relevant articles in detail. For reporting, the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines were used (19).

The following data were extracted from all included articles: study period, study center, study design, etiology of Cushing's syndrome, number of patients, treatment of Cushing's syndrome, age, sex, duration of follow-up, type and number of control subjects, quality of life questionnaires and cognitive functioning tests used, and outcomes of these tests. If available, separate outcomes were extracted for patients in remission and patients not in remission after treatment.

If data were only presented according to categories (e.g., remission status or sex), the data were combined into one outcome score using a fixed effects meta-analysis for the main analyses. Combination scores were calculated from the subscale scores for the following questionnaires: CushingQoL (15), Symptom Rating Test (SRT) (20), Multidimensional Fatigue Inventory-20 (MFI-20) (21), Nottingham Health Profile (NHP) (22), and the Hospital Anxiety and Depression Scale (HADS) (22). For one article, the scores after treatment were calculated using the scores before treatment and the difference between before and after treatment, imputing the standard deviation (SD) from before treatment as the best estimate of the SD after treatment (23). If estimate and 95% confidence interval (CI), but not SD, were given for a single group, the following formula was used to calculate SD: ((CI)/2)/TINV(0.05;n-1)*SQRT(n), with n=number of patients. If data for two groups were combined as described above, SD was calculated using the following formula: SQRT((((n1-1)*SD1^2)+((n2-1)*SD2^2))/((n1+n2)-2)), with n1=number of patients in group 1, SD1=SD in group 1, n2=number of patients in group 2, and SD2=SD in group 2. From one article two questionnaires were excluded (Short Form health survey-36 [SF-36] and Beck Depression Inventory [BDI]) (24), and from another article one questionnaire was excluded (State Trait Anxiety Inventory [STAI]) (25), due to highly improbable or impossible outcomes (e.g. STAI score <20 points).

Risk of bias assessment

A component approach was used to assess risk of bias for all included studies. Components that could potentially bias a reported association between treatment for Cushing's syndrome and quality of life or cognitive functioning were included as follows:

1. Loss to follow-up <5% was considered low risk of bias for follow-up studies; similarly, missing quality of life or cognitive functioning data in <5% of patients was considered low risk of bias for cross-sectional studies.
2. Inclusion of patients: consecutive inclusion of all eligible patients or a random sample was considered low risk of bias.
3. Criteria for diagnosis of Cushing's syndrome: at least one of the following biochemical parameters had to be increased for low risk of bias: 24-h urinary free cortisol or midnight salivary cortisol.
4. Criteria for remission of Cushing's syndrome: at least normalization of biochemical hypercortisolism had to be measured for low risk of bias.
5. Test quality: number of cognitive domains assessed, validation of used questionnaires and tests, reporting of test instructions for cognitive tests, and reporting of sequence of cognitive tests were described for each study. Low risk of bias is considered use of only validated questionnaires and tests and reporting both test instructions and sequence of cognitive tests.

Risk of bias assessment was used to explore potential heterogeneity. Confounding was assessed by comparing baseline characteristics (age, sex, duration of follow-up, and treatment methods) for all included studies, as well as by comparing study group characteristics per study with a direct comparison before versus after treatment, or between patients after treatment and healthy controls. These assessments were made based on study level data.

Study endpoints

Quality of life scores were pooled for generic, disease-specific, and domain-specific (per domain) questionnaires separately. Analysed domains were anxiety, depression, and fatigue, as these were the only domains with enough data for analysis. Cognitive functioning was analysed in the following categories: intelligence (including concept formation), executive functioning (i.e. visuomotor tracking, inhibition, and mental flexibility), attention (i.e. divided, sustained), and memory (i.e. auditory, visual). In the category intelligence, the following tests were analysed: 1. Wechsler Adult Intelligence Scale – Revised (WAIS-R), 2. Similarities, and 3. Raven's Progressive Matrices (RPM). In the category executive functioning, the following tests were analysed: 1. Trail Making Test (TMT, trail A-D), and 2. Fluency tests (Verbal fluency, Word fluency, and the FAS test). In the category attention, the following tests were

analysed: 1. Substitution tests (Digit Symbol [Substitution] Test [D(S)ST], Digit symbol coding, and Letter-Digit Substitution Test [LDST]), and 2. Digit span. For the category memory, no analyses could be performed due to the large variety in used memory tests.

Per analysis, all quality of life questionnaires and cognitive functioning tests were included, with notifications for studies with (partially) overlapping populations using different questionnaires or tests. Separate analyses were performed per questionnaire or test (including subscales if provided) reported by at least two articles. Stratified analyses were performed for longitudinal and cross-sectional studies. Main analyses were performed in all included studies. Subgroup analyses were performed for patients with Cushing's disease only, and for patients in remission and patients not in remission after treatment.

Data were displayed separately for quality of life and cognitive functioning scores of patients before treatment for Cushing's syndrome, after treatment for Cushing's syndrome, and for a healthy control population. Notifications were added stating for which questionnaires and tests a higher score represents a lower quality of life or worse cognitive functioning.

Statistical analysis

Primary study outcomes were the standardized mean differences (SMD) before versus after treatment, as well as treated patients versus healthy controls, within studies. As a rule of thumb for the interpretation of the SMD, an effect size of 0.2 represents a small effect, 0.5 represents a moderate effect, and 0.8 represents a large effect (26). A random-effects model was used, as no fixed effect could be assumed due to the heterogeneity in questionnaires or tests; a fixed-effect model was used for analyses per questionnaire or test including <5 articles as in this case the between-study variance cannot be estimated reliably. All SMD scores were accompanied by 95% CI. No overall scores were presented per analysis with various tests/questionnaires, because a different number of (sub)scales per included article resulted in unintentional inequality in assigned weights per study, leading to incorrect effect estimates and confidence intervals. For meta-analyses only, questionnaires and tests in which a higher score represents a lower quality of life or worse cognitive functioning were reversed by multiplying the outcome with -1, ensuring that all outcomes were in the same direction. For three articles, '±' was interpreted as SD in the analyses, as this remained unclear after reading the articles (24, 27-28). The D(S)ST and LDST were included in the analyses in items/second, the TMT in seconds, and fluency tests in number/minute. All analyses were performed in Stata 14.2 (Stata Corp., College Station, TX, USA).

Results

Study selection

The initial search yielded 717 potentially relevant articles. After searching through references of included articles and repeating the search in PubMed in May 2018, another nine articles were added, providing 726 articles. After screening the articles by title and abstract, 603 articles were excluded, leaving 123 articles for detailed review. In total, 47 articles were included in this review, of which 32 reported on quality of life only, ten on cognitive functioning only, and five reported on both quality of life and cognitive functioning. Reasons for excluding articles are summarized in Figure 1.

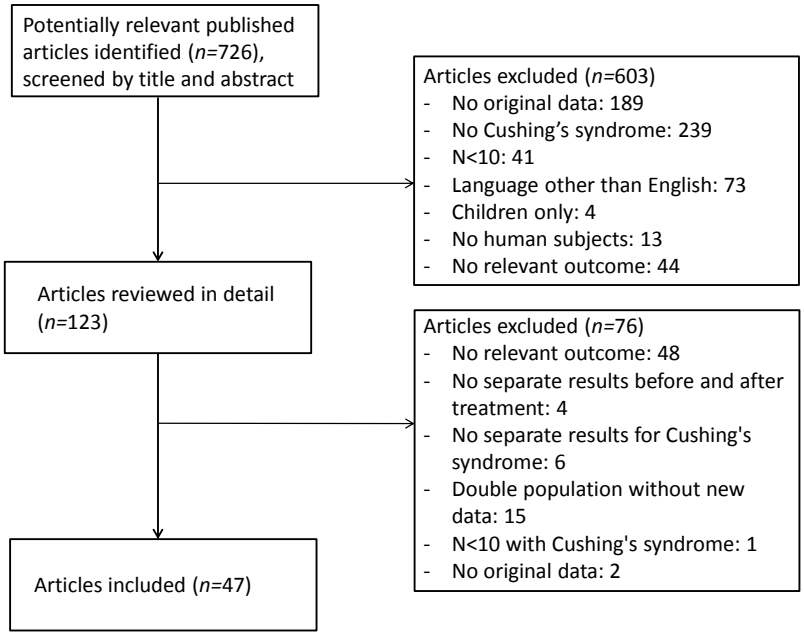

Figure 1: Flow-chart of inclusion of articles in this systematic review.

Study characteristics (Supplemental Data 2)

We included 27 cross-sectional studies (6, 22, 24-25, 27, 29-50), sixteen cohort studies (28, 51-65), one article that included both a cross-sectional study part and a cohort study part (66), one single-arm trial (67), and two articles about the same randomized controlled trial (pasireotide 600 µg versus pasireotide 900 µg) (23, 68). Studies were published between 1985 and 2017. Of the included studies, 28 reported on Cushing's disease only, one on adrenal Cushing's syndrome only, seventeen described a mixed population, and one study included a cohort of patients with

Cushing's disease only, as well as a mixed population cross-sectional study part. In total, the included studies described 2,643 patients, partially from overlapping populations.

Seventeen articles included a healthy control group (n=2,335, also partially from overlapping populations), of which fifteen studies matched controls on at least age and sex, and nine articles used normative data from the general population or literature reference values. Studies comparing patients before and after treatment by design included the same population for both measurement times, reducing risk of confounding, although bias remains possible through loss to follow-up, as described below.

Baseline characteristics varied between all included studies. As data were insufficient to estimate risk of confounding, and inclusion of articles for meta-analysis differed per analysis and per domain, baseline characteristics data were summarized for all articles. Average age was between 33.6 and 57.0 years. Percentage female patients varied between 40% and 100%. Average duration of follow-up for cohort studies was 6 to 54 months. Four studies, which were not included in any meta-analysis, did not present data after treatment. Of the remaining 43 studies, 23 used multimodality treatment, fifteen used surgical procedures only, three only used pharmaceutical treatment, and two did not describe the nature of the treatment.

Risk of bias assessment (Supplemental Data 2)
Loss to follow-up was reported by fourteen out of twenty cohort studies and trials, with a range of 0-74% loss to follow-up. Only four studies reported a loss to follow-up <5%. Of the 27 cross-sectional studies, 6 (22%) reported missing data for quality of life or cognitive functioning ≥5%. Fourteen articles (30%) explicitly stated including consecutive patients. Criteria for diagnosis of Cushing's syndrome were reported adequately by 21 studies (45%). Criteria for remission of Cushing's syndrome were reported adequately by 26 studies out of 43 with postoperative measurements (61%).

Study outcomes
Quality of life was reported in 37 articles, using eleven different generic questionnaires, two disease-specific questionnaires (i.e., CushingQoL and Tuebingen CD-25), and 21 domain-specific questionnaires (including amongst others five anxiety, six depression, and five fatigue questionnaires). Twelve studies reported quality of life both before and after treatment. Quality of life data were reported for patients with Cushing's syndrome before treatment by fifteen studies, for patients with Cushing's syndrome after treatment by 34 studies, and for a healthy control population by seventeen studies.

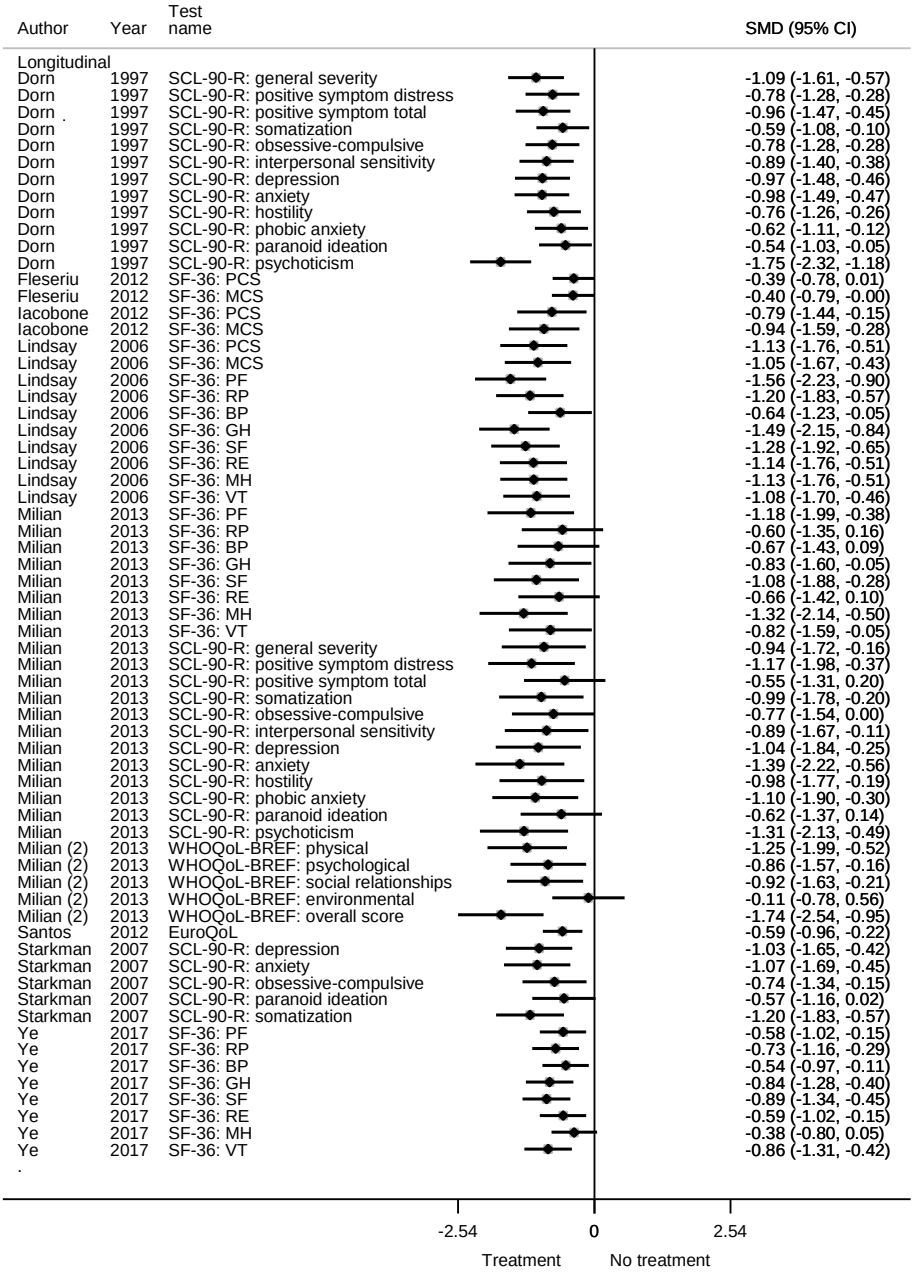

Figure 2: Generic quality of life before versus after treatment for Cushing's syndrome.

Cognitive functioning was reported in fifteen articles, using 35 different tests (including four tests on intelligence and concept formation, six on executive functioning, ten on attention, and fourteen on memory). Only six studies reported

cognitive functioning both before and after treatment. Cognitive functioning scores were reported for patients with Cushing's syndrome before treatment by eight studies, for patients with Cushing's syndrome after treatment by thirteen studies, and for a healthy control population by twelve studies. Detailed study outcomes and an overview of all included questionnaires and tests with abbreviations can be found in Supplemental Data 2.

Meta-analyses of improvement of quality of life and cognitive functioning

Quality of life and cognitive functioning improved after treatment in all studied categories (generic, disease-specific, domain-specific: anxiety, and domain-specific: depression quality of life, and the cognitive functions intelligence, executive functioning and attention). Generic quality of life improved by a SMD of 0.11 to 1.75 in all included studies (see Figure 2). Disease-specific quality of life improved by a SMD of 0.16 to 1.57 in all included studies (see Figure 3). For domain-specific quality of life and cognitive functioning, studies showed SMDs of 0.08 to 0.86, indicating improvement in all aspects of quality of life and cognitive functioning.

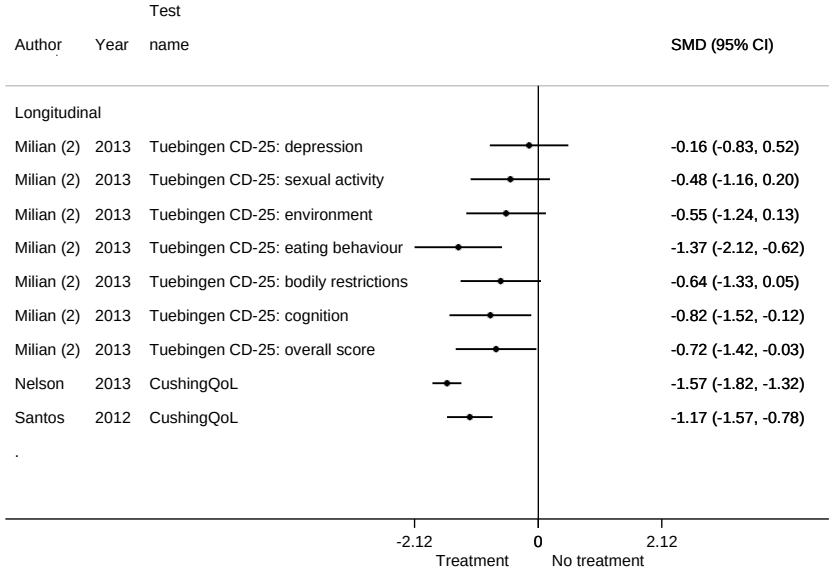

Figure 3: Disease-specific quality of life before versus after treatment for Cushing's syndrome.

Table 1 shows a summary of all meta-analyses regarding improvement in quality of life and cognitive functioning, including subgroup analyses for patients with Cushing's disease only, and analyses stratified by remission status after treatment. Supplemental Data 3-6 show the raw figures for the main analyses, analyses for patients with Cushing's disease only, analyses according to remission status, and analyses according to remission status for patients with Cushing's disease only, respectively.

Table 1: Summary of results of improvement in and normalization of quality of life and cognitive functioning in Cushing's syndrome.

	Main analysis (all etiologies included)	Cushing's disease only	Stratified by remission status: remission	Stratified by remission status: no remission	Cushing's disease only and stratified by remission status: remission	Cushing's disease only and stratified by remission status: no remission
Generic quality of life						
1. Improvement? (Do patients after treatment score better than before treatment?)	Yes (l, s=9, t=65): SMD 0.11 (a) to 1.75 (a)	Yes (l, s=5, t=48): SMD 0.11 (a) to 1.74 (a)	Yes (l, s=5, t=25): SMD 0.11 (a) to 1.75 (a)	Insufficient data for meta-analysis	Yes (l, s=2, t=10): SMD 0.11 (a) to 1.74 (a)	Insufficient data for meta-analysis
2. Normalization? (Do patients after treatment score as well as or better than healthy controls?)	No (c, s=7, t=59): SMD 0.05 (a) to 1.61 (h)	No (c, s=6, t=58): SMD 0.05 (a) to 1.61 (h)	No (c, s=5, t=53): SMD 0.05 (a) to 1.61 (h)	No (c, s=2, t=28): SMD 0.40 (a) to 2.25 (h)	No (c, s=4, t=52): SMD 0.05 (a) to 1.61 (h)	No (c, s=2, t=28): SMD 0.40 (a) to 2.25 (h)
Disease-specific quality of life						
1. Improvement? (Do patients after treatment score better than before treatment?)	Yes (l, s=3, t=9): SMD 0.16 (a) to 1.57 (a)	Yes (l, s=2, t=8): SMD 0.16 (a) to 1.57 (a)	Yes (l, s=3, t=9): SMD 0.16 (a) to 1.37 (a)	Yes (l, s=2, t=2): SMD 0.63 (a) to 1.60 (a)	Yes (l, s=2, t=8): SMD 0.16 (a) to 1.37 (a)	Insufficient data for meta-analysis
2. Normalization? (Do patients after treatment score as well as or better than healthy controls?)	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis
Quality of life, domain: anxiety						
1. Improvement? (Do patients after treatment score better than before treatment?)	Yes (l, s=2, t=3): SMD 0.25 (a) to 0.59 (a)	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis
2. Normalization? (Do patients after treatment score as well as or better than healthy controls?)	No (c, s=3, t=4): SMD: 0.50 (h) to 0.96 (h)	No (c, s=3, t=4): SMD: 0.50 (h) to 0.96 (h)	No (c, s=3, t=4): SMD: 0.50 (h) to 0.96 (h)	Insufficient data for meta-analysis	No (c, s=3, t=4): SMD: 0.50 (h) to 0.96 (h)	Insufficient data for meta-analysis

Table 1: Summary of results of improvement in and normalization of quality of life and cognitive functioning in Cushing's syndrome (continued).

	Main analysis (all etiologies included)	Cushing's disease only	Stratified by remission status: remission	Stratified by remission status: no remission	Cushing's disease only and stratified by remission status: remission	Cushing's disease only and stratified by remission status: no remission
Quality of life, domain: depression						
1. Improvement? (Do patients after treatment score better than before treatment?)	Yes (l, s=2, t=2): SMD 0.35 (a) to 0.51 (a)	Insufficient data for meta-analysis	Yes (l, s=2, t=2): SMD 0.35 (a) to 0.41 (a)	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis
2. Normalization? (Do patients after treatment score as well as or better than healthy controls?)	No (c, s=4, t=5): SMD 0.33 (h) to 1.20 (h)	No (c, s=4, t=5): SMD 0.33 (h) to 1.20 (h)	No (c, s=4, t=5): SMD 0.33 (h) to 1.20 (h)	Insufficient data for meta-analysis	No (c, s=4, t=5): SMD 0.33 (h) to 1.20 (h)	Insufficient data for meta-analysis
Quality of life, domain: fatigue						
1. Improvement? (Do patients after treatment score better than before treatment?)	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis
2. Normalization? (Do patients after treatment score as well as or better than healthy controls?)	No (c, s=2, t=7): SMD 0.20 (h) to 1.08 (h)	Insufficient data for meta-analysis	No (c, s=2, t=7): SMD 0.20 (h) to 1.08 (h)	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis
Cognitive function: intelligence						
1. Improvement? (Do patients after treatment score better than before treatment?)	Yes (l, s=3, t=6): SMD 0.08 (a) to 0.77 (a)	Insufficient data for meta-analysis	Yes (l, s=3, t=6): SMD 0.08 (a) to 0.77 (a)	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis
2. Normalization? (Do patients after treatment score as well as or better than healthy controls?)	Partially (l, s=3, t=6): SMD 0.55 (a) to 0.78 (h)	Insufficient data for meta-analysis	Partially (l, s=3, t=6): SMD 0.55 (a) to 0.78 (h)	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis

Table 1: Summary of results of improvement in and normalization of quality of life and cognitive functioning in Cushing's syndrome (continued).

	Main analysis (all etiologies included)	Cushing's disease only	Stratified by remission status: remission	Stratified by remission status: no remission	Cushing's disease only and stratified by remission status: remission	Cushing's disease only and stratified by remission status: no remission
Cognitive function: executive functioning						
1. Improvement? (Do patients after treatment score better than before treatment?)	Yes (l, s=3, t=6): SMD 0.19 (a) to 0.86 (a)	Yes (l, s=2, t=2): SMD 0.19 (a) to 0.78 (a)	Yes (l, s=3, t=6): SMD 0.19 (a) to 0.86 (a)	Insufficient data for meta-analysis	Yes (l, s=2, t=2): SMD 0.19 (a) to 0.78 (a)	Insufficient data for meta-analysis
2. Normalization? (Do patients after treatment score as well as or better than healthy controls?)	Yes (l, s=2, t=4): SMD: 0.48 (a) to 0.31 (h) Unclear (c, s=4, t=9): SMD 0.00 to 0.31 (h)	Unclear (c, s=2, t=3): SMD 0.00 to 0.11 (h)	Yes (l, s=2, t=4): SMD: 0.48 (a) to 0.31 (h) Unclear (c, s=3, t=7): SMD 0.00 to 0.33 (h)	Insufficient data for meta-analysis	Unclear (c, s=2, t=3): SMD 0.00 to 0.33 (h)	Insufficient data for meta-analysis
Cognitive function: attention						
1. Improvement? (Do patients after treatment score better than before treatment?)	Yes (l, s=2, t=4): SMD 0.53 (a) to 0.71 (a)	Insufficient data for meta-analysis	Yes (l, s=2, t=4): SMD 0.53 (a) to 0.71 (a)	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis
2. Normalization? (Do patients after treatment score as well as or better than healthy controls?)	Yes (l, s=2, t=4): SMD 0.25 (a) to 0.14 (h) No (c, s=3, t=5): SMD 0.16 (h) to 0.31 (h)	No (c, s=2, t=2): SMD 0.21 (h) to 0.31 (h)	Yes (l, s=2, t=4): SMD 0.25 (a) to 0.14 (h) Unclear (c, s=2, t=2): SMD 0.11 (a) to 0.31 (h)	Insufficient data for meta-analysis	Unclear (c, s=2, t=2): SMD 0.11 (a) to 0.31 (h)	Insufficient data for meta-analysis
Cognitive function: memory						
1. Improvement? (Do patients after treatment score better than before treatment?)	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis
2. Normalization? (Do patients after treatment score as well as or better than healthy controls?)	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Insufficient data for meta-analysis

a=indicates that patients after treatment performed better than before treatment, b=indicates that patients before treatment performed better than patients after treatment, c=cross-sectional, h=indicates that healthy controls performed better than patients after treatment, l=longitudinal, s=number of studies included, SMD=standardized mean difference, t=number of (sub)tests or (sub)questionnaires included

Meta-analyses of normalization of quality of life and cognitive functioning

Quality of life did not normalize after treatment for Cushing's syndrome. For generic quality of life SMDs varied across included studies from 0.05 in favor of patients after treatment to 1.61 in favor of healthy controls (see Figure 4). For domain-specific quality of life, SMDs varied from 0.20 to 1.20, indicating that healthy controls consistently have higher quality of life than patients after treatment for Cushing's syndrome.

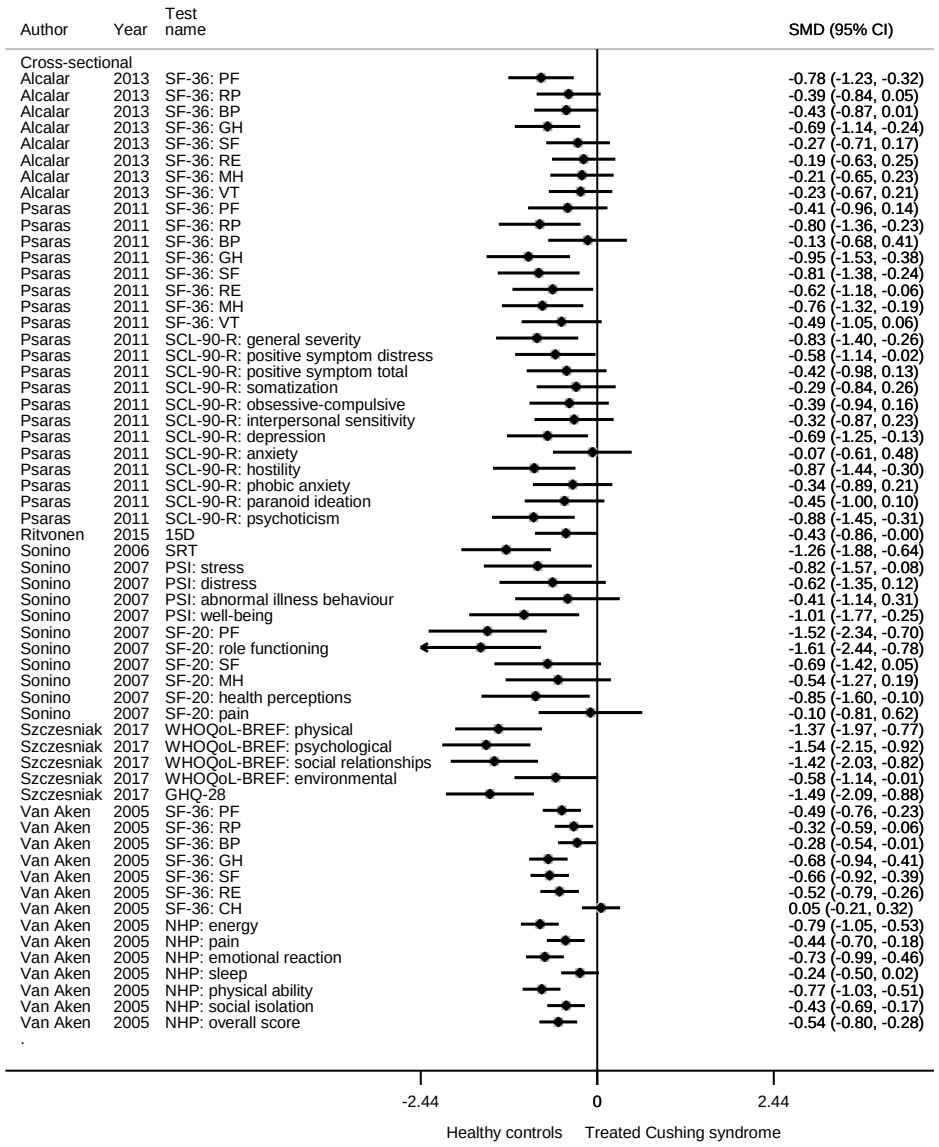

Figure 4: Generic quality of life after treatment for Cushing's syndrome versus healthy controls.

Cognitive functioning partially normalized after treatment for Cushing's syndrome. SMDs for intelligence varied across studies from 0.55 in favor of patients after treatment to 0.78 in favor of healthy controls. Executive functioning and attention were tested in both longitudinal as well as cross-sectional studies, which showed conflicting results. The longitudinal studies showed normalization of both domains of cognitive functioning, with SMDs varying between 0.48 in favor of patients after treatment and 0.31 in favor of healthy controls. The cross-sectional studies showed SMDs of 0.00 to 0.31 in favor of healthy controls, suggesting that no normalization of cognitive functioning had occurred. More detailed results regarding normalization of quality of life and cognitive functioning can be found in Table 1 and Supplemental Data 3-6, including subgroup analyses.

Discussion

The present systematic review and meta-analysis shows that quality of life and cognitive functioning improve after treatment for Cushing's syndrome. However, quality of life does not normalize, and only partial normalization occurs in cognitive functioning. These results demonstrate that biomedical treatment of Cushing's syndrome is the first step towards improvement in quality of life and cognitive functioning, but that room for further improvement remains in aiming to establish normalization of quality of life and cognitive functioning.

The present observations are in line with the results of a previous literature review by Feelders *et al.*, which described that health-related quality of life improved in patients with Cushing's disease during biochemical remission, but that it remained impaired compared to healthy controls. The same study also found that cognitive functioning did not improve short-term, and suggested that there may be a delay between correction of hypercortisolism and recovery of impairments in cognitive functioning (13). This is in contrast with our findings, since we found improvement and partial normalization of cognitive functioning. However, no truly short-term studies with only patients up to one year follow-up were included in this review, preventing extensive analyses according to follow-up time for both cognitive functioning as well as quality of life. This might explain the different findings regarding cognitive functioning improvement, and it would also support the suggestion of a delay between correction of hypercortisolism and recovery of impairments in cognitive functioning. Our findings are in accordance with another systematic review, which described quality of life in patients with a pituitary adenoma in general, and found that patients with Cushing's disease showed the smallest improvement and no normalization after treatment. They also reported room for further improvement in quality of life, potentially by psychosocial

interventions as well as optimal medical treatment (14). Two articles were published after our last search in May 2018. Our results were in accordance with the first one by Valassi *et al.*, which demonstrated that quality of life, as assessed with the EQ-5D and CushingQoL, improved after treatment for Cushing's syndrome (69). The second article by Osswald *et al.* compared quality of life, as assessed with the SF-36, CushingQoL, and Tuebingen CD-25, between patients in remission of ectopic Cushing's syndrome and patients with remitted Cushing's disease, and observed that female patients with ectopic Cushing's syndrome reported a better quality of life compared to female patients with Cushing's disease. This difference was not observed in male patients. Comparing the quality of life scores of these patients to the quality of life scores reported in our included studies, it can be observed that the patients in the study of Osswald *et al.* scored better on all three questionnaires. As this study included a small population (n=69), their results would have meant a small change towards better quality of life in the average that we found for analyses including these questionnaires after treatment for Cushing's syndrome only (70).

Although quality of life and cognitive functioning have been addressed before separately in systematic reviews, this is the first study investigating both quality of life as well as cognitive functioning. Furthermore, not only patients with Cushing's disease were included, but all patients with Cushing's syndrome. The following study limitations need to be taken into account when interpreting the results. Included studies showed heterogeneity regarding etiology of Cushing's syndrome, treatment strategy, and remission status after treatment. Results were consistent across the subgroup analyses for Cushing's disease only and the subgroup analyses stratified by remission status. Due to lack of sufficient data per category, no separate analyses stratified by treatment strategy could be performed. As longitudinal studies were expected to differ less in treatment strategy and follow-up time between individual patients than cross-sectional studies, analyses were performed separately for longitudinal versus cross-sectional studies. Only two cognitive functioning domains were tested by enough longitudinal and cross-sectional studies to perform and compare both analyses, hindering extensive comparison between the two study designs. Studies directly comparing different treatment strategies should be performed to determine the effect of treatment strategy on quality of life and cognitive functioning improvement and normalization.

As there were already few articles included per category, no sensitivity analysis with only low risk of bias studies could be performed. Only two of the included articles had low risk of bias on all components. Most of the included articles were not low risk of bias because they had too high loss to follow-up or because they selected patients based on remission status. High loss to follow-up could have caused bias in longitudinal studies. As it is most likely that patients who perform worse find it

important to participate in quality of life research, our results may be too pessimistic, meaning that the improvement after treatment is actually larger than we observed. Publication bias was minimized by searching for otherwise unpublished meeting abstracts in Embase, Web of Science, and COCHRANE Library. This did not lead to additional data.

As glucocorticoid excess is known to cause not only physical symptoms, but also reduced quality of life and cognitive symptoms (2-3), improvement in quality of life and cognitive functioning after treatment of Cushing's syndrome could be explained by the normalization of cortisol concentrations with accompanying reduction in physical symptoms of Cushing's syndrome. Lack of normalization of quality of life and cognitive functioning after treatment might be explained by the structural and functional brain abnormalities observed in patients with active Cushing's syndrome, that even persist after long-term remission of Cushing's syndrome (5). The partial normalization in cognitive functioning found in this study has not been described previously. Only the results from two small cohort studies showed clear normalization in cognitive functioning (54, 59). Larger cohort or cross-sectional studies, or (randomized controlled) trials comparing different treatment methods, are necessary to confirm the normalization in cognitive functioning observed in these two small cohort studies. Theoretically, partial normalization in cognitive functioning might be explained by the involvement of different brain regions in cognitive functioning tasks that showed normalization, than the brain regions affected by structural and functional abnormalities as described above.

In conclusion, treatment of Cushing's syndrome is the first effective step in improving quality of life and cognitive functioning. However, the most effective treatment regimen for Cushing's syndrome regarding improvement in quality of life and cognitive functioning is still unknown, and probably consists of a multidisciplinary approach of at least endocrinology, surgery, and psychology, as well as early diagnosis to minimize permanent structural and functional brain abnormalities. As no normalization could be achieved in quality of life and part of the cognitive functioning domains, patients require special attention from the clinician for quality of life as well as for cognitive functioning after effective treatment for Cushing's syndrome. Interventions for further improvement and possibly normalization of quality of life and cognitive functioning should be investigated with priority.

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Chapter 10

General discussion and summary

Introduction

Hypercortisolism is the cause of numerous and potentially severe complications, which are often underestimated and not well recognized in clinical practice (1-3). There are two main causes for hypercortisolism. Firstly, exogenous hypercortisolism through corticosteroid use, which is highly prevalent, since around 1% of the general population uses corticosteroids (4, 5). Secondly, endogenous Cushing's syndrome, which is a rare condition, but the disease burden is considerable through its increased morbidity and mortality risks (2, 3, 6). To increase our knowledge on the effects of endogenous hypercortisolism, large-scale studies are necessary, as single-center cohort studies often have insufficient power due to the small patient population. In this thesis, we present several meta-analyses, one population-based cohort study from Denmark, and combined data from two single-center cohorts from tertiary referral centers in Leiden and Berlin.

Part I: Complications of corticosteroid use

Corticosteroids are widely used for various conditions, such as inflammatory disease, malignancies, and organ transplantation, in order to suppress an inflammatory response, although many complications and side effects are known (5, 7). In this thesis, **Chapter 2**, **Chapter 3**, and **Appendix I** discuss potential complications of exogenous hypercortisolism due to corticosteroid use.

Corticosteroid use is the most common cause of adrenal insufficiency, by suppression of the hypothalamus-pituitary-adrenal (HPA) axis. Adrenal insufficiency is a serious and potentially life-threatening situation (8). In **Chapter 2** and **Appendix I**, the proportion of patients that develop adrenal insufficiency after use of corticosteroids was studied in a systematic review. Results were stratified by route of administration, underlying disease, treatment dose, and treatment duration, to identify patients with high risk of adrenal insufficiency. From the literature, 74 articles with 3,753 patients were included in this meta-analysis. Percentage of adrenal insufficiency varied widely, from 4.2% for nasal administration to 52.2% for intra-articular administration. There was no administration form, treatment dose, treatment duration, or underlying disease for which adrenal insufficiency could certainly be excluded. The lowest risk of adrenal insufficiency (1.4%) was for patients after short-term use of corticosteroids, and the highest risk (60.0%) for patients with hematological malignancies. Therefore, in clinical practice, both patients and clinicians should be informed of the risk and symptoms of adrenal insufficiency after use of corticosteroids, and the threshold to test corticosteroid users for adrenal

insufficiency should be low. However, how to test for adrenal insufficiency in a population of corticosteroid users, and when to expect improvement, remains difficult, especially if the dose of corticosteroids is reduced but not withdrawn. The importance of testing for adrenal insufficiency and the need for treatment, despite imperfect tests, is discussed further in **Appendix II**.

To identify a potentially increased mortality risk in patients using corticosteroids compared to non-users, patients with the same disease should be compared to minimize an effect of the underlying disease per se on mortality risk. **Chapter 3** describes a population-based cohort study, situated in Denmark (n=5,289,261), on mortality risk in patients with perforated diverticular disease comparing patients that use corticosteroids with non-users. The study included 4,640 patients with perforated diverticular disease, of whom 19.3% had used corticosteroids for various underlying diseases. Mortality risk was doubled for patients with recent corticosteroid use after full adjustment for potential confounders. Highest mortality risk was 52.5% mortality within one year for patients who started using corticosteroids within 90 days before hospital admission. To assess potential confounding by indication for corticosteroid use on mortality, several sensitivity analyses were performed excluding high-risk patient populations, e.g. patients with malignancies. These sensitivity analyses showed similar results to the main analysis, suggesting confounding by indication was not fully explaining mortality risk after corticosteroid use in patients with perforated diverticular disease. Hence, use of corticosteroids should be regarded as an important risk factor for mortality in clinical practice among patients with perforated diverticular disease.

It was already well known that corticosteroid use is the cause of various complications and side effects (1). Our studies added information to the knowledge of complications of corticosteroid use regarding adrenal insufficiency and mortality risk. However, it is a common misconception that low-dose, short-term use of corticosteroids, or the use of only inhalation corticosteroids, is risk-free (9). Risk of adrenal insufficiency exceeds 50% in long-term (>1 year), high-dose corticosteroid use, and is higher in systemic use rather than inhalation use only, but even patients with short-term (<1 month), low-dose use, or inhalation only, are at risk of adrenal insufficiency. Mortality risk in patients with perforated diverticular disease is increased similarly regardless of corticosteroid dose, and it is increased in inhalation corticosteroid use only as well, although less pronounced. Given the mechanism of action of corticosteroids, it is likely that this increased mortality risk is not restricted to patients with perforated diverticulitis.

Besides adrenal insufficiency and increased mortality risk, patients using corticosteroids are at risk of osteoporosis and fractures, diabetes mellitus,

cardiovascular disease, myopathy, cataracts and glaucoma, neuropsychiatric disturbances, and immunosuppression (10). Considering these risks of severe complications, all corticosteroid-using patients should be monitored adequately, which includes periodical physical check-ups and (laboratory) measurements (e.g. lipids, glucose) for adults every 3-6 months in the first year, and subsequently every 12 months (10). Currently, there is no clinical guideline available specifically for the evaluation of possible adrenal insufficiency after use of corticosteroids, and therefore, clear guidance on recovery of the HPA-axis is lacking. In general, screening for adrenal insufficiency should be performed at least 18-24 hours after last short-acting corticosteroid dose (i.e. hydrocortisone) by measuring early morning serum cortisol, and by performing an adrenocorticotropic hormone (ACTH) stimulation test if morning cortisol concentration is between 3 and 15 µg/dL (i.e. 83-414 nmol/L) (11). We suggest screening at least those patients with nonspecific symptoms after cessation of corticosteroid use. Because recognition and screening of complications of corticosteroid use in clinical practice is currently insufficient, the use of corticosteroids should be minimized if clinically feasible.

Part II: Treatment outcome in Cushing's syndrome

In contrast to exogenous hypercortisolism, endogenous hypercortisolism is very rare, with an incidence of 1.2-2.4 per million persons each year (12). Cushing's disease, caused by a pituitary adenoma, and other causes of endogenous Cushing's syndrome, most often caused by an adrenal adenoma or an ectopic ACTH-producing tumor, are characterized by severe hypercortisolism with increased morbidity and mortality rates, if left untreated (2, 3, 6). Therefore, patients should be treated without delay (13). **Chapters 4 to 6** describe the results of our studies on treatment options for Cushing's disease and other causes of endogenous Cushing's syndrome.

Cushing's disease is the most prevalent underlying cause of endogenous hypercortisolism (12). For Cushing's disease, the first-choice treatment option is transsphenoidal selective adenomectomy (14). For this surgical procedure, two techniques are in use: microscopy and endoscopy. Microscopic surgery is the older procedure, in which the neurosurgeon observes a direct, three-dimensional image of the pituitary by looking through the microscope (15). Endoscopic surgery is newer, and, by means of a camera attached to the end of the endoscope, allows the neurosurgeon a closer look at the pituitary, including angles that are impossible to visualize with the microscope, however losing the three-dimensional vision (16). In **Chapter 4**, we described both techniques in our cohort of patients treated in the Leiden University Medical Center (LUMC) to assess potential differences regarding remission rate, mortality risk, or short- and long-term complications. We included

137 patients, of whom 87 were treated microscopically, and 50 endoscopically. We found no clear advantage of either technique for the treatment of Cushing's disease. When the results were stratified by tumor size, patients with macroadenomas had a lower risk of recurrence after microscopic surgery than after endoscopic surgery. Theoretically however, endoscopic surgery is expected to perform better, especially in patients with large or invasive tumors, because the entire tumor can be visualized, which is not always possible with microscopy. Possibly, the advantage of microscopic surgery for patients with macroadenomas found in our study was due to the selected population of patients with macroadenomas, as patients with large and invasive tumors may have been more often referred to our center than patients with non-invasive macroadenomas. Patients with large and invasive tumors have a worse prognosis despite treatment, and they are more often treated endoscopically than patients with non-invasive and smaller tumors, which can lead to biased results. Selective patient referral resulted in eight endoscopically treated (50%) versus five microscopically treated (28%) patients with macroadenomas and cavernous sinus invasion in our cohort. However, excluding these patients in a sensitivity analysis did not largely alter the results.

To compare the results from our center to the results reported in literature, in **Chapter 5**, we performed a systematic review and meta-analysis on the same topic, microscopic versus endoscopic transsphenoidal adenomectomy. We included 97 articles with 6,695 patients in total, of whom 5,711 were treated microscopically and 984 endoscopically. We found no clear differences in remission, recurrence, or mortality rates. Most complications occurred similarly in both surgical techniques. Cerebrospinal fluid leakage occurred more often in endoscopic surgery, whereas transient diabetes insipidus occurred more often in microscopic surgery. If only microadenomas were considered, no difference between both techniques regarding remission or recurrence rate was found, in accordance with our cohort study. However, for macroadenomas only, we found a higher remission rate and lower recurrence rate after endoscopy compared to microscopy, opposing the results from our cohort study. The difference in outcome between our cohort study and systematic review underlines the importance of standardized outcome measures, internationally shared definitions, and combining data from multiple centers before making recommendations for clinical practice.

As endoscopic surgery is the newer technique, there is on average less experience with this technique than with microscopic surgery. Especially in the first years after switching to endoscopy in a specific center, worse results may be expected, which will improve with increasing experience (17-20). This should be taken into account in interpreting comparisons between both techniques. In **Chapter 4**, we assessed this potential learning curve in our cohort study by comparing the first years of endoscopy

with subsequent years. However, no learning curve was found for endoscopic surgery, which may be due to the small population size and thus low statistical power. For clinical practice, the results of **Chapter 5** suggest that endoscopic surgery is the preferred method for treating patients with Cushing's disease and a macroadenoma. Microscopic surgery can be used based on neurosurgeon's preference, but for macroadenomas referral to a center specialized in endoscopic surgery should be considered. No transient worsening of outcomes was seen in our center during the transition to endoscopic surgery, which may be reassuring for other centers considering transitioning, although evaluation of this new method should be performed per treatment center.

Although transsphenoidal adenomectomy is the preferred treatment method for Cushing's disease, other treatment options are in practice for patients with a contraindication for transsphenoidal surgery, persistent or recurrent disease, and for patients who refuse surgery. These treatment options include cortisol-lowering medical therapy (steroidogenesis inhibitors, glucocorticoid receptor antagonists, cabergoline, and pasireotide), radiotherapy, and bilateral adrenalectomy, and can likewise be used for patients with endogenous Cushing's syndrome (13, 21). Bilateral adrenalectomy always leads to complete adrenal insufficiency, necessitating life-long hydrocortisone and fludrocortisone replacement therapy (8). A common complication of radiotherapy is hypopituitarism with corresponding hormone replacement therapy, and additionally radiotherapy usually takes several months before the first beneficial effects become apparent (13). Therefore, both bilateral adrenalectomy and radiotherapy are not recommended as first-line treatment options (13), and medical treatment is increasingly initiated. To estimate the effectiveness of medical treatment, we performed a systematic review and meta-analysis in **Chapter 6**. We included 35 articles with 1,520 patients with Cushing's disease or other causes of endogenous Cushing's syndrome using six different kinds of cortisol-lowering medical treatment. Average duration of follow-up per study was 2 weeks to 11.5 years, with a majority of short-term studies. Cortisol secretion normalization ranged from 35.7% (cabergoline) to 81.8% (mitotane). However, medical agents with higher effectiveness regarding cortisol normalization also led to a higher percentage of patients with side effects, and vice versa. Patients using multiple medical agents simultaneously, or consecutively, showed a higher percentage of cortisol normalization (65.7%) than patients on monotherapy (49.4%). The percentage of patients with Cushing's disease with normalized cortisol is lower than after first-line transsphenoidal surgery, but it is comparable with remission rates after repeat transsphenoidal surgery, as described in **Chapter 5**. This suggests that medical treatment is a valuable alternative to transsphenoidal surgery for patients with a contraindication for surgery, persistent or recurrent disease, and for patients who refuse surgery. However, long-term effectiveness and side effects have not been investigated in detail, including the

effects on quality of life. This is relevant for these patients, as they will need life-long cortisol-lowering medication if this treatment strategy is considered necessary.
Even after the introduction of a treatment method or medical agent, assumptions about effectiveness and side effects or complications should be confirmed or rejected. For Cushing's disease, our meta-analysis suggests that the presumed advantage of endoscopic surgery exists only for macroadenomas, but not for microadenomas. We also found that medical treatment is as effective and safe as repeat transsphenoidal surgery, extending the range of reasonable treatment options for patients with Cushing's disease. However, medical treatment must be administered life-long and will never normalize pulsatile hormone secretion, which may be important for long-term morbidity and quality of life of these patients. Knowledge on the optimal treatment method as well as a broad range of effective treatment options are essential in reducing the high disease burden due to the severity of untreated Cushing's disease and Cushing's syndrome. To optimize use of existing treatment methods in clinical practice, future studies could investigate which cortisol-lowering medical agent should be administered first, and which combination of medical agents is most effective and safe in the treatment of Cushing's disease and Cushing's syndrome. Furthermore, patient registries with longer-term follow-up are needed to investigate long-term effectiveness and safety of cortisol-lowering agents, including effects on long-term morbidity and quality of life.

Part III: Clinical outcome in Cushing's syndrome

The clinical consequences of Cushing's disease and Cushing's syndrome, both before and after effective treatment, can be severe, and it is often unknown which patients are at a higher risk of specific morbidities. **Chapters 7 to 9** describe a range of clinical outcomes in patients with Cushing's disease and Cushing's syndrome. To determine which patients are at risk of specific clinical outcomes, prediction models can be used to find risk factors and stratified analysis can be performed to compare categories of patients. We decided to investigate sex as a potential risk marker for specific clinical outcomes. Sex-based differences were hypothesized due to the different clinical pictures for males and females encountered in clinical practice. After investigating a potential risk marker for clinical outcomes, we studied two clinical outcomes known to be affected before treatment: quality of life and cognitive functioning. We were interested if successful treatment of Cushing's syndrome is also effective in improving, or even normalizing, quality of life and cognitive functioning. Furthermore, we chose to investigate the occurrence of adrenal crisis after treatment, as this is a severe potential complication after effective treatment of Cushing's disease or Cushing's syndrome. Theoretically,

occurrence of adrenal crisis may differ between patients previously exposed to hypercortisolism and other adrenal insufficient patients. Due to the previous hypercortisolism, the activity of the HPA-axis may have altered the ability to respond to cortisol deficiency in patients with treated Cushing's syndrome. Furthermore, previous hypercortisolism can facilitate the occurrence of the corticosteroid withdrawal syndrome in the presence of acceptable cortisol concentrations, meaning that these patients need higher hydrocortisone replacement doses than other adrenal insufficient patients (22). This may lead to a higher incidence of adrenal crises in patients with treated Cushing's syndrome due to reduced awareness of symptoms, and insufficient replacement doses in case of a withdrawal syndrome.

For Cushing's syndrome, different treatment methods are recommended per etiology, but no further individualized treatment is currently implemented in clinical practice. However, Cushing's disease is more prevalent in females than males, and males are thought to have a higher risk of ectopic Cushing's syndrome (23). Whether treatment decisions should therefore be sex-specific is uncertain. In **Chapter 7**, we performed a cohort study of patients with ACTH-dependent Cushing's syndrome from Leiden and Berlin, comparing males and females before and after surgery, to assess sex as a risk marker for disease severity and complications. We included 130 patients, of whom 37 were male and 93 female patients. Although both sexes had similar serum and urinary cortisol concentrations, ACTH concentrations were higher in males than females at time of diagnosis. Given previous reports in the literature of high ACTH in male patients with Cushing's syndrome (24-27), a higher proportion of ectopic Cushing's syndrome and/or pituitary macroadenomas was expected in male patients, as these have also been associated with high ACTH concentrations (28, 29). However, we found no differences regarding etiology of Cushing's syndrome (i.e. Cushing's disease or ectopic Cushing's syndrome) or pituitary tumor size between both sexes, nor did we find a difference in diagnostic or therapeutic strategy, or surgical outcome including remission and recurrence rate. Males had more often osteoporosis both before and after surgery, with accompanying vertebral fractures, and more often anemia directly after surgery than females. In view of the similar surgical outcome and lack of differences regarding etiology and pituitary tumor size, no sex-based diagnostic strategy or treatment for Cushing's disease or Cushing's syndrome is recommended. However, extra attention should be given to bone mineral density in male patients to diagnose osteoporosis in time, in order to prevent (further) complications, such as vertebral fractures. This may be equally important for males with hypercortisolism due to other causes than endogenous Cushing's syndrome, e.g. corticosteroid use.

In evaluating treatment effect for Cushing's disease, normalization of cortisol concentrations, and if possible, cortisol secretion, is generally considered the most

important treatment outcome. However, comorbidity and complications due to hypercortisolism before treatment may persist after and despite treatment. Therefore, presence of comorbidity and occurrence of complications (e.g. osteoporosis) should also be considered as important markers for successful treatment. Mortality risk is high in patients with untreated Cushing's disease (6). Even after successful treatment, mortality risk in patients with Cushing's disease remains increased compared to the general population (30). One of the reasons for the increased mortality risk after successful treatment for Cushing's disease may be the occurrence of an adrenal crisis. Adrenal crisis is a potentially life-threatening complication of adrenal insufficiency due to acute and severe glucocorticoid deficiency, which can develop after any illness or psychological stress (31). Patients with previous hypercortisolism due to Cushing's syndrome are at risk of adrenal crisis if they are adrenal insufficient after successful surgery, but the extent of this risk is unknown. Furthermore, the diagnosis of adrenal crisis is inconsistent in existing literature due to lack of a common definition, which decreases comparability between existing studies. **Chapter 8** describes a cohort study including patients with Cushing's disease and Cushing's syndrome due to an adrenal adenoma with adrenal insufficiency after successful surgical treatment from Leiden and Berlin. We included 106 patients, of whom 19 had a total of 41 adrenal crises. Nine adrenal crises (95% confidence interval: 6.7-12.0) occurred per 100 patient-years at risk. The risk of adrenal crisis was higher for patients with previous Cushing's disease than for patients with adrenal Cushing's syndrome. As a higher risk of adrenal insufficiency and therefore adrenal crisis might be expected after adrenalectomy, the lower risk suggests greater awareness in patients with adrenal Cushing's syndrome, and thereby timely treatment of early symptoms of adrenal crisis. This may be due to better education after adrenalectomy than after transsphenoidal surgery. Another explanation may be the presence of other pituitary hormone deficiencies in patients treated for Cushing's disease, which seemed to increase the vulnerability for adrenal crisis in our study. The risk of adrenal crisis in previous Cushing's disease may partially explain the increased mortality risk after successful treatment for Cushing's disease, although in the current study we did not investigate mortality due to the low number of deaths. This also means that mortality due to adrenal crisis is relatively low in patients with treated Cushing's syndrome. A systematic review found six deaths related to adrenal crisis among 203 patients during 29-235 months of follow-up (32). However, mortality risk due to adrenal crisis in patients with treated Cushing's syndrome needs to be investigated further in larger patient registries. Previous crisis was a risk factor for recurrent crisis. Patients with at least one adrenal crisis had more complications after surgery than patients without any adrenal crisis, including anterior pituitary hormone deficiencies and diabetes insipidus. Effectively educating patients and endocrine nursing staff is essential in preventing adrenal crisis in patients treated for hypercortisolism. Further research to find risk factors for

adrenal crisis in patients with previous hypercortisolism can aid focusing education in preventing adrenal crisis on the proper patients.

Considering that patients with previous hypercortisolism are at risk of developing new complications (i.e. adrenal crisis) after successful treatment, and that mortality risk is still increased after treatment, other clinical outcomes may remain equally impaired after treatment. The glucocorticoid excess in patients with Cushing's disease and Cushing's syndrome is associated with impaired quality of life and reduced cognitive functioning (3, 33, 34). In **Chapter 9**, we performed a systematic review and meta-analysis evaluating quality of life and cognitive functioning after treatment for Cushing's disease and Cushing's syndrome. We compared scores after treatment with scores before treatment to assess improvement, and we compared scores after treatment with scores from a healthy control population to assess normalization of quality of life and cognitive functioning. We included 47 articles with a total of 2,643 patients. Both quality of life and cognitive functioning improved after treatment. However, quality of life did not normalize, and cognitive functioning only partially normalized after treatment compared to a healthy control population, which may be explained by long-lasting, or even irreversible effects of hypercortisolism on the brain (33). Considering the lack of normalization of quality of life and partially of cognitive functioning, further research is required to develop effective interventions for further improvement, and possibly normalization. Until then, clinicians should pay extra attention to quality of life and cognitive functioning after treatment of Cushing's disease and Cushing's syndrome.

The before described outcomes and risk marker for adverse clinical outcomes were selected based on the literature, clinical experience, and known biological rationale. Although we selected these carefully, our expectations were not always confirmed in a clinical study, e.g. sex-based diagnostic and treatment decisions for Cushing's syndrome could not be recommended based on our study results. Besides etiology and sex, more risk factors for adverse clinical outcomes in Cushing's syndrome should be investigated to increase the potential for individualized treatment. In the case of Cushing's disease, potential risk factors could be the size of the pituitary adenoma and preoperative comorbidity, such as diabetes mellitus. However, even if differences between study groups are confirmed, this does not automatically mean that different treatment strategies should be recommended. Whereas it is not always predictable on which characteristics clinical outcomes depend, treatment of hypercortisolism does not seem to completely resolve morbidity and mortality rate seen without treatment. Therefore, treatment of patients with Cushing's syndrome should expand beyond treatment of hypercortisolism to further reduce morbidity and mortality rates.

Future research perspectives

The studies in this thesis on various aspects of hypercortisolism emphasize the importance of suppressing cortisol secretion to physiological ranges. Both hypercortisolism and adrenal insufficiency have enormous, and sometimes deleterious, impact, both on physical and psychological functioning. Treatment of hypercortisolism and adrenal insufficiency attempting to restore normal cortisol concentrations representing the physiological circadian rhythm does not eliminate risk of adverse outcomes.

To provide patients with rare endocrine conditions, such as Cushing's syndrome, with equal expert specialized care throughout Europe, the European Reference Network on Rare Endocrine Conditions (Endo-ERN) was recently established (35). Via an Endo-ERN reference center, a multidisciplinary expert team can be consulted to provide an advice based on combined specialist expertise throughout Europe (Figure 1) (36). This facilitates the correct identification of the underlying disease in hypercortisolemic patients, thereby reducing delay in the diagnostic process, and promoting earlier referral to a specialized center. Furthermore, the multidisciplinary team can aid with treatment decisions, e.g. for patients with persistent or recurrent Cushing's disease, or ectopic Cushing's syndrome without a clear source of ACTH-production.

What Endo-ERN represents for patient care, international patient registries are for clinical research. For improvement of knowledge and expertise on rare endocrine conditions, international exchange of information through these patient registries is crucial. This increases the population size and therefore accuracy of gained knowledge through research compared to single-center cohort studies. Unfortunately, awareness of and participation rate in these international registries is relatively low. Recently, the European Registries for Rare Endocrine Conditions (EuRRECa, https://eurreca.net/), which will be fed by, and coupled to, Endo-ERN, was also funded by the European Union's health programme. Besides developing new international registries for endocrine conditions that are not covered in a registry yet, awareness of and participation in existing registries should increase to optimize functioning of both the Endo-ERN and patient registries, which is a process under development (37). Patient registries provide information on specific rare diseases, which can be supplemented with knowledge from the Endo-ERN on unusual cases regarding clinical picture, diagnosis or treatment. Combined, they contribute effectively to research on rare endocrine diseases.

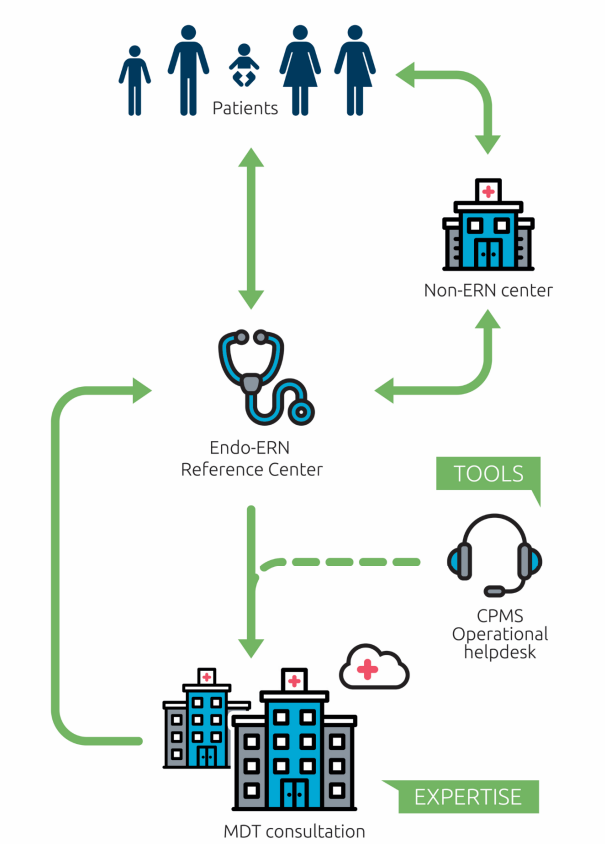

Figure 1: Consultation of a multidisciplinary team (MDT) by an European Reference Network on rare endocrine conditions (Endo-ERN) reference center on behalf of the patient, bringing expert specialized care to all patients in Europe through the Clinical Patient Management System (CPMS), derived from the Endo-ERN website (36).

To effectively increase our knowledge concerning hypercortisolism, it is of paramount importance to clearly define and recognize all states of cortisol excess and cortisol deficiency, as well as all clinical outcomes considered. For some diagnoses classification is especially challenging, e.g. in case of adrenal insufficiency, considering the non-specific symptoms, and the multiple steps necessary for establishing the correct diagnosis (presence of hypocortisolism, level of HPA-axis dysfunction, and exact cause of adrenal insufficiency). Only if all research groups use the same definitions, data can be compared to each other, both directly in meta-analyses, and indirectly in separate trials and cohort studies. Furthermore, definitions used in research should match clinical definitions, to ensure study outcomes are applicable to clinical practice. Future studies should focus on which combination of tests (serum cortisol, urinary cortisol, salivary cortisol, cortisol suppression tests, and -stimulation tests) is ideal for diagnosing Cushing's syndrome,

and what the diagnostic consequences are of inconsistent test results. Moreover, future research should also concentrate on how to differentiate between Cushing's syndrome and pseudo-Cushing states, which show increased cortisol concentrations as well as varying degrees of symptoms associated with Cushing's syndrome. These results can be used in collectively deciding on an improved definition for Cushing's syndrome that can be used in both research settings as well as clinical practice. Future meta-analyses, which include only studies that use the same definitions for diagnosis of disease and clinical outcomes, will be more valuable and easy to interpret than the presently available meta-analyses. However, as this is currently impossible due to use of various definitions in individual studies, authors of meta-analyses should consider carefully which conclusions can be drawn from the data and to which patients these conclusions are applicable, which we were able to do in all meta-analyses presented in this thesis.

Consistent definitions are essential for the optimal functioning of research and patient care. International patient registries supply a large collection of data on rare diseases that is impossible for a single center to assemble. If consistent definitions are used by all participating centers, these registries provide an excellent opportunity to perform large-scale investigations on rare diseases with rare outcomes, e.g. to find risk factors for adrenal crisis after treatment for Cushing's syndrome. However, data collection should match potential research questions, and addition of data should be possible. E.g. in the European Register on Cushing's Syndrome (ERCUSYN) no data on adrenal crisis are available, and retrospective addition of data is difficult, if not impossible (23). Furthermore, current patient registries are only suited to investigate a specific rare disease, such as Cushing's syndrome, whereas a registry for comparing patients with different underlying diseases but similar clinical presentations is still lacking. Such a registry could allow us to compare patients with hypercortisolism from various etiologies. Understanding of differences and similarities between the underlying diseases can lead to a more accurate adaptation of clinical guidelines for specific patient categories with various forms of hypercortisolism. Future research on patients with Cushing's syndrome using these large-scale patient registries should primarily aim to directly compare cortisol-lowering medical agents and look into long-term effectiveness and side effects. Furthermore, randomized controlled trials should be performed to directly compare the most promising medical agents with the highest effectiveness and lowest risk of side effects. Knowledge on preferred medical agent, or combination of agents, can improve treatment options for patients with persistent or recurrent Cushing's syndrome, or for those with a contra-indication for surgery.

Finally, increasing knowledge on the effects of both endogenous Cushing's syndrome and exogenous hypercortisolism is likely to improve patient care beyond the endocrinology department, as these conditions serve as a model for long-term exposure to stress, which is a highly prevalent condition. Insight into the potential consequences of long-term stress exposure, both during periods of stress as well as after abrogation of the stress-inducing situation, can aid all individuals exposed to long-term stress, including patients with chronic disease or long-term hospital admission.

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Chapter 11

Discussie en samenvatting

Introductie

Hypercortisolisme veroorzaakt veel en mogelijk ernstige complicaties, die in de klinische praktijk vaak onderschat en slecht herkend worden (1-3). Er zijn twee belangrijke oorzaken voor hypercortisolisme. Ten eerste, exogeen hypercortisolisme door gebruik van glucocorticoïden, met een hoge prevalentie doordat ongeveer 1% van de bevolking glucocorticoïden gebruikt (4, 5). Ten tweede, het endogene syndroom van Cushing, een zeldzame aandoening met een hoge ziektelast door de verhoogde risico's op morbiditeit en mortaliteit (2, 3, 6). Om onze kennis over de effecten van endogeen hypercortisolisme te vergroten zijn grootschalige studies nodig, want monocentrische cohortstudies hebben vaak onvoldoende bewijskracht door de kleine patiëntenpopulatie. In dit proefschrift presenteren wij enkele meta-analyses, een cohortstudie op bevolkingsniveau uit Denemarken en gecombineerde data van twee cohortstudies van tertiaire referentiecentra uit Leiden en Berlijn.

Deel I: Complicaties door gebruik van glucocorticoïden

Glucocorticoïden worden veelvuldig gebruikt voor de behandeling van patiënten met een inflammatoire ziekte, maligniteit of orgaantransplantatie om een inflammatoire respons te onderdrukken, ondanks de bekende hoeveelheid complicaties en bijwerkingen (5, 7). In **Hoofdstuk 2**, **Hoofdstuk 3** en **Appendix I** van dit proefschrift worden potentiële complicaties van exogeen hypercortisolisme als gevolg van glucocorticoïdgebruik besproken.

Gebruik van glucocorticoïden onderdrukt de hypothalamus-hypofyse-bijnier (HPA) as en is de meest voorkomende oorzaak van bijnierschorsinsufficiëntie. Bijnierschorsinsufficiëntie is een ernstige en potentieel levensbedreigende situatie (8). In **Hoofdstuk 2** en **Appendix I** bestudeerden wij de proportie patiënten met bijnierschorsinsufficiëntie na gebruik van glucocorticoïden in een systematische review. De resultaten zijn gestratificeerd per toedieningsvorm, onderliggende ziekte, behandelduur en behandeldosis, om patiënten te identificeren met een verhoogd risico op bijnierschorsinsufficiëntie. Wij includeerden 74 artikelen uit de literatuur in deze meta-analyse met in totaal 3.753 patiënten. Het percentage patiënten met bijnierschorsinsufficiëntie varieerde per toedieningsvorm van 4,2% bij nasale toediening tot 52,2% bij intra-articulaire toediening. Er was geen toedieningsvorm, behandelduur, behandeldosis of onderliggende ziekte waarvoor bijnierschors-insufficiëntie met zekerheid uitgesloten kon worden. Het laagste risico op bijnierschorsinsufficiëntie (1,4%) hadden patiënten die glucocorticoïden gedurende korte termijn gebruikten en het hoogste risico (60,0%) patiënten met hematologische

maligniteiten. In de klinische praktijk zouden zowel patiënten als artsen geïnformeerd moeten worden over de risico's en symptomen van bijnierschorsinsufficiëntie ten gevolge van glucocorticoidgebruik. De drempel om gebruikers van glucocorticoïden te testen op bijnierschorsinsufficiëntie zou laag moeten zijn. Echter, hoe te testen op bijnierschorsinsufficiëntie in een populatie glucocorticoïdgebruikers, en wanneer verbetering te verwachten is, blijft lastig, vooral als de dosis glucocorticoïden verlaagd is zonder volledig te stoppen. Het belang van testen op bijnierschorsinsufficiëntie en de noodzaak van behandeling, ondanks imperfecte tests, wordt verder bediscussieerd in **Appendix II**.

Om een potentieel verhoogd mortaliteitsrisico op te sporen bij patiënten die glucocorticoïden gebruiken ten opzichte van niet-gebruikers, moeten patiënten met dezelfde ziekte bestudeerd worden om een effect van de onderliggende ziekte zelf op mortaliteitsrisico te minimaliseren. **Hoofdstuk 3** beschrijft een cohortstudie op populatieniveau uit Denemarken (n=5.289.261), waarin mortaliteitsrisico voor patiënten met geperforeerde divertikelziekte vergeleken wordt tussen patiënten die glucocorticoïden gebruikten en niet-gebruikers. Deze studie bevatte 4.640 patiënten met geperforeerde divertikelziekte, van wie 19,3% glucocorticoïden gebruikten voor uiteenlopende onderliggende ziekten. Na correctie voor potentiële verstorende variabelen bleek het mortaliteitsrisico verdubbeld voor patiënten die recent glucocorticoïden gebruikten. Het hoogste mortaliteitsrisico was 52,5% mortaliteit binnen een jaar voor patiënten die met glucocorticoïdgebruik begonnen in de laatste 90 dagen voor ziekenhuisopname. Om mogelijke verstoring van het effect door de indicatie voor glucocorticoïdgebruik te beoordelen hebben wij verschillende sensitiviteitsanalyses uitgevoerd, waarbij we hoog-risico populaties hebben uitgesloten, bijvoorbeeld patiënten met maligniteiten. De resultaten van deze sensitiviteitsanalyses waren vergelijkbaar met de hoofdanalyse, wat suggereert dat verstoring van het effect door indicatie voor glucocorticoïdgebruik geen afdoende verklaring is voor mortaliteitsrisico bij patiënten met geperforeerde divertikelziekte. Daarom moet gebruik van glucocorticoïden beschouwd worden als een belangrijke risicofactor voor mortaliteit in de klinische praktijk bij patiënten met geperforeerde divertikelziekte.

Het was al bekend dat gebruik van glucocorticoïden leidt tot verschillende complicaties en bijwerkingen (1). Onze studies hebben informatie toegevoegd over complicaties van glucocorticoïdgebruik wat betreft bijnierschorsinsufficiëntie en risico op mortaliteit. Echter, het is een veelvoorkomend misverstand dat gebruik van glucocorticoïden in lage doseringen, of het gebruik van alleen inhalatieglucocorticoïden, geen risico's zou opleveren (9). Het risico op bijnierschorsinsufficiëntie overstijgt 50% bij lange-termijn gebruik (>1 jaar) van hoog gedoseerde glucocorticoïden, en is hoger bij gebruik van systemische dan inhalatieglucocorticoïden,

maar toch houden zelfs mensen met korte-termijn (<1 maand) gebruik van laag gedoseerde glucocorticoïden, of alleen inhalatieglucocorticoïden, risico op bijnierschorsinsufficiëntie. De verhoging van het mortaliteitsrisico bij patiënten met geperforeerde divertikelziekte is onafhankelijk van de gebruikte dosis glucocorticoïden. Het mortaliteitsrisico is tevens verhoogd bij patiënten met geperforeerde divertikelziekte die alleen inhalatieglucocorticoïden gebruiken, alhoewel minder uitgesproken. Gegeven het werkingsmechanisme van glucocorticoïden is het waarschijnlijk dat het verhoogde mortaliteitsrisico niet beperkt is tot patiënten met geperforeerde divertikelziekte.

Naast bijnierschorsinsufficiëntie en een verhoogd mortaliteitsrisico hebben patiënten die glucocorticoïden gebruiken risico op osteoporose en fracturen, diabetes mellitus, cardiovasculaire ziekte, myopathie, cataract en glaucoom, neuropsychiatrische klachten en immuunsuppressie (10). Gezien deze risico's op ernstige bijwerkingen zouden alle patiënten die glucocorticoïden gebruiken adequaat gemonitord moeten worden, wat voor volwassenen inhoudt dat periodieke controles door een arts inclusief (lab)metingen (bijvoorbeeld lipiden en glucose) plaatsvinden, iedere 3-6 maanden in het eerste jaar en daarna jaarlijks (10). Op dit moment is er geen klinische richtlijn voor de evaluatie van mogelijke bijnierschorsinsufficiëntie na gebruik van glucocorticoïden en daardoor is er geen duidelijk advies over herstel van de HPA-as. In het algemeen moet screening op bijnierschorsinsufficiëntie ten minste 18-24 uur na de laatste dosis kortwerkende glucocorticoïden, zoals hydrocortison, plaatsvinden door het meten van het serumcortisol in de vroege ochtend en een adrenocorticotroop hormoon (ACTH) stimulatietest bij een serumcortisol tussen de 3 en 15 µg/dL (dit is 83-414 nmol/L) (11). Wij stellen voor in ieder geval de patiënten met aspecifieke symptomen na afbouw van de glucocorticoïden te screenen. Omdat herkenning van en screening op complicaties van glucocorticoïdgebruik op dit moment onvoldoende is in de klinische praktijk, zou gebruik van glucocorticoïden moeten worden geminimaliseerd indien klinisch haalbaar.

Deel II: Behandeluitkomsten bij het syndroom van Cushing

In tegenstelling tot exogeen hypercortisolisme is endogeen hypercortisolisme extreem zeldzaam, met een incidentie van 1,2-2,4 per miljoen personen per jaar (12). De ziekte van Cushing, veroorzaakt door een hypofyseadenoom, en andere oorzaken van het endogene syndroom van Cushing, meestal veroorzaakt door een bijnieradenoom of een ectopische ACTH-producerende tumor, worden gekenmerkt door ernstig hypercortisolisme met verhoogde mortaliteit en morbiditeit indien onbehandeld (2, 3,

6). Daarom moeten deze patiënten zo snel mogelijk behandeld worden (13). **Hoofdstukken 4 tot 6** beschrijven de resultaten van onze studies over behandelopties voor de ziekte van Cushing en andere oorzaken van het endogene syndroom van Cushing.

De ziekte van Cushing is de meest voorkomende oorzaak van endogeen hypercortisolisme (12). Voor de ziekte van Cushing is transsfenoïdale selectieve adenomectomie de eerste keus behandeloptie (14). Voor deze chirurgische behandeling zijn twee technieken in gebruik: microscopie en endoscopie. Microscopische chirurgie is de oudere techniek, waarbij de neurochirurg een direct, driedimensionaal beeld van de hypofyse krijgt door te kijken door de microscoop (15). Endoscopische chirurgie is nieuwer en levert een beeld op dichterbij de hypofyse middels een camera aan het uiteinde van de endoscoop, waarbij echter het driedimensionale beeld verloren gaat. Met de endoscoop kan de hypofyse ook onder hoeken bekeken worden die met de microscoop onmogelijk zijn (16). In **Hoofdstuk 4** beschreven wij beide technieken in ons cohort patiënten uit het Leids Universitair Medisch Centrum (LUMC) om potentiële verschillen te beoordelen wat betreft remissie, mortaliteit en korte- en lange-termijn complicaties. We includeerden 137 patiënten, van wie 87 microscopisch en 50 endoscopisch behandeld werden. We vonden geen duidelijk voordeel van een van beide technieken voor de behandeling van de ziekte van Cushing. Bij stratificatie van de resultaten naar tumorgrootte bleek dat patiënten met macroadenomen een lager risico op recidief hadden na microscopische chirurgie dan na endoscopische chirurgie. Echter, in theorie zou endoscopie betere resultaten moeten opleveren, vooral bij grote of invasieve tumoren, omdat de volledige tumor in beeld kan worden gebracht, wat met microscopie niet altijd mogelijk is. Mogelijk was in onze studie microscopische chirurgie een voordeel voor patiënten met macroadenomen door selectie, omdat patiënten met grote en invasieve tumoren mogelijk vaker naar ons centrum verwezen werden dan patiënten met niet-invasieve macroadenomen. Patiënten met grote en invasieve tumoren hebben een slechtere prognose ondanks behandeling en zij worden vaker endoscopisch behandeld dan patiënten met niet-invasieve en kleinere tumoren, wat kan leiden tot vertekende resultaten. Selectieve verwijzing van patiënten resulteerde in acht endoscopisch behandelde (50%) en vijf microscopisch behandelde (28%) patiënten met macroadenomen en invasie van de sinus cavernosus in ons cohort. Echter, het excluderen van deze patiënten in een sensitiviteitsanalyse veranderde de resultaten niet wezenlijk.

Om de resultaten van ons centrum te vergelijken met de resultaten gerapporteerd in de literatuur hebben we in **Hoofdstuk 5** een systematische review en meta-analyse uitgevoerd over hetzelfde onderwerp, microscopische versus endoscopische transsfenoïdale adenomectomie. We includeerden 97 artikelen met in totaal 6.695

patiënten, van wie 5.711 microscopisch en 984 endoscopisch behandeld waren. We vonden geen duidelijk verschil in remissie, recidief of mortaliteit. De meeste complicaties kwamen bij beide technieken ongeveer even vaak voor. Liquorlekkage kwam vaker voor na endoscopie, terwijl tijdelijke diabetes insipidus vaker voorkwam na microscopie. Als alleen naar patiënten met microadenomen gekeken werd, was er geen verschil tussen beide technieken wat betreft remissie of recidiefkans, conform de bevindingen van onze cohortstudie. Echter, als alleen naar patiënten met macroadenomen gekeken werd, vonden we een hoger percentage patiënten in remissie en een lagere recidiefkans na endoscopie dan na microscopie, wat tegenstrijdig is met de resultaten van onze cohortstudie. Het verschil in uitkomst tussen onze cohortstudie en systematische review benadrukt het belang van gestandaardiseerde uitkomstmaten, internationaal geaccepteerde definities en het combineren van data uit verschillende centra voordat aanbevelingen worden gedaan voor de klinische praktijk.

Doordat endoscopie nieuwer is, is er minder ervaring met deze techniek dan met microscopische chirurgie. Vooral in de eerste jaren na overstappen op endoscopie in een bepaald centrum kunnen slechtere resultaten geanticipeerd worden, die zullen verbeteren met een toename aan ervaring (17-20). Hier dient rekening mee gehouden te worden bij de interpretatie van vergelijkingen tussen beide technieken. In **Hoofdstuk 4** onderzochten we deze potentiële leercurve in ons cohort door endoscopie in de vroegere jaren te vergelijken met navolgende jaren. We vonden echter geen leercurve voor endoscopische chirurgie, mogelijk door de kleine populatie en daardoor lage statistische bewijskracht. Voor de klinische praktijk suggereren de resultaten van **Hoofdstuk 5** dat endoscopische chirurgie de beste behandelmethode is voor patiënten met de ziekte van Cushing en een macroadenoom. Microscopie kan gebruikt worden als de neurochirurg hier een voorkeur voor heeft, maar dan moet overwogen worden patiënten met een macroadenoom te verwijzen naar een ander centrum gespecialiseerd in endoscopische chirurgie. In ons centrum zagen wij geen tijdelijke verslechtering van de resultaten gedurende de transitie naar endoscopische chirurgie, wat voor andere centra die deze overstap overwegen geruststellend kan zijn, alhoewel deze nieuwe methode per behandelcentrum geëvalueerd moet worden.

Alhoewel transsfenoïdale adenomectomie de eerste keus behandelmethode is voor patiënten met de ziekte van Cushing, zijn er andere methoden beschikbaar voor patiënten met een contra-indicatie voor transsfenoïdale chirurgie, persisterende ziekte of recidief, en voor patiënten die chirurgie weigeren. Deze behandelopties zijn cortisolverlagende medicatie (steroïdgenese-remmers, glucocorticoïdreceptor-antagonisten, cabergoline en pasireotide), radiotherapie en bilaterale adrenalectomie, en deze kunnen ook gebruikt worden voor patiënten met het syndroom van

Cushing (13, 21). Bilaterale adrenalectomie leidt altijd tot volledige bijnierschors-insufficiëntie, waardoor levenslange substitutietherapie met hydrocortison en fludrocortison nodig is (8). Een veelvoorkomende complicatie van radiotherapie is hypofyseuitval, waardoor hormoonsubstitutietherapie noodzakelijk is. Bovendien duurt het normaal gesproken enige maanden voordat de eerste gunstige effecten van radiotherapie bemerkt worden (13). Daarom worden zowel bilaterale adrenalectomie als radiotherapie niet aanbevolen als eerstelijns behandeloptie (13), en wordt behandeling met medicatie steeds vaker geïnitieerd. Om de effectiviteit van cortisolverlagende medicatie te schatten hebben we een systematische review en meta-analyse uitgevoerd in **Hoofdstuk 6**. We includeerden 35 artikelen met 1.520 patiënten met de ziekte of andere oorzaken van het endogene syndroom van Cushing, die zes verschillende soorten cortisolverlagende medicijnen gebruikten. Gemiddeld per studie werden de patiënten 2 weken tot 11,5 jaar gevolgd, met een meerendeel aan korte-termijn studies. Normalisatie van de cortisolsecretie varieerde van 35,7% (cabergoline) tot 81,8% (mitotane). Echter, medicijnen met een grotere effectiviteit wat betreft normalisatie van de cortisolsecretie leidden tot een hoger percentage patiënten met bijwerkingen en vice versa. Patiënten die meerdere medicijnen tegelijkertijd of achtereenvolgens gebruikten lieten vaker normalisatie van de cortisolsecretie zien (65,7%) dan patiënten die slechts één medicijn gebruikten (49,4%). Het percentage patiënten met de ziekte van Cushing met een genormaliseerd cortisol door cortisolverlagende medicatie is lager dan na eerstelijns transsfenoïdale chirurgie, maar is vergelijkbaar met het percentage patiënten in remissie na herhaalde transsfenoïdale chirurgie, zoals beschreven in **Hoofdstuk 5**. Dit suggereert dat medicatie een waardevol alternatief is voor transsfenoïdale chirurgie voor patiënten met een contra-indicatie voor chirurgie, persisterende ziekte of recidief, en voor patiënten die chirurgie weigeren. Echter, lange-termijn effectiviteit en bijwerkingen zijn nog niet in detail onderzocht, inclusief de effecten op kwaliteit van leven. Dit is relevant voor deze patiënten, aangezien zij levenslang cortisolverlagende medicijnen nodig zullen hebben als deze behandelstrategie noodzakelijk geacht wordt.

Ook na de introductie van een nieuwe behandelmethode of medicijn moeten aannames over effectiviteit en bijwerkingen of complicaties bevestigd of weerlegd worden. Voor de ziekte van Cushing suggereert onze meta-analyse dat het verwachte voordeel van endoscopische chirurgie alleen bestaat voor macroadenomen, maar niet voor microadenomen. Tevens vonden wij dat medicamenteuze behandeling net zo effectief en veilig is als herhaalde transsfenoïdale chirurgie, wat de behandelmogelijkheden voor patiënten met de ziekte van Cushing uitbreidt. Echter, cortisolverlagende medicatie moet levenslang gebruikt worden en zal nooit de pulsatiele hormoonafgifte normaliseren, wat belangrijk kan zijn voor lange-termijn morbiditeit en kwaliteit van leven van deze patiënten. Kennis omtrent de optimale

behandelmethode en een breed aanbod aan effectieve behandelopties zijn essentieel in het verminderen van de hoge ziektelast van onbehandelde ziekte van Cushing en syndroom van Cushing. Om het gebruik van bestaande behandelmethoden in de klinische praktijk te optimaliseren kunnen toekomstige studies onderzoeken welk cortisolverlagend medicijn als eerst toegediend zou moeten worden en welke combinatie van medicijnen het meest effectief en veilig is in de behandeling van de ziekte en het syndroom van Cushing. Bovendien zijn patiëntenregisters die patiënten langdurig vervolgen nodig om de lange-termijn effectiviteit en veiligheid van cortisolverlagende medicijnen te onderzoeken, inclusief het effect op lange-termijn morbiditeit en kwaliteit van leven.

Deel III: Klinische uitkomsten bij het syndroom van Cushing

De klinische consequenties van de ziekte en het syndroom van Cushing kunnen ernstig zijn, zowel voor als na behandeling, en het is vaak onbekend welke patiënten een hoger risico op specifieke morbiditeit hebben. **Hoofdstukken 7 tot 9** beschrijven een reeks klinische uitkomsten bij patiënten met de ziekte en het syndroom van Cushing. Om te bepalen welke patiënten risico lopen op specifieke klinische uitkomsten kunnen predictiemodellen gebruikt worden om risicofactoren te vinden en met gestratificeerde analyses kunnen categorieën patiënten vergeleken worden. We besloten geslacht te onderzoeken als potentiële risicomarker voor specifieke klinische uitkomsten. Geslachtsafhankelijke verschillen werden verondersteld op basis van het verschillende klinische beeld dat bij mannen en vrouwen gezien wordt in de kliniek. Na het bestuderen van een potentiële risicomarker voor klinische uitkomsten, onderzochten we twee klinische uitkomstmaten die voorafgaand aan behandeling aangedaan zijn: kwaliteit van leven en cognitief functioneren. We wilden weten of succesvolle behandeling van het syndroom van Cushing ook effectief is in het verbeteren, of zelfs normaliseren, van kwaliteit van leven en cognitie. Daarnaast kozen we ervoor het optreden van Addisonse crisis na behandeling te onderzoeken, aangezien dit een ernstige mogelijke complicatie na effectieve behandeling van de ziekte of het syndroom van Cushing is. Theoretisch kan het optreden van Addisonse crisis verschillen tussen patiënten met voormalig hypercortisolisme en andere bijnierschorsinsufficiënte patiënten. Door het voormalige hypercortisolisme is mogelijk het vermogen van de activiteit van de HPA-as om te reageren op cortisoldeficiëntie veranderd bij patiënten die behandeld zijn voor het syndroom van Cushing. Bovendien kan voormalig hypercortisolisme het optreden van het glucocorticoïdonttrekkingssyndroom faciliteren in aanwezigheid van acceptabele cortisolconcentraties, waardoor deze patiënten hogere hydrocortisonsubstitutiedoses

nodig hebben dan andere bijnierschorsinsufficiënte patiënten (22). Dit kan leiden tot een hogere incidentie van Addisonse crisen bij patiënten die behandeld zijn voor het syndroom van Cushing door een verminderde gewaarwording van de symptomen en onvoldoende substitutie in het geval van het onttrekkingssyndroom.

Voor het syndroom van Cushing worden verschillende behandelmethoden aanbevolen per etiologie, maar momenteel wordt er geen verdere individualisatie van behandeling toegepast in de klinische praktijk. Echter, de ziekte van Cushing komt vaker voor bij vrouwen dan bij mannen en van mannen wordt gedacht dat zij een groter risico hebben op het ectopische syndroom van Cushing (23). Of als gevolg daarvan behandelkeuzes geslachtsafhankelijk zouden moeten zijn is onbekend. In **Hoofdstuk 7** hebben wij een cohortstudie gedaan naar patiënten met ACTH-afhankelijk syndroom van Cushing uit Leiden en Berlijn, waarbij we mannen en vrouwen voor en na chirurgische behandeling hebben vergeleken om te beoordelen of geslacht een risicomarker is voor ernst van de ziekte en complicaties. We hebben 130 patiënten geïncludeerd, waaronder 37 mannen en 93 vrouwen. Alhoewel beide geslachten vergelijkbare cortisolconcentraties in het serum hadden, waren bij diagnose de ACTH-concentraties hoger bij mannen dan bij vrouwen. Gezien onze bevindingen overeenkomen met vermeldingen in de literatuur van hoog ACTH bij mannelijke patiënten met het syndroom van Cushing (24-27), verwachtten wij tevens een hogere proportie van het ectopische syndroom van Cushing en/of hypofysaire macroadenomen bij mannen, aangezien deze ook geassocieerd worden met hoge ACTH-concentraties (28, 29). Echter, wij vonden geen verschil wat betreft etiologie van het syndroom van Cushing (in dit geval de ziekte van Cushing of het ectopische syndroom van Cushing) of tumorgrootte van het hypofyseadenoom tussen beide geslachten. Ook vonden wij geen verschil in diagnostische of therapeutische strategie of in uitkomst van chirurgie inclusief remissie en recidief. Mannen hadden vaker osteoporose, zowel voor en na chirurgie, met begeleidende vertebrale fracturen, en ze hadden vaker anemie direct na chirurgie dan vrouwen. Vanwege de vergelijkbare behandeluitkomst en het gebrek aan verschil in etiologie en tumorgrootte van het hypofyseadenoom, wordt geen geslachtsafhankelijke diagnostiek of behandeling aanbevolen voor de ziekte of het syndroom van Cushing. Echter, er zou extra aandacht geschonken moeten worden aan de botdichtheid bij mannelijke patiënten om osteoporose tijdig te diagnosticeren, zodat (verdere) complicaties zoals vertebrale fracturen voorkomen kunnen worden. Dit is mogelijk ook belangrijk voor mannen met hypercortisolisme door andere oorzaken dan endogeen syndroom van Cushing, zoals gebruik van glucocorticoïden.

Bij de evaluatie van de effectiviteit van behandeling voor de ziekte van Cushing wordt normalisatie van de cortisolconcentraties, en indien mogelijk, cortisolsecretie, in het algemeen als belangrijkste behandeluitkomst beschouwd. Echter,

comorbiditeit en complicaties door hypercortisolisme voorafgaand aan behandeling kunnen persisteren na en ondanks behandeling. Daarom zouden aanwezigheid van comorbiditeit en optreden van complicaties (zoals osteoporose) ook als belangrijke markers voor succesvolle behandeling moeten worden beschouwd. Mortaliteitsrisico is hoog in patiënten met onbehandelde ziekte van Cushing (6). Zelfs na succesvolle behandeling blijft het mortaliteitsrisico verhoogd in patiënten met de ziekte van Cushing in vergelijking met de algemene bevolking (30). Een van de redenen voor de verhoogde mortaliteit na succesvolle behandeling voor de ziekte van Cushing zou het optreden van een Addisonse crisis kunnen zijn. Een Addisonse crisis is een potentieel levensbedreigende complicatie van bijnierschorsinsufficiëntie door acute en ernstige glucocorticoïddeficiëntie, wat zich kan ontwikkelen na elke ziekte of psychologische stress (31). Patiënten met voormalig hypercortisolisme door het syndroom van Cushing lopen risico op een Addisonse crisis als zij bijnierschorsinsufficiënt zijn na succesvolle chirurgische behandeling, maar de rijkwijdte van dit risico is onbekend. Bovendien wordt Addisonse crisis inconsistent gediagnosticeerd in de bestaande literatuur door gebrek aan een algemeen geaccepteerde definitie, wat de vergelijkbaarheid tussen bestaande studies vermindert. **Hoofdstuk 8** beschrijft een cohortstudie bestaande uit patiënten uit Leiden en Berlijn met de ziekte van Cushing en patiënten met het syndroom van Cushing door een bijnieradenoom, die bijnierschorsinsufficiënt zijn na succesvolle chirurgische behandeling. We includeerden 106 patiënten, van wie 19 in totaal 41 Addisonse crisen meemaakten. Negen Addisonse crisen (95% betrouwbaarheidsinterval: 6,7-12,0) deden zich voor per 100 patiëntjaren met risico op Addisonse crisis. Het risico op Addisonse crisis was hoger voor patiënten met de ziekte van Cushing dan voor patiënten met het syndroom van Cushing door een bijnieradenoom. Aangezien een hoger risico op bijnierschorsinsufficiëntie en daardoor Addisonse crisis verwacht kan worden na adrenalectomie, suggereert het lagere risico een grotere gewaarwording bij patiënten met het syndroom van Cushing door een bijnieradenoom en daardoor tijdige behandeling van vroege symptomen van een Addisonse crisis. Dit kan komen door een betere educatie na adrenalectomie dan na transsfenoïdale chirurgie. Een andere mogelijke verklaring zou de aanwezigheid van andere hypofysehormoon-deficiënties kunnen zijn bij patiënten die behandeld zijn voor de ziekte van Cushing, wat de kwetsbaarheid voor Addisonse crisis in onze studie leek te vergroten. Het risico op een Addisonse crisis in patiënten met voorheen de ziekte van Cushing verklaart mogelijk deels de verhoogde mortaliteit na succesvolle behandeling voor de ziekte van Cushing, alhoewel we in de huidige studie mortaliteit niet onderzocht hebben door een laag aantal overlijdens. Dit betekent ook dat mortaliteit door Addisonse crisis relatief laag is in patiënten behandeld voor het syndroom van Cushing. Een systematische review vond zes overlijdens gerelateerd aan Addisonse crisis onder 203 patiënten gedurende 29-235 maanden follow-up (32). Echter, mortaliteit door Addisonse crisis in patiënten behandeld voor het syndroom van

Cushing zou verder onderzocht moeten worden in grotere patiëntenregisters. Eerdere Addisonse crisis was een risicofactor voor een recidief crisis. Patiënten met ten minste één crisis hadden meer complicaties na chirurgie dan patiënten zonder crisis, namelijk hypofysevoorkwabsdeficiënties en diabetes insipidus. Effectieve educatie van patiënten en endocrinologieverpleegkundigen is essentieel in het voorkomen van Addisonse crisis bij patiënten behandeld voor hypercortisolisme. Toekomstig onderzoek naar risicofactoren voor Addisonse crisis bij patiënten met voormalig hypercortisolisme kan helpen de educatie om Addisonse crisis te voorkomen op de juiste patiënten te richten.

Aangezien patiënten met voormalig hypercortisolisme risico lopen op het ontwikkelen van nieuwe complicaties (zoals Addisonse crisis) na succesvolle behandeling, en aangezien mortaliteit verhoogd blijft na behandeling, blijven andere klinische uitkomsten mogelijk evenzeer aangedaan na behandeling. Het teveel aan glucocorticoïden bij patiënten met de ziekte en het syndroom van Cushing is geassocieerd met verminderde kwaliteit van leven en verlaagd cognitief functioneren (3, 33, 34). In **Hoofdstuk 9** beschrijven wij een systematische review en meta-analyse, waarin kwaliteit van leven en cognitief functioneren na behandeling voor de ziekte en het syndroom van Cushing geëvalueerd worden. We vergelijken scores na behandeling met scores voor behandeling om eventuele verbetering te beoordelen en we vergelijken scores na behandeling met scores van een gezonde controlepopulatie om eventuele normalisatie van kwaliteit van leven en cognitief funtioneren te beoordelen. We includeerden 47 artikelen met in totaal 2.643 patiënten. Zowel kwaliteit van leven als cognitief functioneren verbeterde na behandeling. Echter, kwaliteit van leven normaliseerde niet, terwijl cognitief functioneren slechts gedeeltelijk normaliseerde na behandeling in vergelijking met een gezonde controlepopulatie, wat mogelijk verklaard kan worden door langdurige of zelfs irreversibele effecten van hypercortisolisme op het brein (33). Gezien het gebrek aan normalisatie van kwaliteit van leven en gedeeltelijk ook van cognitief functioneren, is vervolgonderzoek noodzakelijk om effectieve interventies te ontwikkelen voor verdere verbetering en mogelijk normalisatie. Vooralsnog moeten clinici extra aandacht schenken aan kwaliteit van leven en cognitief functioneren na behandeling van de ziekte en het syndroom van Cushing.

De hiervoor beschreven uitkomsten en risicomarker voor negatieve klinische uitkomsten zijn geselecteerd op basis van de literatuur, klinische ervaring en bekende biologische rationale. Alhoewel we ze zorgvuldig geselecteerd hebben, werden onze verwachtingen niet altijd bevestigd in een klinische studie, bijvoorbeeld geslachtsafhankelijke diagnostiek en behandelkeuzes voor het syndroom van Cushing konden niet worden aanbevolen gebaseerd op onze studieresultaten. Naast etiologie en geslacht zouden meer risicofactoren voor ongunstige klinische uitkomsten bij het

syndroom van Cushing moeten worden onderzocht om het potentieel voor geïndividualiseerde behandeling te vergroten. Bij de ziekte van Cushing zouden potentiële risicofactoren de grootte van het hypofyseadenoom en preoperatieve comorbiditeit, zoals diabetes mellitus, kunnen zijn. Echter, zelfs als verschillen tussen studiegroepen bevestigd worden betekent dit niet automatisch dat verschillende behandelstrategieën aanbevolen moeten worden. Alhoewel het niet altijd voorspelbaar is van welke factoren klinische uitkomsten afhankelijk zijn, lijkt behandeling van hypercortisolisme risico op morbiditeit en mortaliteit niet volledig weg te nemen. Daarom zou behandeling van patiënten met het syndroom van Cushing zich verder moeten uitstrekken dan alleen behandeling van hypercortisolisme om mortaliteit en morbiditeit verder te verminderen.

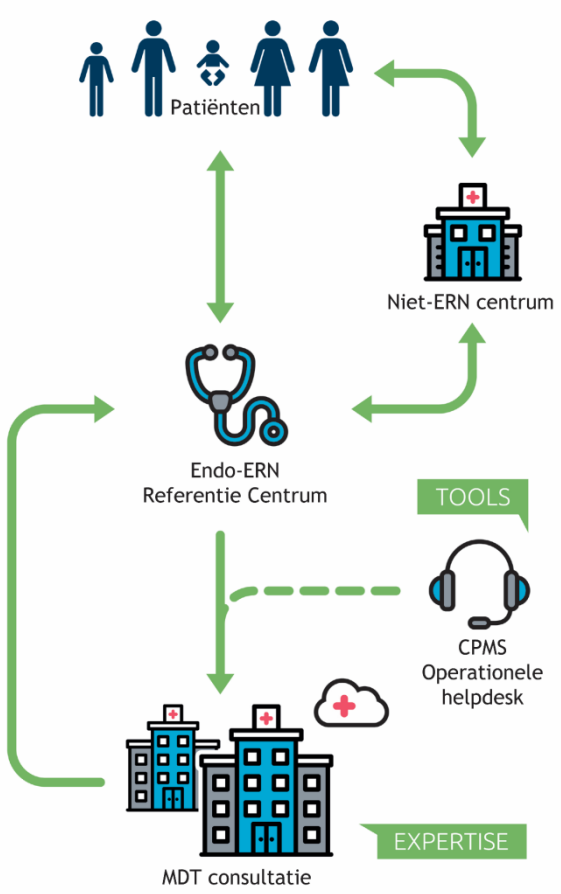
Toekomstperspectief

De studies in dit proefschrift over verschillende aspecten van hypercortisolisme benadrukken het belang van suppressie van de cortisolsecretie tot in het fysiologische bereik. Zowel hypercortisolisme als bijnierschorsinsufficiëntie hebben enorme, en soms zeer schadelijke, impact op zowel fysiek als psychisch functioneren. Behandeling van hypercortisolisme en bijnierschorsinsufficiëntie met als doel het herstellen van normale cortisolconcentraties met een fysiologisch circadiaan ritme sluit het risico op ongunstige uitkomsten niet uit.

Om patiënten met zeldzame endocriene stoornissen, zoals het syndroom van Cushing, gelijkwaardige deskundige gespecialiseerde zorg te bieden in heel Europa, is recent het Europees Referentie Netwerk voor zeldzame endocriene ziekten (Endo-ERN) opgericht (35). Via een Endo-ERN referentiecentrum kan een multidisciplinair team van experts geconsulteerd worden voor een advies gebaseerd op gecombineerde specialistische expertise vanuit heel Europa (Figuur 1) (36). Dit ondersteunt de correcte identificatie van de onderliggende ziekte bij patiënten met hypercortisolisme, waarmee vertraging in het diagnostische proces vermindert, en vroegere verwijzing naar een gespecialiseerd centrum kan worden bevorderd. Bovendien kan het multidisciplinaire team helpen bij behandelkeuzes, zoals bij patiënten met persisterende ziekte of recidief, of bij patiënten met het ectopische syndroom van Cushing zonder duidelijke bron van ACTH-productie.

Wat Endo-ERN is voor patiëntenzorg, zijn internationale patiëntenregisters voor klinisch onderzoek. Voor het verbeteren van kennis en expertise wat betreft zeldzame endocriene ziekten is internationale uitwisseling van informatie middels deze patiëntenregisters cruciaal. Dit vergroot de populatie en daarmee de nauwkeurigheid van de verworven kennis door onderzoek vergeleken met

monocentrische cohortstudies. Helaas is bewustzijn van en deelname aan deze internationale registers relatief laag. Recent is het Europese register voor zeldzame endocriene ziekten (EuRRECa, https://eurreca.net/), wat gevoed zal worden door, en gekoppeld zal worden aan, Endo-ERN, mede gefinancierd door het gezondheidsprogramma van de Europese Unie. Naast het ontwikkelen van nieuwe internationale registers voor endocriene ziekten waarvoor tot nu toe nog geen register bestaat, moet bewustzijn van en deelname aan bestaande registers vergroten om optimaal functioneren van zowel Endo-ERN als patiëntenregisters te garanderen, wat een proces in ontwikkeling is (37). Patiëntenregisters bieden informatie over specifieke zeldzame ziekten, wat kan worden aangevuld met kennis vanuit de Endo-ERN over buitengewone gevallen wat betreft klinisch beeld, diagnose of behandeling. Gecombineerd kunnen zij effectief bijdragen aan onderzoek over zeldzame endocriene ziekten.



Figuur 1: Consultatie van een multidisciplinair team (MDT) door een referentiecentrum van het Europees Referentie Netwerk voor zeldzame endocriene ziekten (Endo-ERN) namens een patiënt, waarmee deskundige gespecialiseerde zorg naar alle patiënten in Europa wordt gebracht middels het Klinische Patiënt Management Systeem (CPMS), aangepast van de Endo-ERN website (36).

Om onze kennis wat betreft hypercortisolisme effectief te vergroten is het uiterst belangrijk om alle statussen van cortisolexces en cortisoldeficiëntie, evenals alle klinische uitkomsten, duidelijk te definiëren en herkennen. Voor sommige diagnosen is de classificatie extra uitdagend, bijvoorbeeld in het geval van bijnierschors-insufficiëntie, gezien de aspecifieke symptomen en de combinatie van stappen die nodig zijn om een juiste diagnose te stellen (aanwezigheid van hypocortisolisme, niveau van dysfunctie van de HPA-as en precieze oorzaak van bijnier-schorsinsufficiëntie). Alleen als alle onderzoeksgroepen dezelfde definities gebruiken kunnen data met elkaar vergeleken worden, zowel direct in meta-analyses, als indirect in losstaande klinische studies. Bovendien moeten definities gebruikt in onderzoek overeenkomen met klinische definities om te verzekeren dat studieuitkomsten toepasbaar zijn in de klinische praktijk. Toekomstige studies zouden moeten kijken naar welke combinatie van testen (serumcortisol, urinecortisol, speekselcortisol, cortisol suppressietesten en -stimulatietesten) optimaal is voor het diagnosticeren van het syndroom van Cushing en wat de diagnostische consequenties zijn van inconsistente testresultaten. Verder zou toekomstig onderzoek zich ook moeten concentreren op hoe te differentiëren tussen het syndroom van Cushing en pseudo-Cushing, waarbij verhoogde cortisol-concentraties gezien worden, evenals verschillende gradaties van symptomen die passen bij het syndroom van Cushing. Deze resultaten kunnen gebruikt worden bij het collectief kiezen voor een verbeterde definitie van het syndroom van Cushing, die in zowel onderzoek als de klinische praktijk gebruikt kan worden. Toekomstige meta-analyses, die alleen studies meenemen waarin dezelfde definities voor diagnose en klinische uitkomsten zijn gebruikt, zullen waardevoller en makkelijker te interpreteren zijn dan bestaande meta-analyses. Echter, aangezien dit op het moment niet uitvoerbaar is door gebruik van verschillende definities in individuele studies, moeten auteurs van meta-analyses zorgvuldig zijn bij het bepalen welke conclusies getrokken kunnen worden uit de data en voor welke patiënten deze conclusies geldig zijn, wat wij hebben kunnen doen in alle meta-analyses gepresenteerd in dit proefschrift.

Consistente definities zijn essentieel voor het optimaal functioneren van onderzoek en patiëntenzorg. Internationale patiëntenregisters leveren een grote hoeveelheid data over zeldzame ziekten, die onmogelijk door een enkel centrum verzameld kan worden. Als consistente definities door alle deelnemende centra gebruikt worden, bieden deze registers een uitstekende mogelijkheid grootschalige studies naar zeldzame ziekten met zeldzame uitkomsten uit te voeren, zoals het vinden van risicofactoren voor Addisonse crisis na behandeling voor het syndroom van Cushing. Echter, dataverzameling moet in overeenstemming zijn met potentiële onderzoeks-vragen en data achteraf toevoegen moet mogelijk zijn. Bijvoorbeeld in het Europees Register voor het Syndroom van Cushing (ERCUSYN) zijn geen data beschikbaar over

Addisonse crisis en retrospectief data toevoegen is vrijwel onmogelijk (23). Bovendien zijn huidige patiëntenregisters alleen geschikt voor het onderzoeken van een specifieke zeldzame ziekte, zoals het syndroom van Cushing, terwijl een register voor het vergelijken van patiënten met verschillende onderliggende ziekten maar vergelijkbare klinische presentatie ontbreekt. Een dergelijk register zou ons in staat kunnen stellen patiënten met hypercortisolisme door verschillende onderliggende ziekten met elkaar te vergelijken. Kennis over de verschillen en overeenkomsten tussen de onderliggende ziekten kan leiden tot een zorgvuldigere bewerking van klinische richtlijnen voor specifieke patiëntengroepen met verschillende vormen van hypercortisolisme. Toekomstig onderzoek naar patiënten met het syndroom van Cushing kan deze grootschalige patiëntenregisters gebruiken voor een directe vergelijking van verschillende cortisolverlagende medicijnen, waarbij tevens gekeken wordt naar lange-termijn effectiviteit en bijwerkingen. Bovendien zouden gerandomiseerde gecontrolleerde studies moeten worden uitgevoerd om de veelbelovendste medicijnen met de hoogste effectiviteit en laagste risico op bijwerkingen direct te kunnen vergelijken. Kennis omtrent medicijn, of combinatie van medicijnen, van voorkeur kan de behandelopties verbeteren voor patiënten met persisterende ziekte of recidief, of voor diegenen met een contra-indicatie voor chirurgie.

Tot slot kan extra kennis over de effecten van zowel endogeen syndroom van Cushing als exogeen hypercortisolisme waarschijnlijk de patiëntenzorg ook buiten de afdeling Endocrinologie verbeteren, aangezien deze aandoeningen model staan voor lange-termijn blootstelling aan stress, wat een veelvoorkomende conditie is. Inzicht in de potentiële consequenties van lange-termijn blootstelling aan stress, zowel tijdens perioden van stress als na opheffing van de stressinducerende situatie, kan alle individuen blootgesteld aan lange-termijn stress helpen, inclusief patiënten met chronische ziekte of lange-termijn ziekenhuisopname.

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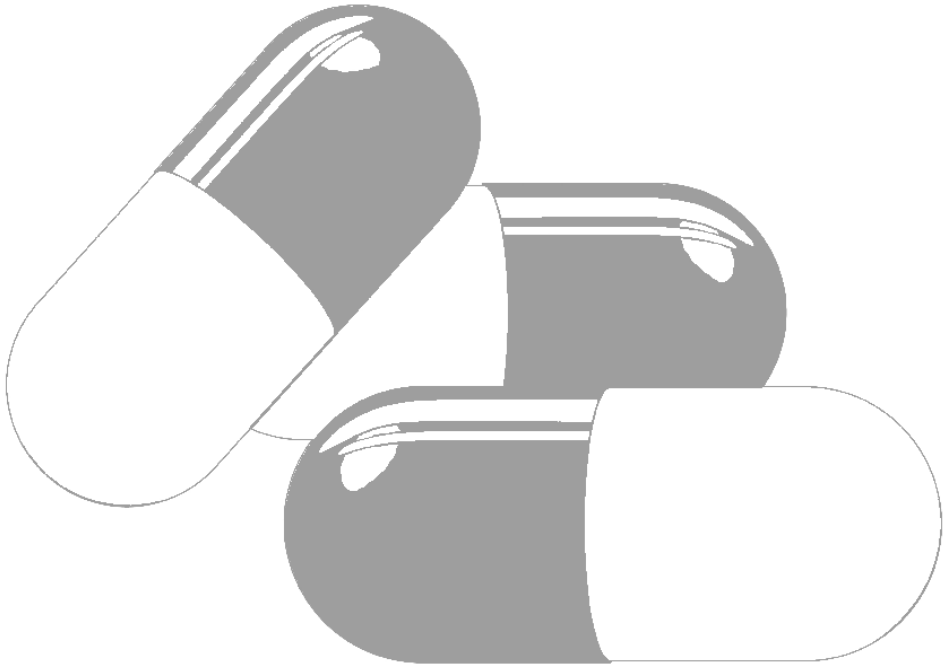
Appendix I

Bijnierschorsinsufficiëntie bij gebruik glucocorticoïden: een systematische review en meta-analyse



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Samenvatting

Doel
Het schatten van het risico op bijnierschorsinsufficiëntie na behandeling met glucocorticoïden, en het stratificeren van de resultaten naar toedieningsvorm, ziekte, behandelduur en dosering.

Opzet
Systematische review en meta-analyse.

Methode
In PubMed, MEDLINE, Embase, Cochrane, CENTRAL, Web of Science, CINAHL en Academic Search Premier zochten we naar relevante studies. We includeerden originele artikelen die bijnierschorsinsufficiëntie diagnosticeerden bij volwassen gebruikers van glucocorticoïden.

Resultaten
We includeerden 74 artikelen met in totaal 3.753 deelnemers. Gestratificeerd naar toedieningsvorm varieerde het percentage patiënten met bijnierschorsinsufficiëntie van 4,2% bij nasale toediening (95%-betrouwbaarheidsinterval [BI]: 0,5%-28,9%) tot 52,2% bij intra-articulaire toediening (95%-BI: 40,5%-63,6%). Gestratificeerd naar ziekte varieerde het percentage patiënten met bijnierschorsinsufficiëntie van 6,8% bij astmapatiënten die alleen inhalatieglucocorticoïden gebruikten (95%-BI: 3,8%-12,0%) tot 60,0% bij patiënten met hematologische maligniteiten (95%-BI: 38,0%-78,6%). Bij astmapatiënten varieerde het risico op bijnierschorsinsufficiëntie per dosering van 2,4% bij een lage dosering (95%-BI: 0,6%-9,3%) tot 21,5% bij een hoge dosering (95%-BI: 12,0%-35,5%), en per behandelduur van 1,4% bij een behandelduur <28 dagen (95%-BI: 0,3%-7,4%) tot 27,4% (95%-BI: 17,7%-39,8%) bij behandelingen >1 jaar.

Conclusie
Bijnierschorsinsufficiëntie komt vaak voor bij patiënten die glucocorticoïden hebben gebruikt. Er is geen toedieningsvorm, dosering, behandelduur of onderliggende ziekte waarbij bijnierschorsinsufficiëntie met zekerheid kan worden uitgesloten, hoewel hogere doses en een langere behandelduur een hoger risico geven. In de klinische praktijk moeten gebruikers van glucocorticoïden laagdrempelig getest worden op bijnierschorsinsufficiëntie, zeker patiënten met aspecifieke symptomen na het staken van glucocorticoïden.

Introductie

Glucocorticoïden worden gebruikt voor de behandeling van patiënten met een inflammatoire ziekte, maligniteit of orgaantransplantatie. Behandeling met glucocorticoïden is gericht op inhibitie van een inflammatoire respons (1-3). Het gebruik van deze medicijnen is gerelateerd aan talloze bijwerkingen en het is de meest voorkomende oorzaak van bijnierschorsinsufficiëntie (4, 5). Bijnierschors-insufficiëntie kan ontstaan doordat chronisch gebruik van glucocorticoïden via negatieve feedback de hypothalamus-hypofyse-bijnier-as remt (4, 6).

Bijnierschorsinsufficiëntie is een ernstige, mogelijk levensbedreigende bijwerking van het gebruik van glucocorticoïden. Om die reden hebben patiënten glucocorticoïd-vervangende therapie nodig ten tijde van stress, zoals bij trauma, operaties of acute ziekte, totdat de functie van de bijnierschors volledig hersteld is. Soms is chronische vervangende therapie met fysiologische doses glucocorticoïden geïndiceerd (7-9).

Noch de dosering en behandelduur, noch de toedieningsvorm lijken accurate voorspellers voor het ontstaan van bijnierschorsinsufficiëntie na gebruik van glucocorticoïden (10, 11). Daarnaast is niet duidelijk hoe groot het risico is op het ontstaan van deze bijwerking. Omdat veel patiënten glucocorticoïden gebruiken, is het klinisch relevant om kennis te vergaren over het risico dat het gebruik van deze medicijnen geeft op het ontwikkelen van bijnierschorsinsufficiëntie.

Het doel van deze studie was inzicht te krijgen in het risico op bijnierschors-insufficiëntie na glucocorticoïdgebruik. Dit deden we met een systematische review van de beschikbare literatuur en een meta-analyse van het percentage patiënten dat bijnierschorsinsufficiëntie ontwikkelt na gebruik van glucocorticoïden. Nevendoelen waren het stratificeren van de onderzoeksresultaten naar toedieningsvorm, onderliggende ziekte, dosering en behandelduur.

Methode

Inclusie van artikelen

We zochten in PubMed, MEDLINE, Embase, Cochrane, CENTRAL, Web of Science, CINAHL en Academic Search Premier naar mogelijk relevante studies uit de periode 1975-februari 2014. Originele studies die bijnierschorsinsufficiëntie diagnosticeerden bij volwassen gebruikers van glucocorticoïden waren geschikt voor inclusie. De diagnose 'bijnierschorsinsufficiëntie' moest gesteld zijn op basis van een van de volgende testen: de insulinetolerantietest, de stimulatietest met ACTH (0,5 µg, 1 µg

of 250 µg), de corticotropine-'releasing'-hormoon (CRH)-test of de metyrapontest. Er waren geen restricties in dosis, behandelduur, type glucocorticoïd of toedienings-vorm, met uitzondering van intraveneuze toediening.

Definitie van bijnierschorsinsufficiëntie
De afkapwaarde van de serumcortisolconcentratie voor het definiëren van bijnier-schorsinsufficiëntie was ≤500 nmol/l of hoger, bijvoorbeeld ≤550 nmol/l (12-14). Bij de metyrapontest moest de concentratie 11-deoxycortisol ten minste 200 nmol/l zijn (12). We voerden een sensitiviteitsanalyse uit voor artikelen waarin ≥24 uur na de laatste dosering glucocorticoïden getest was op bijnierschorsinsufficiëntie (12).

Risico op bias
Voor alle geïncludeerde studies werd het risico op bias beoordeeld. Artikelen met een hoog risico op bias werden niet geëxcludeerd, aangezien dat zou leiden tot een klein aantal beschikbare studies voor de systematische review en meta-analyse.

Statistische analyse
De primaire uitkomstmaten van deze meta-analyse waren de gepoolde percentages patiënten die bijnierschorsinsufficiëntie kregen na gebruik van glucocorticoïden. Hierbij werd gestratificeerd naar toedieningsvorm, ziekte, dosering en behandelduur. De percentages werden gepoold in een logistisch regressiemodel.

De analyse waarin werd gestratificeerd naar toedieningsvorm was gebaseerd op de toedieningsvorm die gebruikt werd, op het moment dat de bijnierschorsfunctie werd getest.

Bij stratificatie naar ziekte werden de volgende ziektegroepen of indicaties onderscheiden: astma, inclusief chronische obstructieve longziekte (COPD), waarbij de behandeling bestond uit alleen inhalatieglucocorticoïden; astma, inclusief COPD, met ook andere toedieningsvormen; allergische rhinitis en rinosinusitis; dermatologische ziekten, zoals psoriasis, atopisch eczeem en lichen planus; reumatische ziekten, waaronder artrose en reumatoïde artritis; niertransplantatie; hematologische maligniteiten, waaronder myeloom, lymfoom, acute lymfatische leukemie en de ziekte van Hodgkin; nasale polypose; cystische fibrose; en de ziekte van Crohn. Ziektes of indicaties die slechts bestudeerd waren in een enkele studie, werden niet geïncludeerd in de analyse waarin naar ziekte werd gestratificeerd.

De behandelduur werd als volgt gecategoriseerd: <28 dagen gebruik als 'korte termijn', 28 dagen-1 jaar als 'gemiddelde termijn' en >1 jaar als 'lange termijn'. De dosering werd gecategoriseerd aan de hand van de aanbevolen doseringen, waarbij doses tussen de onderste en bovenste grens van de aanbeveling werden gecodeerd als

'gemiddelde dosis', doses onder de onderste grens als 'lage dosis' en doses boven de bovenste grens als 'hoge dosis'. Aangezien de meeste doses die gebruikt werden suprafysiologisch waren, werden deze niet geclassificeerd naar fysiologische en suprafysiologische dosis. Voor de gebruikte grenzen voor het bepalen van de dosiscategorie verwijzen wij naar het originele artikel (15).

De analyses van het risico op bijnierschorsinsufficiëntie per dosering en per behandelduur werden alleen bij astmapatiënten uitgevoerd om zo een homogene patiëntenpopulatie te creëren. Studiegroepen waarbij herhaaldelijk tests werden uitgevoerd na het staken van glucocorticoïden werden eveneens apart geanalyseerd.

Voor verdere details omtrent inclusiecriteria, definitie van bijnierschorsinsufficiëntie en beschouwing van het risico op bias, en voor informatie over de zoekstrategie, gegevensverzameling en statistische analyse, zie het originele artikel (15).

Resultaten

Studieselectie
De initiële zoekstrategie leverde 3.600 unieke artikelen op. Door in de referenties van belangrijke artikelen te zoeken, vonden we nog 16 artikelen. Dit leidde tot een totaal van 3.616 artikelen. Na het screenen op titel en samenvatting bleven er 365 artikelen over voor een gedetailleerde beschouwing. De redenen voor exclusie staan in het originele artikel (15). Uiteindelijk includeerden we 74 artikelen in de meta-analyse, met gegevens van 136 studiegroepen. Voor referenties van de geïncludeerde studies, zie het originele artikel (15).

Studie-eigenschappen
Uitgebreide informatie over de studie-eigenschappen staat genoemd in het originele artikel (15). De geïncludeerde studies waren gepubliceerd in de periode 1975-februari 2014. Van de 74 artikelen waren er 36 gerandomiseerde gecontroleerde trials, 23 cohortstudies en 15 dwarsdoorsnedeonderzoeken. De 74 studies bestonden uit in totaal 136 studiegroepen en 3.753 deelnemers, onder wie 124 gezonde vrijwilligers.

Risico op bias
Een gedetailleerde beschouwing van het risico op bias per studie is te vinden in het originele artikel (15).

Uitkomsten van de studies
Van de 3.753 deelnemers werd bij 1.190 de diagnose 'bijnierschorsinsufficiëntie' gesteld. De stimulatietest met ACTH 250 µg werd gebruikt door 103 studiegroepen.

Bij 79 studiegroepen was de tijd tussen de laatste gift glucocorticoïden en de test op bijnierschorsinsufficiëntie ≥24 uur. Bij 7 studiegroepen, met in totaal 199 patiënten, was het gebruik van glucocorticoïden als comedicatie toegestaan. Voor details over de studie-uitkomsten en de gebruikte testen, zie het originele artikel (15).

Symptomen van bijnierschorsinsufficiëntie

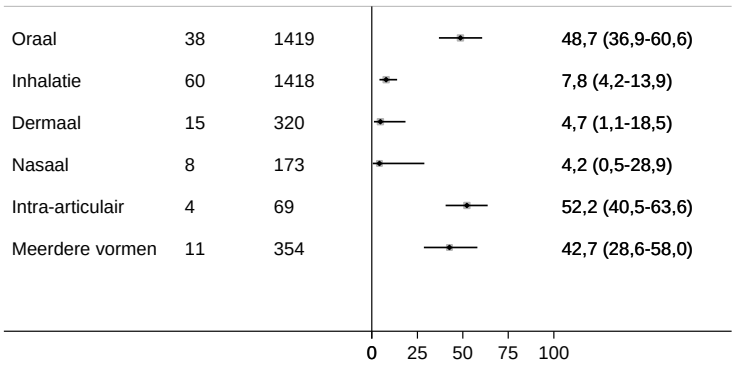
Voor slechts 10 studiegroepen werden de symptomen van bijnierschorsinsufficiëntie gerapporteerd. In totaal meldden 10 van de 521 patiënten (1,9%) symptomen van bijnierschorsinsufficiëntie. Deze symptomen werden in geen van de artikelen systematisch gescoord. Na het testen op bijnierschorsinsufficiëntie werd de diagnose bij 98 patiënten (18,8%) uit de 10 studiegroepen gesteld. Zodoende zouden 88 patiënten (89,8%) gemist zijn als alleen patiënten met symptomen van bijnierschorsinsufficiëntie waren getest.

Gepoolde analyses

We stratificeerden de onderzoeksresultaten naar toedieningsvorm, onderliggende ziekte, dosering, behandelduur en naar studies die meerdere keren op bijnierschorsinsufficiëntie testten.

Tabel 1: Absolute risico op bijnierschorsinsufficiëntie na glucocorticoïdgebruik, uitgesplitst naar toedieningsvorm.

Toedieningsvorm	Studies	Patiënten	Absoluut risico (95%-BI)
Oraal	38	1419	48,7 (36,9-60,6)
Inhalatie	60	1418	7,8 (4,2-13,9)
Dermaal	15	320	4,7 (1,1-18,5)
Nasaal	8	173	4,2 (0,5-28,9)
Intra-articulair	4	69	52,2 (40,5-63,6)
Meerdere vormen	11	354	42,7 (28,6-58,0)



Toedieningsvorm

Van de patiënten die werden behandeld met orale glucocorticoïden had 48,7% (95%-betrouwbaarheidsinterval [BI]: 36,9%-60,6%) bijnierschorsinsufficiëntie (Tabel 1). De risico's op bijnierschorsinsufficiëntie bij gebruik van andere toedieningsvormen waren: 7,8% (95%-BI: 4,2%-13,9%) bij inhalatie, 4,7% (95%-BI: 1,1%-18,5%) bij dermale toediening, 4,2% (95%- BI: 0,5%-28,9%) na nasale toediening en 52,2% (95%-BI: 40,5%-63,6%) bij patiënten die intra-articulaire glucocorticoïden kregen. Van de patiënten

die glucocorticoïden in meerdere toedieningsvormen tegelijkertijd gebruikten, had 42,7% bijnierschorsinsufficiëntie (95%-BI: 28,6%-58,0%).

Ziekte

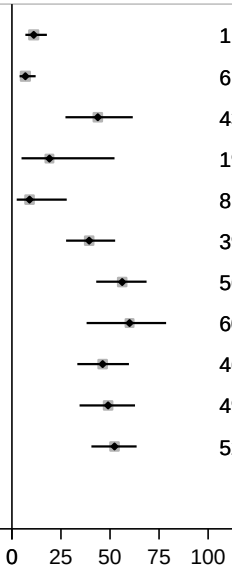
De gepoolde percentages bijnierschorsinsufficiëntie per ziekte of indicatie staan in Tabel 2. De gepoolde percentages varieerden van 6,8%-60,0%. Van de astmapatiënten had 11,1% (95%-BI: 6,8%-17,7%) bijnierschorsinsufficiëntie. Dit percentage was lager voor astmapatiënten die alleen inhalatieglucocorticoïden gebruikten (6,8%; 95%-BI: 3,8%-12,0%) dan voor astmapatiënten die andere toedieningsvormen gebruikten (43,7%; 95%-BI: 27,3%-61,6%).

Dosering en behandelduur

De analyse per dosering en behandelduur werd alleen uitgevoerd bij astmapatiënten, zodat de populatie relatief homogeen was (Tabel 3). Het gebruik van glucocorticoïden in een lage, gemiddelde en hoge dosering leidde tot bijnierschorsinsufficiëntie bij respectievelijk 2,4% (95%-BI: 0,6%-9,3%), 8,5% (95%-BI: 4,2%-16,8%) en 21,5% van de astmapatiënten (95%-BI: 12,0%-35,5%). Gebruik van glucocorticoïden gedurende korte, gemiddelde of lange termijn veroorzaakte bijnierschorsinsufficiëntie bij respectievelijk 1,4% (95%-BI: 0,3%-7,4%), 11,9% (95%-BI: 5,8%-23,1%) en 27,4% van de astmapatiënten (95%-BI: 17,7%-39,8%).

Tabel 2: Absolute risico op bijnierschorsinsufficiëntie na glucocorticoïdgebruik, uitgesplitst naar ziekte of indicatie.

Ziekte	Studies	Patiënten	Absoluut risico (95%-BI)
Astma	68	1692	11,1 (6,8-17,7)
Astma: alleen inhalatie	54	1317	6,8 (3,8-12,0)
Astma: andere toedieningsvorm	14	375	43,7 (27,3-61,6)
Rinitis of rinosinusitis	8	195	19,0 (4,8-52,2)
Psoriasis/atopisch eczeem/lichen planus	12	273	8,9 (2,4-27,9)
Reumatische ziekte	8	236	39,4 (27,5-52,6)
Niertransplantatie	8	176	56,2 (42,9-68,6)
Hematologische maligniteit	4	20	60,0 (38,0-78,6)
Nasale polypose	2	52	46,2 (33,2-59,7)
Cystische fibrose	3	49	49,0 (34,4-62,7)
Ziekte van Crohn	2	69	52,2 (40,5-63,6)

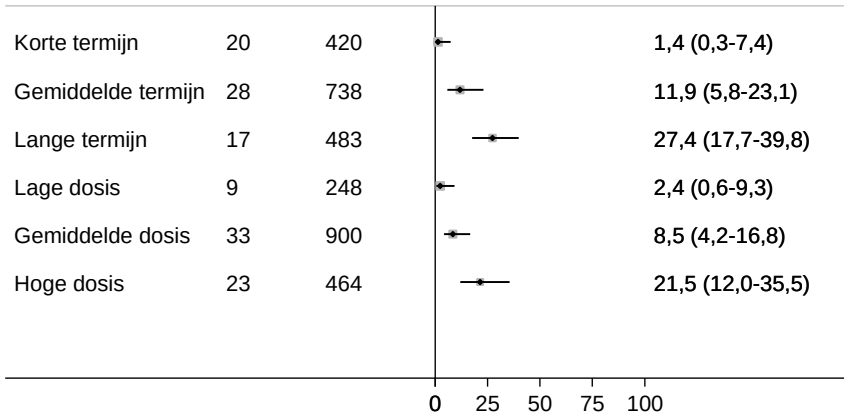


Na opnieuw testen

We splitsten de onderzoeksresultaten in studies die opnieuw op bijnierschorsinsufficiëntie testten na 4 weken bij patiënten die voornamelijk gedurende een korte termijn hoge doses glucocorticoïden gebruikten, en studies die opnieuw testten na 6 maanden bij patiënten die voornamelijk gedurende een lange termijn gemiddelde doses glucocorticoïden gebruikten (Tabel 4). Bij studies die opnieuw testten na 4 weken had 38,7% van de patiënten (95%-BI: 21,7%-58,8%) bijnierschorsinsufficiëntie na de eerste test. Na 4 weken was dit percentage afgenomen tot 14,9% (95%-BI: 6,8%-29,5%). Studies die opnieuw testten na 6 maanden lieten zien dat 56,4% van de patiënten (95%-BI: 38,2%-72,9%) bijnierschorsinsufficiëntie had na de eerste test. Na 6 maanden was het percentage nog steeds 25,3% (95%-BI: 19,4%-32,3%).

Tabel 3: Absolute risico op bijnierschorsinsufficiëntie bij astmapatiënten na glucocorticoïdgebruik, uitgesplitst naar behandelduur en dosering.

Factor	Studies	Patiënten	Absoluut risico (95%-BI)
Korte termijn	20	420	1,4 (0,3-7,4)
Gemiddelde termijn	28	738	11,9 (5,8-23,1)
Lange termijn	17	483	27,4 (17,7-39,8)
Lage dosis	9	248	2,4 (0,6-9,3)
Gemiddelde dosis	33	900	8,5 (4,2-16,8)
Hoge dosis	23	464	21,5 (12,0-35,5)

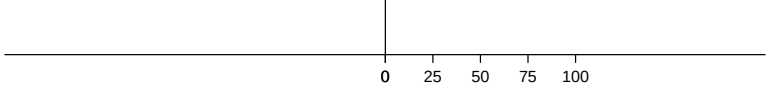


Sensitiviteitsanalyses

Voor de sensitiviteitsanalyses combineerden we alle studies met astmapatiënten als referentiegroep; hierbij was het percentage bijnierschorsinsufficiëntie 11,1% (95%-BI: 6,8%-17,7%). Als alleen studies werden geïncludeerd die expliciet vermeldden dat de tijd tussen de laatste dosis glucocorticoïden en het moment van testen ≥24 uur was, was het percentage patiënten met bijnierschorsinsufficiëntie iets lager (6,6%; 95%-BI: 2,2%-18,3%). Als alleen studies werden geanalyseerd die gebruikmaakten van de stimulatietest met ACTH 250 µg, had 8,5% (95%-BI: 4,7%-14,8%) van de patiënten bijnierschorsinsufficiëntie.

Tabel 4: Absolute risico op bijnierschorsinsufficiëntie na glucocorticoïdgebruik, uitgesplitst naar moment van testen

Testmoment	Studies	Patiënten	Absoluut risico (95%-BI)
Kortetermijnbehandeling met hoge dosering			
Eerste test	6	141	38,7 (21,7-58,8)
Test na 4 weken	6	141	14,9 (6,8-29,5)
Langetermijnbehandeling met gemiddelde dosering			
Eerste test	5	174	56,4 (38,2-72,9)
Test na 4 weken	5	174	25,3 (19,4-32,3)



Beschouwing

Wij voerden een systematische review en meta-analyse uit om het percentage patiënten te schatten dat bijnierschorsinsufficiëntie ontwikkelt na gebruik van glucocorticoïden. Afhankelijk van de toedieningsvorm varieerde het percentage patiënten met bijnierschorsinsufficiëntie van 4,2% bij nasaal toegediende glucocorticoïden tot 52,2% bij intra-articulaire glucocorticoïden. Gestratificeerd naar ziekte varieerde het percentage van 6,8% bij astmapatiënten die alleen inhalatieglucocorticoïden gebruikten tot 60% bij patiënten met hematologische maligniteiten. Bij astmapatiënten varieerde het percentage bijnierschors-insufficiëntie afhankelijk van de dosis van 2,4% (lage dosis) tot 21,5% (hoge dosis), en afhankelijk van de behandelduur van 1,4% (<28 dagen) tot 27,4% (>1 jaar). Dit betekent dat er geen toedieningsvorm, ziekte, dosering of behandelduur was waarbij het risico op bijnierschorsinsufficiëntie veilig kon worden uitgesloten. Hoewel het percentage patiënten met bijnierschorsinsufficiëntie na gebruik van glucocorticoïden in de tijd afnam, hield een substantieel aantal patiënten bijnierschorsinsufficiëntie na 6 maanden.

Dit is de eerste meta-analyse die een brede blik biedt op het risico op bijnierschorsinsufficiëntie na gebruik van verschillende typen glucocorticoïden bij verschillende ziekten. Er is slechts 1 eerdere meta-analyse gepubliceerd over bijnierschorsinsufficiëntie bij astmapatiënten; deze studie rapporteert percentages bijnierschorsinsufficiëntie van 5,5%-13,3% (16). Dit komt overeen met de resultaten

uit onze meta-analyse, namelijk het percentage bijnierschorsinsufficiëntie van 6,8% bij astmapatiënten die alleen inhalatieglucocorticoïden gebruikten.

De geïncludeerde studies vertoonden heterogeniteit in type glucocorticoïd, onderliggende ziekte, dosering, behandelduur en toedieningsvorm. Het is belangrijk te beseffen dat deze heterogeniteit de klinische praktijk weerspiegelt. Men moet er ook rekening mee houden dat de ziekte, dosering, behandelduur en toedieningsvorm onderling gerelateerd zijn. In onze gestratificeerde analyses hebben wij niet gecorrigeerd voor alle onderling afhankelijke factoren, met name omdat deze factoren ook in de klinische praktijk gerelateerd zijn, maar ook omdat meta-regressietechnieken deze factoren niet uit elkaar kunnen halen zonder de beschikbaarheid van individuele-patiëntengegevens.

Een hogere dosis van en een langere behandelduur met glucocorticoïden veroorzaken een hogere serumconcentratie hiervan en daarmee een hoger risico op bijnierschorsinsufficiëntie (17). Zo resulteert het gebruik van orale glucocorticoïden in hogere systemische concentraties glucocorticoïden dan het gebruik van inhalatieglucocorticoïden, of dermale of nasale glucocorticoïden, waardoor patiënten die orale glucocorticoïden gebruiken een hoger risico hebben op bijnierschors-insufficiëntie (18).

Ook intra-articulaire glucocorticoïden worden in hoge doses toegediend en hiervan is bekend dat zij binnen 24-48 uur de cortisolsecretie onderdrukken, die pas herstelt na 1-4 weken (19). Dit vormt mogelijk een verklaring voor het frequente voorkomen van bijnierschorsinsufficiëntie bij deze patiënten. Het hoge risico op bijnierschors-insufficiëntie wordt mogelijk ook verklaard doordat glucocorticoïdinjecties depots vormen. Hierdoor zijn er in het lichaam continu glucocorticoïden aanwezig en deze hoeveelheid neemt geleidelijk af, niet abrupt.

De meeste studies die we includeerden gaven geen informatie over de therapietrouw. Hierdoor was het niet mogelijk om te kijken naar de impact van therapietrouw of therapieontrouw op het risico op bijnierschorsinsufficiëntie. De geïncludeerde studies toonden ook heterogeniteit in de bepaling van de cortisolconcentratie en in het type test dat gebruikt werd voor evaluatie van de cortisolreserve. De sensitiviteitsanalyses toonden geen wezenlijk verschil in het percentage bijnierschorsinsufficiëntie als alleen artikelen werden geïncludeerd die radio-immunoassay gebruikten, of als alleen artikelen geïncludeerd werden waarin de stimulatietest met ACTH 250 µg was gebruikt.

Glucocorticoïden worden gebruikt door ten minste 1% van de bevolking (3). Onze meta-analyse toont dat het risico dat deze patiënten bijnierschorsinsufficiëntie

ontwikkelen 1,4%-60,0% is. Verschijnselen van lichte tot matige bijnierschors-insufficiëntie, zoals vermoeidheid en buikklachten, zijn aspecifiek en worden dus niet zonder meer toegeschreven aan bijnierschorsinsufficiëntie. Daarnaast ontbreken accurate voorspellers om onderscheid te maken tussen patiënten die bijnierschorsinsufficiëntie zullen ontwikkelen en zij die dat niet ontwikkelen. Ook is er onvoldoende bewijs dat enig afbouwschema na gebruik van glucocorticoïden efficiënt of veilig is (20).

Daarom is het aan te bevelen alle patiënten met onverklaarde symptomen na het afbouwen van glucocorticoïden te testen op mogelijke bijnierschorsinsufficiëntie. Patiënten met een onvoldoende cortisolrespons moeten worden behandeld met fysiologische doseringen hydrocortison.

Conclusie

Alle patiënten die behandeld worden met glucocorticoïden lopen risico op het ontwikkelen van bijnierschorsinsufficiëntie. In de eerste plaats impliceert dit dat behandelaars patiënten die glucocorticoïden gebruiken moeten informeren over het risico op en de symptomen van bijnierschorsinsufficiëntie. Daarnaast moeten behandelaars overwegen patiënten te testen op bijnierschorsinsufficiëntie na het staken van hoge doses glucocorticoïden of een langetermijnbehandeling met deze medicijnen. Ten slotte moet met name laagdrempelig op bijnierschorsinsufficiëntie worden getest bij patiënten die aspecifieke symptomen hebben na het staken van glucocorticoïden.

Leerpunten

1. Bijnierschorsinsufficiëntie is een veelvoorkomende bijwerking na het gebruik van glucocorticoïden.
2. Van de ten minste 1% van de bevolking die glucocorticoïden gebruikt, ontwikkelt 1,4%-60% bijnierschorsinsufficiëntie, waarbij het risico afhangt van de behandelduur, toedieningsvorm, dosering en onderliggende ziekte.
3. Als een patiënt met glucocorticoïden wordt behandeld, is er geen enkele toedieningsvorm, ziekte, dosering of behandelduur waarbij het risico op bijnierschorsinsufficiëntie kan worden uitgesloten.
4. Patiënten dienen bij het begin van een behandeling met glucocorticoïden geïnformeerd te worden over het risico op het ontstaan van bijnierschorsinsufficiëntie.

5. Vanwege de aspecifieke symptomen die bijnierschorsinsufficiëntie kan veroorzaken, is het raadzaam om patiënten met onverklaarde symptomen na het staken van glucocorticoïden te testen op mogelijke bijnierschors-insufficiëntie.

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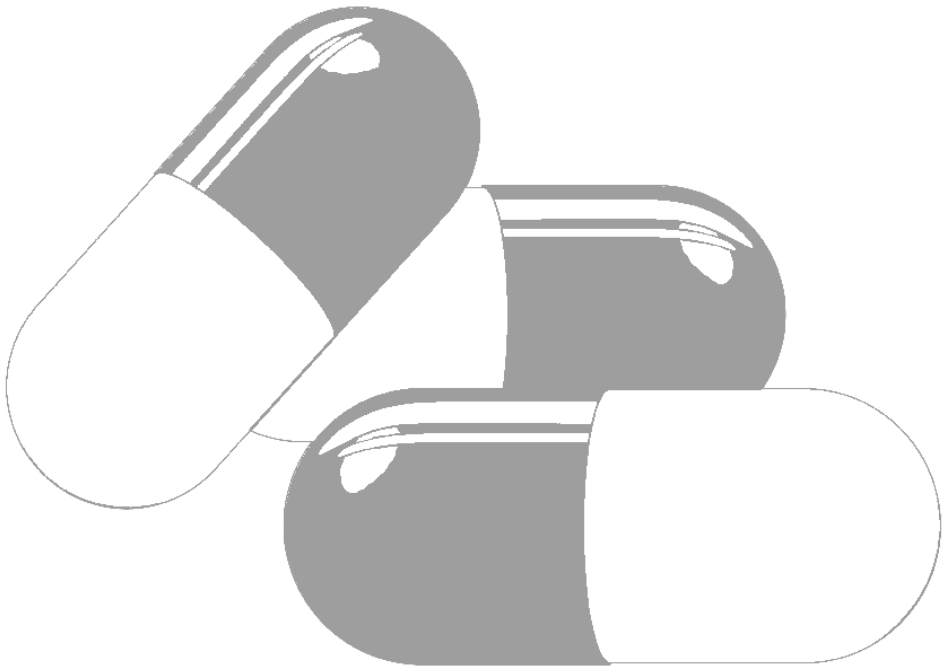
Appendix II

Response to the Letter by Lindholm, *et al*.



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and Olaf M. Dekkers

J Clin Endocrinol Metab. 2015 Aug;100(8):L66-7

We thank Dr. Lindholm for his interest in our paper and for drawing attention to two important issues: first, the definition of adrenal insufficiency; and second, the association between the result of a biochemical test for adrenal insufficiency and the need for hormone replacement.

Adrenal insufficiency could be defined as insufficient cortisol secretion in response to stress, of which medical emergencies clearly pose the highest risk. At present we can only assess adrenal function through biochemical measurements and dynamic tests. It is well known that no test — including the ACTH test — is infallible. It might also be that tests perform differently according to the specific condition (pituitary or adrenal disease, glucocorticoid users, patients on intensive care, etc.). Also, cutoff values can be debated (1). When performing a systematic review, one has to be pragmatic, and it is an empirical fact that the ACTH test is the most frequently used; for that simple reason, our study relies largely on the ACTH test.

We fully agree that an inadequate response to the ACTH test does not automatically demand glucocorticoid replacement. For good reasons, there are limited data on the risk of glucocorticoid not being replaced, and pharmaco-epidemiological studies should therefore be undertaken to address this important clinical issue. For the time being, the decision about glucocorticoid replacement is therefore at the discretion of the physician in charge.

The main clinical message from our study is that some degree of secondary adrenocortical failure is detectable in many former glucocorticoid users (2). This is the reason that in glucocorticoid users admitted to the intensive care unit, high-dose glucocorticoid replacement is started immediately (3). But we argue that knowledge of this risk is also of relevance apart from the medical emergencies because adrenal insufficiency is directly related to a decreased quality of life, which will improve after substitution. Awareness of this risk in glucocorticoid users is important, given the high prevalence of such users and the nonspecificity of symptoms of adrenal insufficiency that can be difficult to disentangle from symptoms of underlying diseases or treatment. Physicians should therefore utilize a low threshold to test for adrenal insufficiency in glucocorticoid users, although these tests are not perfect.

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Curriculum vitae

Leonarda Hubertina Alagonda Broersen werd geboren op 11 januari 1992 te Amsterdam. Zij behaalde in 2010 haar gymnasiumdiploma aan het Stedelijk Gymnasium te Haarlem, waarna zij datzelfde jaar startte met de studie Geneeskunde aan de Universiteit Leiden. Tijdens haar Master deed zij haar wetenschapsstage bij de afdelingen Klinische Epidemiologie en Endocrinologie van het Leids Universitair Medisch Centrum (LUMC). Na het behalen van haar artsexamen in 2016 zette zij haar wetenschappelijk onderzoek bij deze afdelingen voort in het kader van haar promotieonderzoek, onder leiding van Prof. dr. A.M. Pereira, Prof. dr. O.M. Dekkers en Prof. dr. N.R. Biermasz. Voor het voltooien van haar promotieonderzoek ontving zij een tweejarige beurs van de Raad van Bestuur van het LUMC. In 2017 verhuisde zij naar Berlijn, waar zij verschillende onderdelen van haar promotieonderzoek uitvoerde met data uit zowel Leiden als Berlijn. Hiervoor ontving zij twee studiebeurzen, van het Leids Universitair Fonds / Mulder Hamelers Fonds en de Van Leersum beurs van de Koninklijke Nederlandse Akademie van Wetenschappen (KNAW). Naast haar promotieonderzoek doet zij sinds 2018 wetenschappelijk onderzoek bij de afdelingen Neurologie en het Center for Stroke Research Berlin (CSB) aan het Charité Universitätsmedizin Berlin. Tevens werkt zij sinds 2019 mee als methodologische ondersteuning aan het ontwikkelen van de Europese richtlijn ‘Hypofysestoornissen in de zwangerschap’.

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Velen hebben bijgedragen bij de totstandkoming van dit proefschrift. Graag wil ik mijn promotors, collega's in Leiden en in Berlijn, familie en vrienden bedanken voor hun begeleiding, inspiratie, steun en interesse.

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