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Cycle Characterization and Clinical Complications in Patients with Cyclic Cushing's Syndrome: Insights From An International Retrospective Cohort Study

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Authors' contributions:

E.N. and M.R. designed the study, had direct access to and verified the underlying data reported in the manuscript, and conducted the data analysis. E.N., M.R., and M.Fl. carried out the literature search, recruited centers, collected data, and contributed to data interpretation. E.N. drafted the initial version of the manuscript, with M.R. and M.Fl. providing critical content revisions. All authors were involved in patient investigations, data curation and collection. All authors reviewed and revised the manuscript. All authors had full access to the data presented in the study and had final responsibility for the decision to submit for publication.

Abstract

Background: Cyclic Cushing's syndrome (cCS) features fluctuating cortisol secretion, often causing diagnostic errors/delays, and possibly poorer outcomes. We aimed to identify unpublished cCS cases to identify clinical challenges and guide strategies for improving outcomes by characterizing cycle patterns, peak frequency, and evaluating complications.

Methods: This was a retrospective observational study at 45 endocrine centers in 21 countries, including patients with confirmed CS showing ≥2 hypercortisolemic peaks and ≥1 spontaneous eu/hypocortisolemic trough. Data included clinical/biochemical parameters, diagnostics, treatment, complications, and outcomes.

Findings: Among 110 patients, cCS origin was pituitary in 64% (70/110), ectopic in 23% (25/110), adrenal in 3% (3/110), and occult in 11% (12/110). Cyclicity was primarily determined by 24-hour urinary free cortisol, with median peaks of 7.40 (2.86-28.9) and troughs of 0.31×ULN (IQR 0.15-0.53). The median peak count was 3.0 (IQR 2.0-4.0), most frequent and pronounced in ectopic cCS, and mostly (86%, 55/64) occurring at irregular intervals. Symptoms worsened in 81% (87/108) during peaks and improved in 74% (79/107) during troughs; 28% (31/110) experienced spontaneous adrenal insufficiency. Bilateral inferior petrosal sinus sampling (BIPSS) was performed during troughs in 18% (14/78). Imaging missed tumors in 32% (35/110), and 8% (9/110) underwent unwarranted surgeries at the wrong anatomical site due to misclassification. After 5.8 years median follow-up, 50% (55/110) achieved complete biochemical surgical remission, 6% (7/110) spontaneous remission, 20% (22/110) were medically controlled, 5% (6/110) had partial remission, 10% (10/110) remained uncontrolled, and 8% (9/110) were lost to follow-up. Overall, 3% (3/110) died. Delayed diagnosis and therapy occurred in 41% (45/110) and 43% (47/110), respectively.

Interpretation: Even in specialized centers, cCS diagnosis and management remain challenging with high rates of spontaneous adrenal insufficiency, inappropriate surgeries and poor outcomes. Ectopic cCS showed the most frequent and severe peaks. These findings may help guide imaging localizations or the timing of BIPSS in patients with active occult ACTH-dependent cCS. Hypercortisolism needs to be biochemically confirmed prior to BIPSS to enable correct tumor localization. Patients with suspected or proven cCS should be equipped with salivary cortisol collection kits to capture dynamic changes as well as glucocorticoids prescribed to be used as a precaution.

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Research in context

Evidence before the study

Cushing's syndrome (CS), caused by endogenous cortisol excess, is potentially fatal if left untreated. It typically results from a benign adrenocorticotropin (ACTH)-secreting pituitary tumor, followed by ACTH-independent adrenal origin, and ectopic sources of ACTH excess. Diagnosis is often delayed due to nonspecific symptoms and inconclusive test results. Intraindividual variability in cortisol secretion is common, as demonstrated by prospective studies using urinary free cortisol and late-night salivary cortisol. Cyclic Cushing's syndrome (cCS) refers to a subtype of CS characterized by intermittent hypercortisolism interspersed with spontaneous troughs of physiological or hypocortisolemic cortisol concentrations. These troughs may last for years, during which clinical signs can improve or resolve. In a recent systematic review, we identified 212 cCS cases, with prevalence estimates ranging from 14% (≥3 peaks and 2 troughs) to 18% (≥2 peaks and 1 trough) depending on diagnostic criteria. However, data reporting from previously published case reports and series was often imprecise or uncomplete, impeding detailed analysis of cycle patterns. To overcome this limitation and to guide future diagnostic strategies, we conducted the first international and largest observational study on cCS to date, including previously unpublished cases from expert endocrine and pituitary centers.

Added value of this study

This study analyzed 110 previously unpublished cCS cases, enabling for the first time a comparative assessment of cycle characteristics across different CS etiologies. Patients with ectopic or occult cCS exhibited the most pronounced cortisol elevations and the greatest biochemical deltas between peaks and troughs, alongside the highest rates of hypokalemia compared to pituitary and adrenal cCS. Ectopic cCS also had the highest annual peak frequency, with a median of 1·80 (IQR 1·05–11·3) peaks per year. Across all etiologies, spontaneous adrenal insufficiency occurred in 28% (31/110) of patients, a rate higher than previously recognized, likely reflecting corticotroph suppression during prolonged troughs. Among these, six patients experienced spontaneous adrenal crises. We also identified a higher rate of inappropriate surgeries (9/110, 8%) in patients with cCS due to misleading findings than previously reported. With 41% (45/110) of patients experiencing a delayed diagnosis, and 43% (47/110) receiving delayed therapy, only 50% (55/110) achieved complete therapy-induced biochemical remission after a median follow-up of 5·8 years.

Implications of all available evidence

Our data suggest, that although there is considerable variability both within and between individuals, cyclicity may be more frequent and/or more biochemically pronounced in patients with ectopic compared to other forms of cCS. These observations could guide diagnostic procedures, such as the choice of imaging regions or timing of bilateral inferior petrosal sinus sampling, in patients with occult ACTH-dependent cCS. Confirmation of biochemical cortisol excess prior to inferior petrosal sinus

sampling (e.g., through late night salivary cortisol and/or urinary free cortisol measurements) is essential to enable accurate tumor localization. As most patients experience symptom fluctuation in line with cortisol cycling, we recommend equipping patients suspected of having cCS with salivary cortisol collection kits to capture dynamic changes. Given the high rate of spontaneous adrenal insufficiencies, we furthermore advise that these patients be educated about this risk and equipped with glucocorticoid replacement as a precaution. While prospective studies are underway to define optimal diagnostic timelines, our study lays the groundwork for future molecular investigations. Eventually increased awareness of a cyclic phenotype and improved technologies for more frequent, and ideally continuous cortisol monitoring will improve diagnostic accuracy and consequently therapeutic care.

Introduction

Cushing's syndrome (CS) is a severe endocrine disorder caused by endogenous hypercortisolism, typically due to a benign endocrine tumor. The most frequent form is Cushing's disease, caused by a pituitary adenoma secreting adrenocorticotropic hormone (ACTH), followed by adrenal CS (ACTH-independent) and ectopic CS where ACTH is secreted from a non-pituitary site[1]. In some cases, the source of ACTH hypersecretion is never found (occult). CS is associated with various metabolic and cardiovascular comorbidities and can be fatal if left untreated[2-5]. Although intraindividual variability of glucocorticoid excess has been described in all types of overt CS[6-8], so-called "cyclic Cushing syndrome" (cCS) refers to a subgroup of patients in whom phases of glucocorticoid excess (peaks) alternate with spontaneous phases of low or normal cortisol concentrations (troughs)[9]. First described in 1966[10], cCS has gained increasing recognition since[9,11-14]. Depending on the trough duration, which may last several years, clinical signs of CS can improve or even vanish[15]. In a recent systematic review of 212 cCS cases[15], we identified that cCS may be present in 7-21% of individuals with pituitary CS and in up to 26% of those with micronodular adrenal hyperplasia. Depending on the diagnostic criteria, the prevalence ranges from 14% (≥3 peaks and 2 troughs) to 18% (≥2 peaks and 1 trough)[15]. Due to pronounced cortisol fluctuations, diagnosing cCS is challenging, often delaying diagnosis and treatment compared to "non-cyclic" CS[15].

The switch between hyper- and eu/hypocortisolism appears spontaneously, and the underlying mechanisms are usually not identifiable in individual patients. Potential mechanisms include microhemorrhages, hypothalamic dysregulation, neurotransmitters, and variable feedback disruptions[15].

We aimed to identify previously unpublished cases of cCS to identify common challenges in expert clinical care and define strategies to improve long-term outcomes. Specifically, we sought to characterize clinical and biochemical features, assess peak frequency, and evaluate the impact of cCS diagnosis on complications and overall outcomes.

Methods

Study design and ethical approval

This was an international retrospective cohort study of patients with cCS, approved by the local ethics committee of the leading study center (LMU Hospital Munich; study number: 23-0786) and local institutional review boards. Each participating center also obtained appropriate local approval for data collection and sharing. Written informed consent was obtained unless waived by local regulations. Centers were invited via e-mail, national and international conferences, and public calls for eligible cases during these meetings.

Data collection, quality control and definition of outcome variables

Data collection was performed between December 2023 and February 2025, until a minimum of 100 patients had been enrolled. To ensure data quality, a standardized, pre-defined data collection spreadsheet was used across all participating centers (appendix p22-25). Data were extracted locally from electronic patient records. Key outcome variables were clearly defined, and biochemical data were documented alongside measurement units and local reference ranges. Missing data were systematically categorized as not available, not applicable, or lost to follow-up. The lead author conducted consistency checks across variables and issued data queries when entries were imprecise or required clarification. Outcomes were documented and reported following the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology, https://www.strobe-statement.org/) guidelines. Remission was categorized as complete therapy-induced (i.e. prolonged adrenal insufficiency or response to the 1 mg dexamethasone suppression test (DST) <50 nmol/L following successful tumor resection, targeted radiotherapy, or bilateral adrenalectomy), partial (improvement without full cortisol normalization), and spontaneous (same biochemical criteria as complete remission, without therapeutic intervention). Spontaneous adrenal insufficiency was defined as low morning cortisol and/or need for glucocorticoid supplementation without prior intervention. Adrenal crises were defined according to the criteria established by Bruno Allolio[16]. Patients on medical therapy were considered biochemically controlled if 24-hour urinary free cortisol (UFC) was within the normal range.

Definition and subtyping of cyclic Cushing's syndrome

CS was diagnosed and subtyped according to guideline recommendations[18,19]. In detail, Cushing's disease was diagnosed based on at least one of the following: histopathological confirmation, a positive ACTH gradient on BIPSS and/or a positive confirmatory test (CRH and/or desmopressin stimulation and/or 8 mg DST) during biochemical hypercortisolism. Ectopic CS was diagnosed based on histopathological confirmation and/or remission after NET surgery, or – if occult on imaging – on the absence of an ACTH gradient on BIPSS during hypercortisolism. BIPSS results were considered diagnostic only if performed during hypercortisolism and with adequate catheter placement. Occult ACTH-dependent CS was diagnosed in patients with confirmed CS, in whom the ACTH-source

remained undetermined. ACTH-independent adrenal CS was diagnosed based on low/suppressed ACTH concentrations during peaks as well as positive adrenal imaging and/or histopathological confirmation. cCS was subsequently diagnosed in patients who had experienced at least two spontaneous phases of cortisol excess ("peaks") separated by one spontaneous (i.e., without intervention) phase of low or normal cortisol concentrations ("troughs"). Although diagnostic criteria vary, this study used a uniform definition as above and both peaks and troughs were documented by at least two biochemical tests performed on at least two separate occasions. A cycle was defined as the period spanning one peak and one trough. Accepted biochemical tests included morning and midnight serum cortisol, UFC, late night salivary cortisol (LNSC), hair cortisol, and the 1 mg DST. Plasma ACTH obtained during hypercortisolism distinguished ACTH-dependent from -independent CS. Biochemical data were documented alongside their local reference ranges and reported as multiples the upper limit of normal (ULN).

Statistical analysis

Statistical analysis was performed with Graph Pad Prism Version 10.4. Continuous variables were presented as median with interquartile range (IQR) or full ranges, while categorical variables were expressed as percentages. Percentages were reported alongside the number of individuals with available data. Binary outcomes were analyzed using the Fisher's exact test. Non-normally distributed continuous data were analyzed using the Kruskal-Wallis test for independent group comparisons and the Mann-Whitney test for pairwise comparisons. Non-parametric Spearman correlation was used to assess the association between peak frequency and measures of cortisol excess. A p-value of $<0{\cdot}05$ was considered statistically significant, and no adjustments were made for potential confounders.

Funding source

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Results

Baseline characteristics and tumor origin

Of 116 potentially eligible patients, 110 from 45 centers in 21 countries were included. Six patients were excluded (five due to only one spontaneous peak and trough; one due to suspected non-neoplastic CS). Center-specific patient numbers and ethnicity are shown in the appendix (**p2**). Patients were diagnosed between 1985 and 2024 with more frequent detection in recent years (**appendix p3**). Overall, 107 patients (107/110, 97%) had ACTH-dependent CS, while three (3/110, 3%) patients had ACTH-independent CS (**Table 1**). Pituitary cCS was diagnosed in 70 patients (70/110, 64%), with histological confirmation in 53 (53/70, 76%). Ectopic cCS was diagnosed in 25 patients (25/110, 23%): 8 pulmonary, 6 thymic, 2 pancreatic, 1 small bowel NET, and 8 of unidentified ectopic sources. Additionally, 12 patients (11%, 12/110) had ACTH-dependent tumors of unknown origin that could not be classified as pituitary or ectopic (i.e., occult). Among the three adrenal cCS cases, all had bilateral adrenal macroadenomas. Most patients were female (84/110, 76%), with a median age at diagnosis of 44·0 years ([31·8–58·3]). Typical CS-related comorbidities were present in most patients. Median BMI at first evaluation was 29·3 kg/m^2 (IQR 25·4-33·2), with 69% (72/105) classified as obese (BMI >30 kg/m^2). At diagnosis, 79% (86/109) had hypertension, 38% (41/108) had diabetes, and 35% (36/102) had osteoporosis (T-score < -2.5), with 22% (24/110) having experienced at least one pathological fracture. Depression was reported in 32% (35/110) of patients. Severe or opportunistic infections had occurred in 15% (17/110), and 14% (15/110) had experienced at least one thromboembolic event (**Table 1**).

Biochemical characterization of peaks and troughs and correspondence of clinical signs and symptoms

Biochemical data were analyzed at referrals (**appendix p4 and 14-15**), and during maximum peaks and minimum troughs (**Figures 1A-B and appendix p5-6**). 24h-UFC and LNSC were the main biochemical tests used to assess cortisol excess and periodicity. Overall, 24h-UFC ranged from a median of 7·40 x ULN (range 0·44-299) during peaks to 0·31 x ULN (range 0·02-0·98) during troughs. Likewise, LNSC ranged from 5·74 x ULN (range 0·11-2831) during peaks to 0·46 x ULN (range 0·05-7·89) during troughs (**appendix p5-6**). Patients with ectopic and occult cCS showed the highest cortisol elevations (**Figure 1A**) and largest biochemical deltas between peaks and troughs (**appendix p7**). They were also associated with the highest rates of hypokalemia (ectopic cCS: 22/25, 88%; occult cCS: 8/11 with available data, 73%) compared with pituitary (21/66 with available data, 32%) and adrenal cCS (0/3, 0%). Overall, spontaneous adrenal insufficiency (i.e., without medical/surgical intervention) were documented in 31 cases (31/110, 28%). They occurred in 26% of patients with pituitary cCS (18/70), 28% of patients with ectopic cCS (7/25) and 50% of those with occult cCS (6/12). None was documented in patients with adrenal cCS. Spontaneous adrenal crises occurred in six patients (3 ectopic, 2 occult, 1 pituitary).

During an overall median observation period of 3·4 years (IQR 1·5-8·1) until either therapy-induced remission or the latest follow-up, patients experienced a median of 3·0 (IQR 2·0-4·0) documented hypercortisolemic peaks with higher frequency observed in ACTH-dependent versus ACTH-independent CS (**appendix p8**). In relation to their observation period, patients with ectopic cCS had the highest frequency of peaks per year (1·80 [IQR 1·05-11·3]), followed by occult (1·45 [IQR 0·82-2·68]) and pituitary cCS (0·70 [IQR 0·30-1·78]; **Figure 2A-C and appendix p8**. There was a weak positive correlation between peak frequency and degree of cortisol excess during peaks, with the strongest association observed for LNSC (r=0.49 [95% CI 0.26-0.67]; p<0.0001; **appendix p9**). Cycle regularity was assessed in 64 patients with ≥3 documented peaks separated by spontaneous troughs. Of these, 55 (55/64, 86%) had irregular trough durations between peaks, ranging from days to years. Due to the retrospective design, individual test frequencies and the common use of medical therapy, a detailed analysis of duration of peaks and troughs was not feasible. However, centers were able to classify the trough durations into rough time intervals, namely days, weeks, months, or years during which biochemical tests were repeatedly negative. Most patients (61/110, 55%) experienced trough durations lasting several months. Very rapid cycles with trough durations of only days occurred in 7 patients with pituitary cCS and two patients with ectopic and occult cCS, respectively. Twelve patients had troughs lasting weeks, while 25 experienced trough durations of at least one year (16 pituitary, four ectopic, three occult, and two adrenal cCS). The longest interval (10 years) was seen in a female patient with a pituitary microadenoma.

All patients showed typical CS signs during peaks. While weight gain, muscle weakness and plethora usually worsened during peaks and improved during troughs, central obesity, a round face, and dorsocervical fat pad were most often present regardless of the cycling phase (**appendix p10**). Overall, 81% (87/108) of patients reported worsening of at least one clinical symptom during peaks, while 74% (79/107) experienced symptom improvement during troughs.

Imaging characteristics

Imaging was considered diagnostic for CS localization in 75 cases (75/110, 68%). Amongst patients with pituitary cCS, 40 (40/70, 57%) had a microadenoma, 15 (15/70, 21%) a macroadenoma, and no lesion was visible in 15 patients (15/70, 21%). Of the 25 patients with ectopic tumor origin, a suspicious lesion was detected in 18 cases (18/25, 72%). In ACTH-dependent cCS, bilateral adrenal enlargement was described in 33% (19/57) of pituitary, 38% (9/24) of ectopic, and 50% (6/12) of occult cCS.

Bilateral inferior petrosal sinus sampling (BIPSS)

BIPSS is an invasive diagnostic procedure used to determine the source of excess ACTH production in patients with ACTH-dependent CS. It involves inserting catheters into the inferior petrosal sinuses and collecting blood samples from both the central (pituitary) and peripheral circulation, typically before and after stimulation with corticotropin-releasing hormone or desmopressin. A central-to-peripheral

ACTH gradient of <2 at baseline and <3 after stimulation suggests an ectopic source; however, false-negative and false-positive results have been reported[2,19]. In total, 78 BIPSS were performed in 67 patients with ACTH-dependent CS (67/107, 61%), including 11 patients who underwent the procedure twice as they were not hypercortisolemic at the time of their first BIPSS. The majority (64/78, 82%) were conducted during biochemical hypercortisolism, while 14 (14/78, 18%) were performed during a trough.

To assess the diagnostic accuracy of BIPSS, a subanalysis was conducted in patients with histological tumor proof upon surgery (**Table 2**). Among them, 37 patients (28 pituitary, 9 ectopic) underwent 41 BIPSS procedures, with four patients with pituitary cCS undergoing the test twice. Two BIPSS were deemed non-diagnostic (e.g. due to anatomical vessel variants), leaving 39 for analysis (**Table 2**). Of these, 33 were performed during a biochemical peak and six during a trough. Regardless of the timing, this resulted in an overall positive predictive value for true pituitary origin of 96% and a negative predictive value for true ectopic origin of 67%, with better performance during peaks. During biochemical troughs, four BIPSS showed low ACTH concentrations that did not respond to stimulation despite a true pituitary origin, falsely suggesting an ectopic source. Of those performed during a peak, only one BIPSS was misleading: In a patient with a histologically confirmed thymic NET, BIPSS incorrectly indicated a pituitary source. Ectopic CRH production was suspected but could not be proven.

Therapeutic interventions and histopathological findings

Therapeutic interventions are detailed in the appendix (**p11-13**). Most patients with pituitary cCS (63/70, 90%) underwent transsphenoidal surgery (TSS). Among these, cyclicity was diagnosed prior to TSS in 36 cases (36/63, 57%) and after TSS in 27 cases (27/63, 43%). Only 56% (14/25) of patients with ectopic cCS underwent ectopic tumor resection. Histopathological evidence of tumor infarction or necrosis was reported in just two cases of pituitary cCS and one case of ectopic cCS caused by a thymic NET (an atypical carcinoid with 4-6 mitoses per 10 high-power fields, with necrosis being focal and not extensive). Overall, 13 patients (13/110, 12%) underwent bilateral adrenalectomy, including four (4/13, 31%) with pituitary and nine (9/13, 69%) with an ectopic or occult tumor source. Tumor targeted radiotherapy was administered to 14 patients with pituitary and six patients with ectopic cCS. Adrenal steroidogenesis inhibitors were used in 60 patients (60/110, 54%). The majority of these (38/60, 63%) received a titration regimen, while a block-and-replace approach was used in nine patients (9/60, 15%). In 13 patients (13/60, 22%), both regimens were applied at different times.

Follow-up and outcomes

After a median follow-up duration of 5·8 years (IQR 2·6-10·5) from first to last documented visit, three patients with ectopic cCS had died – one from pneumonia, one from pneumonia and heart failure (both during hypercortisolism before curative surgery or bilateral adrenalectomy could be performed), and one from multi-organ failure due to metastatic thymic NET (after bilateral adrenalectomy, but unrelated

to adrenal insufficiency). Clinical and biochemical outcomes are shown in **Figures 3A-B** and in the appendix (**p11-13)**. Overall, 55 patients (55/110, 50%) had achieved multimodal complete therapy-induced biochemical remission, and seven patients (7/110, 6%) went into spontaneous remission. Eleven patients (11/110, 10%) were biochemically uncontrolled at last follow-up.

Diagnostic challenges and adverse and unanticipated events

Diagnostic challenges regarded the timing of BIPSS, with 18% (14/76) of inconclusive or potentially misleading results due to testing during a trough. Overall, nine patients (9/110, 8%) underwent unwarranted surgeries at the wrong anatomical site due to misclassification of the tumor origin based on misleading imaging and/or BIPSS results. This included five patients with ectopic cCS who underwent inappropriate removal of incidental pituitary microadenomas, and three patients with occult (but later suspected ectopic) cCS who all had inappropriate pituitary surgery – one of them even twice. One of these patients also underwent surgery to remove a peri-carotid paraganglioma, which was not the source of ACTH. Additionally, one patient with pituitary cCS underwent an inappropriate unilateral adrenalectomy due to initial suspicion of ACTH-independent adrenal disease.

Further adverse events included the occurrence of unexpected spontaneous episodes of adrenal insufficiency in 31 cases (31/110, 28%), requiring glucocorticoid supplementation. Notably, six of these patients experienced spontaneous adrenal crises. Overall, centers reported a delayed diagnosis of CS in 45 cases (45/110, 41%), and a delayed initiation of targeted therapy in 47 cases (47/110, 43%), based on self-assessments informed by their clinical experience with potential diagnostic delays in CS in general (**appendix p11-13**).

Discussion

Here, we present the largest and first international cohort of patients with cCS, highlighting the considerable inter- and intraindividual variability in cyclicity. Furthermore, we report substantial diagnostic and therapeutic challenges as encountered by expert endocrine and pituitary centers.

We observed a high prevalence of cardiovascular (arterial hypertension in 86/110, 79%, thromboembolic events in 15/110, 14%), metabolic (obesity in 72/105, 69%, diabetes in 41/108, 38%), psychiatric (35/110, 32%), and infectious (17/110, 16%) comorbidities in our study, consistent with literature findings in patients with CS in general[1,2]. This underscores that cortisol excess, even if only present intermittently or for a limited duration, is associated with a substantial disease burden that requires targeted and effective treatment. However, due to diagnostic challenges in cCS, 43% (47/110) of patients had treatment delays and more noteworthy, 8% (9/110) underwent unwarranted surgery at the wrong anatomical site. This resulted in a poor overall outcome with only 50% (55/110) achieving therapy-induced biochemical remission at 5·8 years. In comparison, remission rates after primary TSS in patients with non-cyclic Cushing disease range between 76-80%[20-22]. A recent study of patients with non-cyclic CS due to different etiologies demonstrated that after a median follow-up of 6·3 years biochemical control was achieved in 93% of patients[23]. The comparatively low remission rate in our cohort likely reflects multiple contributing factors, including diagnostic challenges and prolonged trough durations. Despite this, overall mortality was low, with only two patients dying from CS-related complications (pneumonia).Variability of cortisol secretion is common, if not omnipresent, in conditions of cortisol excess, yet no optimal measurement method exists[24]. Our study is the first to compare cycle patterns across different cCS etiologies. We observed cortisol peaks to be the most frequent and severe in ectopic cCS, less so in pituitary, and least in adrenal cases. These findings are essential when planning diagnostic procedures (e.g., by considering the frequency, duration and severity of peaks when performing BIPSS) or evaluating medical therapy response. Notably, occult ectopic sources were slightly more common in cCS (8/25, 32%) than previously reported in non-cyclic ectopic CS (18·6-20%)[25,26]. In patients with non-cyclic pituitary CS, false negative rates of BIPSS range from 1-10%[27]. False positive and negative findings occur not only related to the overall amplitude of hypercortisolism but importantly, also the duration of cortisol excess[28]. Since the diagnostic accuracy of BIPSS is (consequently) even lower during troughs in patients with cCS, it is important to ensure that cortisol excess is still present at the time of BIPSS[19] to avoid misdiagnosis and repeat invasive procedures. As per expert consensus on Cushing's disease[19], we recommend assessing for hypercortisolism presence (LNSC and/or UFC, depending on availability and turnaround time), shortly before BIPSS. Ideally, morning serum cortisol concentrations on the day of the procedure should also fall within previously observed cortisol excess ranges. However, as a recent onset of hypercortisolism may not allow sufficient time for full hypothalamic-pituitary-adrenal axis suppression, clinical judgment is required when evaluating biochemical results in this context. Importantly, standard diagnostic criteria for Cushing disease –

including suppression/stimulation tests and BIPSS – may be unreliable, as intermittent or recently developed hypercortisolism can result in dynamic test responses that overlap with normal individuals or ectopic ACTH secretion. Thus, all such test results should be interpreted with caution in this setting, and the diagnosis should be supported by repeated biochemical confirmation and careful clinical correlation. If hypercortisolism can be biochemically confirmed on separate occasions (thereby confirming a longer duration), this may increase the likelihood that BIPSS will yield accurate and reliable results. While very highly elevated 24-UFC can accurately distinguish an ectopic from a pituitary source in most patients with non-cyclic CS[29], our findings show for the first time that a similar observation may apply to the management of cCS. Notably, all median cortisol markers – not just median 24h-UFC – were substantially higher in ectopic than in pituitary and adrenal cCS. However, there was a considerable overlap between groups: 79% (19/24 ectopic patients with available UFC data during peaks) had values that overlapped with those observed in pituitary cCS. While 24h-UFC normalized across all entities during biochemical troughs, other biochemical parameters did not always follow, as they only reflect cortisol concentrations at one specific time point. These observations demonstrate the need for repeated biochemical testing and the combination of different methods to obtain accurate information. They also suggest that remission may not have been complete but only partial in some cases. Biochemical cortisol fluctuations often, but not always, aligned with changes in clinical presentation or comorbidity severity. We recommend, therefore, that patients with suspected or confirmed cCS be provided with saliva tubes for LNSC assessment, as it could be particularly suited for detecting short-term fluctuations, especially when CS-related symptoms are worsening. Hair cortisol, on the other hand, may be useful for identifying long-term variations and as a proof-of-concept tool[32].

It is important to consider that the actual peak frequency is likely much higher than reported here. Frequent use of medical therapy, surgical interventions, and the lack of continuous monitoring likely contribute to an underestimation of cycle frequency in our study. Furthermore, we might have to distinguish between patients diagnosed with cyclicity prior to any interventional therapy and those diagnosed afterward. In our cohort, cyclicity was diagnosed postoperatively in 43% of patients with pituitary cCS undergoing TSS. While there is no causal evidence to explain this observation, frequent postoperative biochemical monitoring to assess therapeutic success likely results in higher detection rates of cyclicity.

Another novel finding of our study pertains to a much higher prevalence of spontaneous adrenal insufficiency and even adrenal crisis, likely resulting from suppressed normal corticotrophs, compared to previously reported rates[15]. This discrepancy is presumably due to the more comprehensive data documentation of our well-characterized cohort presented here and a more unified definition. Based on these findings we advise that all patients with cCS be informed about the risk of spontaneous adrenal insufficiency and provided with glucocorticoid replacement as a precaution as well as injectable glucocorticoids for emergencies at home. A low morning serum cortisol (<138-150 nmol/L [<5 µg/dL])

may indicate adrenal insufficiency; however these values should be interpreted as part of a continuum rather than as strict cut-offs, and additional information such as clinical symptoms or results from dynamic testing may aid in diagnosis[33,34]. Whenever medical therapy is adopted in a block-and-replace approach, steroidogenesis inhibitors should be administered at doses sufficient to completely block the adrenal secretion and adjust glucocorticoid doses accordingly to clinical status[35,36]. A titration regimen could be reserved for patients with mild cortisol excess or those unable or unwilling to take additional drugs, however risk of adrenal insufficiency is higher in patients with cCS as demonstrated here.

Our study has several strengths. First, it is the largest and most comprehensively described cohort of patients with cCS to date. Second, it includes all cases from 45 expert endocrine centers, with large international representation across 21 countries on five continents over a diagnostic time frame of 39 years. Third, the study design, which required repeated clinical and biochemical assessments, increased the chances of only including cases with "true" cyclicity. Fourth, the cases are more recent than published monocentric series that include old cases with different diagnostic and therapeutic regimens[14]. Finally, our findings contribute to raise awareness of a cyclic phenotype and should therefore improve care for these patients.

Limitations of our study relate to the retrospective design which precluded a detailed analysis of trough durations and could lead to data bias due to variations in test frequency, methodologies, and overall data quality. However, data consistency was reviewed by the lead author, and additional inquiries were made to each center when necessary. These limitations can only be overcome by multicenter prospective observational studies with frequent, and ideally continuous, biochemical monitoring which can also allow estimating the true prevalence and incidence of cCS. Further limitations include the small number of patients with adrenal cCS, lack of standardized imaging data, and absence of adjustment for multiple testing or potential confounders. Delayed diagnoses and treatment initiation were based on self-assessments by individual centers and should therefore be interpreted with caution, as no formal analysis using reference standards was conducted. Adrenal insufficiency in some patients who ever received medical therapy could have been related to other causes, not just cCS, including prolonged and/or delayed effects of adrenal steroidogenesis inhibitors[1,2,37] or genetic differences in response to medications[38]. Finally, the absence of a contemporary control cohort with "non-cyclic" CS, along with the absence of data on the overall number of patients with CS at each center, limits both the interpretation of cCS-specific features and the ability to estimate its prevalence. In conclusion, our large international patient cohort shows that cCS is associated with a high burden of cortisol-related comorbidities, a substantial risk of spontaneous adrenal insufficiency, a high rate of inappropriate surgeries and repeated invasive procedures, a delay in diagnosis and therapeutic interventions, and overall poorer outcomes. Although cyclicity varies significantly within and between individuals, cycles may be more frequent and/or more biochemically pronounced in patients with ectopic compared to pituitary cCS. These observations, synthesizing collective knowledge from prior experiences to advance understanding,

389 could be useful to guide diagnostic procedures in patients with occult ACTH-dependent cCS. Increased
390 awareness of a cyclic phenotype, along with ongoing research into technologies aiming to enable more
391 frequent – and ideally continuous – cortisol monitoring, such as current investigations into wearable
392 devices[39,40], may support future improvements in diagnostic accuracy and consequently therapeutic care.

Data sharing

De-identified individual participant data underlying the results reported here may be made available upon reasonable request. Requests should be directed to the corresponding author accompanied by a detailed study hypothesis and statistical analysis plan. The corresponding author and lead investigators will review each request to assess its scientific value before approving data access. Approved applicants will be required to sign a data access agreement.

Figure Legends

Figure 1A-B. Maximum and minimum documented biochemical values during the evaluation of cortisol excess (i.e. peaks, A) and normal/low cortisol concentrations (i.e. troughs, B).

All biochemical results are expressed as x-times upper limit of normal (ULN). Response to the 1 mg dexamethasone suppression test (DST) is additionally expressed in SI units. The black horizontal bars on top of the graphs refer to the Kruskal-Wallis test for the overall group comparisons with the asterisks indicating statistically significant findings (****$p<0.0001$, ***<0.001, ns, not significant). The colored horizontal bars within the data points represent medians with extensions indicating the interquartile range (IQR). The dotted line indicates the upper limit of normal (i.e., 1) in all graphs, except for DST values expressed in nmol/L, where it corresponds to 50 nmol/L. In adrenal Cushing's syndrome ACTH concentrations are suppressed (lower) during peaks and higher during troughs. Abbreviations: ACTH, adrenocorticotropic hormone; Adr, Adrenal Cushing's syndrome; DST, dexamethasone suppression test; Ect, ectopic Cushing's syndrome; IQR, interquartile range; LNSC, late night salivary cortisol; Occ, occult Cushing's syndrome; Pit, pituitary Cushing's syndrome; UFC, urinary free cortisol; ULN, upper limit of normal.

Figure 2A-C. Cycle frequency per patient group.

Peaks per year of observation referring to the observation period until therapy-induced remission or the latest follow-up. The dotted line indicates one peak per year. Kruskal-Wallis test for comparison of all four groups with asterisks indicating statistically significant findings ($p<0.05$). A: peaks per observation year in the entire cohort. B: Zoom for differentiation of cycle characterization in the lower (0-5) range. C: Cycle frequency per patient group. Abbreviations: Adr, Adrenal Cushing's syndrome; Ect, ectopic Cushing's syndrome; Occ, occult Cushing's syndrome; Pit, pituitary Cushing's syndrome.

Figure 3A-B. Clinical (A) and biochemical (B) outcomes at the latest available follow-up.

Results are expressed as percentages out of the respective entities. Abbreviations: Adr, Adrenal Cushing's syndrome; All, entire cohort; Ect, ectopic Cushing's syndrome; Occ, occult Cushing's syndrome¸ Pit, pituitary Cushing's syndrome.

References

1. Reincke M, Fleseriu M. Cushing Syndrome: A Review. *JAMA*. Jul 11 2023;330(2):170-181. doi:10.1001/jama.2023.11305

2. Gadelha M, Gatto F, Wildemberg LE, Fleseriu M. Cushing's syndrome. *Lancet*. Dec 9 2023;402(10418):2237-2252. doi:10.1016/S0140-6736(23)01961-X

3. Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. *Lancet*. Aug 29 2015;386(9996):913-27. doi:10.1016/S0140-6736(14)61375-1

4. Valassi E, Tabarin A, Brue T, et al. High mortality within 90 days of diagnosis in patients with Cushing's syndrome: results from the ERCUSYN registry. *Eur J Endocrinol*. Nov 2019;181(5):461-472. doi:10.1530/EJE-19-0464

5. Clayton RN, Jones PW, Reulen RC, et al. Mortality in patients with Cushing's disease more than 10 years after remission: a multicentre, multinational, retrospective cohort study. *Lancet Diabetes Endocrinol*. Jul 2016;4(7):569-76. doi:10.1016/S2213-8587(16)30005-5

6. Petersenn S, Newell-Price J, Findling JW, et al. High variability in baseline urinary free cortisol values in patients with Cushing's disease. *Clin Endocrinol (Oxf)*. Feb 2014;80(2):261-9. doi:10.1111/cen.12259

7. Jahandideh D, Swearingen B, Nachtigall LB, Klibanski A, Biller BMK, Tritos NA. Characterization of cyclic Cushing's disease using late night salivary cortisol testing. *Clin Endocrinol (Oxf)*. Sep 2018;89(3):336-345. doi:10.1111/cen.13758

8. Shapiro MS, Shenkman L. Variable hormonogenesis in Cushing's syndrome. *Q J Med*. Apr 1991;79(288):351-63.

9. Meinardi JR, Wolffenbuttel BH, Dullaart RP. Cyclic Cushing's syndrome: a clinical challenge. *Eur J Endocrinol*. Sep 2007;157(3):245-54. doi:10.1530/EJE-07-0262

10. Brooks RV, Jeffcoate SL, London DR, Prunty FT, Smith PM. Intermittent Cushing's syndrome with anomalous response to dexamethasone. *J Endocrinol*. Sep 1966;36(1):53-61. doi:10.1677/joe.0.0360053

11. Bailey RE. Periodic hormonogenesis--a new phenomenon. Periodicity in function of a hormone-producing tumor in man. *J Clin Endocrinol Metab*. Mar 1971;32(3):317-27. doi:10.1210/jcem-32-3-317

12. Atkinson AB, Kennedy AL, Carson DJ, Hadden DR, Weaver JA, Sheridan B. Five cases of cyclical Cushing's syndrome. *Br Med J (Clin Res Ed)*. Nov 23 1985;291(6507):1453-7. doi:10.1136/bmj.291.6507.1453

13. Atkinson AB, McCance DR, Kennedy L, Sheridan B. Cyclical Cushing's syndrome first diagnosed after pituitary surgery: a trap for the unwary. *Clin Endocrinol (Oxf)*. Mar 1992;36(3):297-9. doi:10.1111/j.1365-2265.1992.tb01447.x

14. Alexandraki KI, Kaltsas GA, Isidori AM, et al. The prevalence and characteristic features of cyclicity and variability in Cushing's disease. *Eur J Endocrinol*. Jun 2009;160(6):1011-8. doi:10.1530/EJE-09-0046

15. Nowak E, Vogel F, Albani A, et al. Diagnostic challenges in cyclic Cushing's syndrome: a systematic review. *Lancet Diabetes Endocrinol*. Aug 2023;11(8):593-606. doi:10.1016/S2213-8587(23)00150-X

16. Allolio B. Extensive expertise in endocrinology. Adrenal crisis. *Eur J Endocrinol*. Mar 2015;172(3):R115-24. doi:10.1530/EJE-14-0824

17. Kienitz T, Bechmann N, Deutschbein T, et al. Adrenal Crisis - Definition, Prevention and Treatment: Results from a Delphi Survey. *Horm Metab Res*. Jan 2024;56(1):10-15. doi:10.1055/a-2130-1938

18. Nieman LK, Biller BM, Findling JW, et al. Treatment of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. Aug 2015;100(8):2807-31. doi:10.1210/jc.2015-1818

19. Fleseriu M, Auchus R, Bancos I, et al. Consensus on diagnosis and management of Cushing's disease: a guideline update. *Lancet Diabetes Endocrinol*. Dec 2021;9(12):847-875. doi:10.1016/S2213-8587(21)00235-7

20. Petersenn S, Beckers A, Ferone D, et al. Therapy of endocrine disease: outcomes in patients with Cushing's disease undergoing transsphenoidal surgery: systematic review assessing criteria used to define remission and recurrence. *Eur J Endocrinol*. Jun 2015;172(6):R227-39. doi:10.1530/EJE-14-0883

21. Abu Dabrh AM, Singh Ospina NM, Al Nofal A, et al. Predictors of Biochemical Remission and Recurrence after Surgical and Radiation Treatments of Cushing Disease: A Systematic Review and Meta-Analysis. *Endocr Pract*. Apr 2016;22(4):466-75. doi:10.4158/EP15922.RA

22. Stroud A, Dhaliwal P, Alvarado R, et al. Outcomes of pituitary surgery for Cushing's disease: a systematic review and meta-analysis. *Pituitary*. Oct 2020;23(5):595-609. doi:10.1007/s11102-020-01066-8

23. Ritzel K, Fazel J, August L, et al. Biochemical Control in Cushing's Syndrome: Outcomes of the Treatment in a Large Single Center Cohort. *J Clin Endocrinol Metab*. Mar 17 2025;110(4):e1038-e1045. doi:10.1210/clinem/dgae337

24. Clarke SA, Eng PC, Comninos AN, et al. Current Challenges and Future Directions in the Assessment of Glucocorticoid Status. *Endocr Rev*. Nov 22 2024;45(6):795-817. doi:10.1210/endrev/bnae016

25. Isidori AM, Kaltsas GA, Pozza C, et al. The ectopic adrenocorticotropin syndrome: clinical features, diagnosis, management, and long-term follow-up. *J Clin Endocrinol Metab*. Feb 2006;91(2):371-7. doi:10.1210/jc.2005-1542

26. Isidori AM, Sbardella E, Zatelli MC, et al. Conventional and Nuclear Medicine Imaging in Ectopic Cushing's Syndrome: A Systematic Review. *J Clin Endocrinol Metab*. Sep 2015;100(9):3231-44. doi:10.1210/JC.2015-1589

27. Findling JW, Kehoe ME, Raff H. Identification of patients with Cushing's disease with negative pituitary adrenocorticotropin gradients during inferior petrosal sinus sampling: prolactin as an index of pituitary venous effluent. *J Clin Endocrinol Metab*. Dec 2004;89(12):6005-9. doi:10.1210/jc.2004-1378

28. Yanovski JAMD, Ph.D.; Cutler, Gordon B. Jr. Pitfalls in the Use of Inferior Petrosal Sinus Sampling for the Differential Diagnosis of ACTH-Dependent Cushing's Syndrome. *The Endocrinologist*. 1994:;4(4):245-251.

29. Lavoillotte J, Mohammedi K, Salenave S, et al. Personalized Noninvasive Diagnostic Algorithms Based on Urinary Free Cortisol in ACTH-dependant Cushing's Syndrome. *J Clin Endocrinol Metab*. Oct 15 2024;109(11):2882-2891. doi:10.1210/clinem/dgae258

30. van der Pas R, de Bruin C, Pereira AM, et al. Cortisol diurnal rhythm and quality of life after successful medical treatment of Cushing's disease. *Pituitary*. Dec 2013;16(4):536-44. doi:10.1007/s11102-012-0452-2

31. Debono M, Harrison RF, Chadarevian R, Gueroult C, Abitbol JL, Newell-Price J. Resetting the Abnormal Circadian Cortisol Rhythm in Adrenal Incidentaloma Patients With Mild Autonomous Cortisol Secretion. *J Clin Endocrinol Metab*. Sep 1 2017;102(9):3461-3469. doi:10.1210/jc.2017-00823

32. Manenschijn L, Koper JW, van den Akker EL, et al. A novel tool in the diagnosis and follow-up of (cyclic) Cushing's syndrome: measurement of long-term cortisol in scalp hair. *J Clin Endocrinol Metab*. Oct 2012;97(10):E1836-43. doi:10.1210/jc.2012-1852

33. Kazlauskaite R, Evans AT, Villabona CV, et al. Corticotropin tests for hypothalamic-pituitary- adrenal insufficiency: a metaanalysis. *J Clin Endocrinol Metab*. Nov 2008;93(11):4245-53. doi:10.1210/jc.2008-0710

34. Beuschlein F, Else T, Bancos I, et al. European Society of Endocrinology and Endocrine Society Joint Clinical Guideline: Diagnosis and Therapy of Glucocorticoid-induced Adrenal

Insufficiency. *J Clin Endocrinol Metab*. Jun 17 2024;109(7):1657-1683. doi:10.1210/clinem/dgae250

35. Castinetti F. Pharmacological Treatment of Cushing's Syndrome. *Arch Med Res*. Dec 2023;54(8):102908. doi:10.1016/j.arcmed.2023.102908

36. Fleseriu M, Auchus RJ, Bancos I, Biller BMK. Osilodrostat Treatment for Adrenal and Ectopic Cushing Syndrome: Integration of Clinical Studies With Case Presentations. *J Endocr Soc*. Mar 3 2025;9(4):bvaf027. doi:10.1210/jendso/bvaf027

37. Ferriere A, Salenave S, Puerto M, Young J, Tabarin A. Prolonged adrenal insufficiency following discontinuation of osilodrostat treatment for intense hypercortisolism. *Eur J Endocrinol*. Jan 3 2024;190(1):L1-L3. doi:10.1093/ejendo/lvad167

38. Shimatsu A, Biller BM, Fleseriu M, et al. Osilodrostat treatment in patients with Cushing's disease of Asian or non-Asian origin: a pooled analysis of two Phase III randomized trials (LINC 3 and LINC 4). *Endocr J*. Dec 2 2024;71(12):1103-1123. doi:10.1507/endocrj.EJ24-0153

39. Upton TJ, Zavala E, Methlie P, et al. High-resolution daily profiles of tissue adrenal steroids by portable automated collection. *Sci Transl Med*. Jun 21 2023;15(701):eadg8464. doi:10.1126/scitranslmed.adg8464

40. Lightman SL, Conway-Campbell BL. Circadian and ultradian rhythms: Clinical implications. *J Intern Med*. Aug 2024;296(2):121-138. doi:10.1111/joim.13795