Clinical consequences of endogenous and exogenous glucocorticoid excess

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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.

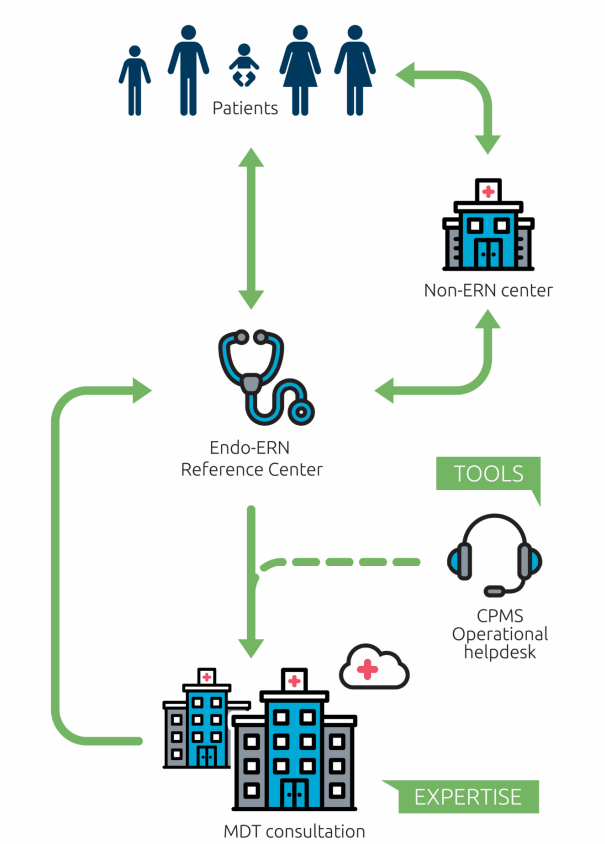

Figure 1: Consultation of a multidisciplinary team (MDT) by an European Reference Network on rare endocrine conditions (Endo-ERN) reference center on behalf of the patient, bringing expert specialized care to all patients in Europe through the Clinical Patient Management System (CPMS), derived from the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