Article:
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Supplement

Table of Content

Supplementary Table S1. Number of contributed patients and their ethnicity per center.

Center	Country	Number of included patients	Asian	European American/ Caucasian	Other
Huashan Hospital, Fudan University	China	10	10	0	0
Sao Paolo	Brazil	9	0	8	1
Budapest Semmelweis University, Department of Internal Medicine and Oncology	Hungary	8	0	8	0
BNI Phoenix	USA	6	0	2	4
Oregon Health & Science University	USA	5	0	5	0
Cambridge	UK	5	0	3	2
Sydney	Australia	4	0	4	0
Munich	Germany	4	0	4	0
Krakow	Poland	4	0	4	0
Brussels	Belgium	3	0	2	1
HCPA-UFRGS	Brazil	3	0	3	0
IECPN	Brazil	3	0	2	1
Zagreb	Croatia	3	0	3	0
Bordeaux	France	3	0	3	0
Sapienza University, Rome	Italy	3	0	3	0
Linz	Austria	2	0	2	0
Helsinki	Finland	2	0	2	0
GR ARETAIEON	Greece	2	0	2	0
Tel Aviv Medical center	Israel	2	0	2	0
Rabin Medical Center	Israel	2	0	2	0
Belgrade	Serbia	2	0	2	0
Barcelona, HCB	Spain	2	0	2	0
Michigan	USA	2	0	2	0
Stanford	USA	2	0	2	0
Vienna	Austria	1	0	1	0
Tallinn	Estonia	1	0	1	0
Marseille	France	1	0	0	1
Frankfurt	Germany	1	0	1	0
Wurzburg	Germany	1	0	1	0
GR Laiko	Greece	1	0	1	0
Athens Gennimatas	Greece	1	0	1	0
Budapest Semmelweis University, Department of Endocrinology	Hungary	1	0	1	0
Soroka Medical Center, Beer Sheva	Israel	1	0	1	0
Turin	Italy	1	0	1	0
Rotterdam	Netherlands	1	0	1	0
Belgrade	Serbia	1	0	1	0
Barcelona, Hospital Germans Trias i Pujol	Spain	1	0	1	0
Madrid	Spain	1	0	1	0
Gothenburg	Sweden	1	0	1	0
Malmoe	Sweden	1	1	0	0
Oxford	UK	1	0	1	0
Sheffield	UK	1	0	1	0
Rochester, Minnesota	USA	1	0	1	0

Supplemental Table S2. Number of included cases with cyclic Cushing's syndrome per five-year periods according to the year of diagnosis.

Decade	Number of cases, n
1985-1990	1
1991-1995	2
1996-1999	2
2000-2005	9
2006-2010	14
2011-2015	26
2016-2020	32
2020-2024	24

Supplemental Table S3. Biochemical assessment of cortisol concentrations at the initial evaluation.

All biochemical results are expressed as x-times upper limit of normal (ULN) with medians and full ranges (min-max). Response to 1 mg DST is additionally expressed in SI units. N refers to the number of available samples. The p-values in the last column refer to the Kruskal-Wallis test for group comparisons between all etiologies. The footnotes indicate statistically significant differences for pairwise comparisons determined by the Mann-Whitey test. Abbreviations: ACTH, adrenocorticotropic hormone; cCS, cyclic Cushing's syndrome; DST, dexamethasone suppression test; IQR, interquartile range; LNSC, late night salivary cortisol; UFC, urinary free cortisol; ULN, upper limit of normal.

	N	All patients	Pituitary cCS	Ectopic cCS	Adrenal cCS	Occult cCS	P-value
Morning serum cortisol x ULN, median (min-max)	106	1·28 (0·33-9·20)	1·05 (0·36-4·46)	1·64 (0·33-5·27)	0·84 (0·41-1·24)	1·34 (0·45-9·20)	0·0009[1]
Plasma ACTH x ULN, median (min-max)	106	1·21 (0·08-17·6)	1·06 (0·15-17·6)	2·08 (1·44-2·70)	0·24 (0·08-0·36)	1·06 (0·38-3·13)	<0·0001[2]
24h-UFC x ULN, median (min-max)	88	3·98 (0·24-189)	3·50 (0·24-39·1)	15·9 (0·45-189)	0·51 (0·33-0·69)	15·3 (0·44-62·8)	0·0004[3]
LNSC x ULN, median (min-max)	43	4·24 (0·67-192)	2·81 (0·67-132)	16·6 (1·01-192)	-	6·16 (0·74-48·7)	0·0324[4]
DST x ULN, median (min-max)	78	9·39 (0·30-40·6)	7·44 (0·30-31·6)	18·1 (1·55-40·60)	2·41 (1·22-5·00)	10·7 (0·50-29·6)	0·0032[5]
DST in nmol/L, median (min-max)	78	469 (15·0-2028)	372 (15·0-1578)	906 (77·5-2028)	120 (61·1-250)	533 (25·0-1480)	0·0032[6]

[1]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -0·59; 95% CI -1·18 to -0·41) and ectopic versus adrenal cCS (median difference: 0·80; 95% CI 0·14 to 2·89)

[2]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -0·95; 95% CI -1·46 to -0·49), pituitary versus adrenal cCS (median difference: 0·85; 95% CI 0·18 to 2·02), ectopic versus adrenal cCS (median difference: 1·88; 95% CI 0·86 to 3·98), occult versus ectopic cCS (median difference: -1·02; 95% CI -1·78 to -0·32), occult versus adrenal cCS (median difference: 0·83; 95% CI 0·21 to 2·65)

[3]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -12·4; 95% CI -42·8 to -5·03), pituitary versus adrenal cCS (median difference: 2·99; 95% CI 0·44 to 11·1), and ectopic versus adrenal cCS (median difference: 15·4; 95% CI 0·16 to 138)

[4]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -13·8; 95% CI -40·9 to -2·32),

[5]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -10·7; 95% CI -17·8 to -3·78), and ectopic versus adrenal cCS (median difference: 15·7; 95% CI 1·94 to 30·0)

[6]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -533; 95% CI -889 to -189), and ectopic versus adrenal cCS (median difference: 785; 95% CI 97·2 to 1500)

Supplemental Table S4. Maximum documented biochemical values during evaluation of cortisol excess (i.e., peaks).

All biochemical results are expressed as x-times upper limit of normal (ULN) with medians and full ranges (min-max). Response to 1 mg DST is additionally expressed in SI units. N refers to the number of available samples. The p-values in the last column refer to the Kruskal-Wallis test for group comparisons between all etiologies. The footnotes indicate statistically significant differences for pairwise comparisons determined by the Mann-Whitey test. Abbreviations: ACTH, adrenocorticotropic hormone; cCS, cyclic Cushing's syndrome; CI, confidence interval; DST, dexamethasone suppression test; IQR, interquartile range; LNSC, late night salivary cortisol; UFC, urinary free cortisol; ULN, upper limit of normal. *In ACTH-independent (adrenal) Cushing's syndrome ACTH concentrations are suppressed (lower) during peak phases.

	N	All patients	Pituitary cCS	Ectopic cCS	Adrenal cCS	Occult cCS	P-value
Morning serum cortisol x ULN, median (min-max)	97	1·71 (0·47-14·9)	1·29 (0·47-6·14)	2·64 (0·84-14·9)	0·81 (0·80-0·95)	2·08 (0·84-3·57)	<0·0001[1]
Plasma ACTH x ULN, median (min-max)	99	1·78 (0·03-17·6)	1·36 (0·29-17·6)	3·68 (0·84-15·3)	0·25 (0·03-0·64)*	2·83 (0·48-6·32)	<0·0001[2]
24h-UFC x ULN, median (min-max)	102	7·40 (0·44-299)	4·58 (0·53-90·9)	44·8 (1·04-292)	1·89 (0·53-2·63)	39·6 (0·44-299)	<0·0001[3]
LNSC x ULN, median (min-max)	61	5·74 (0·01-2831)	3·27 (0·11-12·7)	39·3 (1·21-192)	0·72 (0·72-0·72)	48·7 (3·87-2831)	<0·0001[4]
DST x ULN, median (min-max)	74	9·11 (1·67-63·5)	7·44 (1·67-63·5)	26·8 (6·94-60·6)	4·25 (2·39-6·11)	18·3 (1·89-36·7)	<0·0001[5]
DST in nmol/L, median (min-max)	74	456 (83·3-3175)	372 (83·3-3175)	1339 (347-3028)	213 (119-306)	917 (94·5-1833)	<0·0001[6]

[1]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS, (median difference: -1·35; 95% CI -2·00 to -0·87), pituitary versus occult cCS (median difference: -0·74; 95% CI -1·36 to -0·17), pituitary versus adrenal cCS (median difference: 0·48; 95% CI 0·02 to 1·68), ectopic versus adrenal cCS (median difference: 1·83; 95% CI 0·94 to 4·47), and occult versus adrenal cCS (median difference: 1·26; 95% CI 0·51 to 4·17)

[2]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS, (median difference: -2·33; 95% CI -2·93 to -1·09), pituitary versus occult cCS (median difference: -1·47; 95% CI -2·17 to -0·11), pituitary versus adrenal cCS (median difference: 1·11; 95% CI 0·33 to 2·96), ectopic versus adrenal cCS (median difference: 3·44; 95% CI 1·60 to 6·68), and occult versus adrenal cCS (median difference: 2·58; 95% CI 0·56 to 4·63)

[3]p<0·05 for pairwise comparisons of pituitary cCS versus ectopic cCS (median difference: -40·2; 95% CI -50·9 to -12·6), pituitary versus adrenal cCS (median difference: 2·69; 95% CI 0·36 to 18·7), pituitary versus occult cCS (median difference: -35·0; 95% CI -52·1 to -0·80), and ectopic versus adrenal cCS (median difference: 42·9; 95% CI 7·97 to 174)

[4]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -36·1; 95% CI -44·9 to -19·7) and pituitary versus occult cCS (median difference: -44·2; 95% CI -102 to 2·87)

[5]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -19·3; 95% CI -23·6 to -10·4), and ectopic versus adrenal cCS (median difference: 22·5; 95% CI 4·56 to 54·4)

[6]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -967; 95% CI -1181 to -519), and ectopic versus adrenal cCS (median difference: 1127; 95% CI 228 to 2722)

Supplemental Table S5. Minimum documented biochemical values during evaluation of normal or low cortisol concentrations (i.e., trough).

All biochemical results are expressed as x-times upper limit of normal (ULN) with medians and full ranges (min-max). Response to 1 mg DST is additionally expressed in SI units. N refers to the number of available samples. The p-values in the last column refer to the Kruskal-Wallis test for group comparisons between all etiologies. Abbreviations: ACTH, adrenocorticotropic hormone; cCS, cyclic Cushing's syndrome; DST, dexamethasone suppression test; IQR, interquartile range; LNSC, late night salivary cortisol; UFC, urinary free cortisol; ULN, upper limit of normal.

	N	All patients	Pituitary cCS	Ectopic cCS	Adrenal cCS	Occult cCS	P-value
Morning serum cortisol x ULN, median (min-max)	104	0·38 (0·04-1·23)	0·41 (0·04-1·23)	0·39 (0·05-0·77)	0·46 (0·36-1·03)	0·27 (0·04-0·65)	0·32
Plasma ACTH x ULN, median (min-max)	98	0·44 (0·03-1·70)	0·41 (0·05-1·68)	0·41 (0·08-1·70)	0·58 (0·07-0·83)	0·49 (0·03-0·93)	0·79
24h-UFC x ULN, median (min-max)	92	0·31 (0·02-0·98)	0·36 (0·04-0·98)	0·22 (0·03-0·98)	0·50 (0·13-0·86)	0·29 (0·02-0·51)	0·14
LNSC x ULN, median (min-max)	57	0·46 (0·05-7·89)	0·48 (0·05-0·77)	0·50 (0·34-1·07)	0·24 (0·24-0·24)	0·24 (0·16-1·28)	0·56
DST x ULN, median (min-max)	41	0·83 (0·30-7·41)	0·73 (0·30-2·61)	1·06 (0·67-7·41)	0·67 (0·44-0·89)	0·86 (0·50-1·28)	0·25
DST in nmol/L, median (min-max)	41	41·7 (15·0-371)	36·5 (15·0-131)	52·8 (33·3-77·5)	33·3 (22·2-44·4)	42·9 (25·0-63·9)	0·25

Supplemental Table S6. Delta (Δ) biochemical values referring to the difference between the highest (peak) and lowest (trough) recorded measurements.

All biochemical results are expressed as x-times upper limit of normal (ULN). Response to 1 mg DST is additionally expressed in SI units. N refers to the number of available sample pairs (i.e. minimum and maximum values available). The p-values in the last column refer to the Kruskal-Wallis test for group comparisons between all etiologies. The footnotes indicate statistically significant differences for pairwise comparisons determined by the Mann-Whitey test. Abbreviations: Δ, delta; ACTH, adrenocorticotropic hormone; cCS, cyclic Cushing's syndrome; DST, dexamethasone suppression test; IQR, interquartile range; LNSC, late night salivary cortisol; UFC, urinary free cortisol; ULN, upper limit of normal.

	N	All patients	Pituitary cCS	Ectopic cCS	Adrenal cCS	Occult cCS	P-value
Δ min-max morning serum cortisol x ULN, median (IQR)	95	1·39 (0·59-2·32)	0·96 (0·51-1·76)	2·52 (1·84-4·13)	0·34 (-0·07 to 4·13)	1·88 (1·03-3·33)	<0·0001[1]
Δ min-max plasma ACTH x ULN, median (IQR)	89	1·22 (0·54-2·72)	0·74 (0·40-1·80)	2·24 (1·62-4·84)	-0·19 (-0·34 to -0·03)	2·26 (0·85-4·17)	<0·0001[2]
Δ min-max 24h-UFC x ULN, median (IQR)	88	6·92 (2·38-27·6)	3·64 (2·13-11·1)	41·6 (15·1-77·0)	1·76 (1·03-2·50)	27·10 (2·08-64·80)	<0·0001[3]
Δ min-max LNSC x ULN, median (IQR)	49	5·17 (1·72-22·0)	3·16 (1·48-14·8)	38·4 (20·1-74·1)	0·49 (0·49-0·49)	34·5 (4·25-105)	0·0039[4]
Δ min-max DST x ULN, median (IQR)	32	26·28 (3·02-16·5)	5·94 (2·67-10·8)	30·6 (14·0-35·2)	3·58 (1·50-5·67)	15·1 (2·95-27·3)	0·10
Δ min-max DST in nmol/L, median (IQR)	32	314 (151-825)	297 (133-541)	1531 (700-1760)	179 (75·0-283)	757 (148-1367)	0·10

[1]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -1·56; 95% CI -2·28 to -1·04), pituitary versus occult cCS (median difference: -0·92; 95% CI -1·64 to -0·22), pituitary versus adrenal (median difference: 0·62; 95% CI 0·11 to 1·92), ectopic versus adrenal cCS (median difference: 2·18; 95% CI 1·39 to 4·47), occult versus adrenal cCS (median difference: 1·54; 95% CI 0·49 to 4·26)

[2]p<0·05 for pairwise comparisons of pituitary versus ectopic cCS (median difference: -1·51; 95% CI -2·40 to -0·95), pituitary versus occult cCS (median difference: -1·52; 95% CI -2·11 to -0·15), pituitary versus adrenal (median difference: 0·93; 95% CI 0·50 to 2·79), ectopic versus adrenal cCS (median difference: 2·43; 95% CI 1·66 to 5·56), occult versus adrenal cCS (median difference: 2·44; 95% CI 0·78 to 4·53)

[3]p<0·05 for pairwise comparisons for pituitary versus ectopic cCS (median difference: -38·0; 95% CI -48·6 to -12·9) and ectopic versus adrenal cCS (median difference: 39·8; 95% CI 7·30 to 216)

[3]p<0·05 for pairwise comparisons for pituitary versus ectopic cCS (median difference: -35·2; 95% CI 52·3 to -9·90) and pituitary versus occult cCS (median difference: -31·4; 95% CI -87·8 to -2·10)

Supplemental Table S7. Cycle characterization.

Peaks per year of observation referring to the observation period until therapy-induced remission or the latest follow-up. The p-values in the last column refer to group comparisons between all etiologies using the Kruskal-Wallis test for comparisons of continuous and the Fisher's exact test for binary variables. The footnotes indicate statistically significant differences for pairwise comparisons determined by the Mann-Whitney test. Abbreviations: cCS, cyclic Cushing's syndrome; CI, confidence interval; IQR, interquartile range.

	All patients	**Pituitary cCS**	**Ectopic cCS**	**Adrenal cCS**	**Occult cCS**	**P-value**
Total number of hypercortisolemic peaks, median (IQR)	3·00 (2·00-4·00)	4·00 (3·00-5·75)	3·00 (2·50-5·00)	2·00 (2·00-3·00)	2·00 (2·00-4·00)	0·023[1]
Peaks per year of observation, median (IQR)	1·02 (0·41-2·00)	0·70 (0·30-1·78)	1·80 (1·05-11·3)	0·26 (0·19-0·34)	1·45 (0·82-2·68)	<0·0001[2]
Worsening clinical symptoms during peaks/available data, n/n (%)	87/108 (81%)	54/68 (79%)	22/25 (88%)	3/3 (100%)	8/12 (67%)	0·42
Improvement of clinical symptoms during troughs/available data, n/n (%)	79/107 (74%)	46/67 (69%)	22/25 (88%)	3/3 (100%)	8/12 (67%)	0·18
Spontaneous phases of adrenal insufficiency/available data, n/n (%)	31/110 (28%)	18/70 (26%)	7/25 (28%)	0/3 (0%)	6/12 (50%)	0·27
Spontaneous phases of adrenal crises/available data, n/n (%)	6/31 (19%)	1/18 (6%)	3/7 (43%)	0/0 (0%)	2/6 (33%)	0·044

[1]p<0·05 for pairwise comparisons of occult versus pituitary cCS (median difference: -2·00; 95% CI: -2·00 to 0·00)

[2]p<0·05 for pairwise comparisons pituitary versus ectopic cCS (median difference: -1·11; 95% CI: -1·63 to -0·53), ectopic versus adrenal cCS (median difference: 1·54; 95% CI: 0·58 to 23·7), and occult versus adrenal cCS (median difference: 1·18, 95% CI 0·37 to 5·74)

Supplemental Table S8. Spearman correlation for peak frequency and maximum documented biochemical values during evaluation of cortisol excess (i.e., peaks)

All biochemical results were analyzed as x-times upper limit of normal (ULN). N refers to the number of available sample pairs. The p-values refer to Spearman correlation between peak frequency and measures of cortisol excess. Abbreviations: ACTH, adrenocorticotropic hormone; cCS, cyclic Cushing's syndrome; CI, confidence interval: DST, dexamethasone suppression test; IQR, interquartile range; LNSC, late night salivary cortisol; UFC, urinary free cortisol.

	Serum cortisol	**Plasma ACTH**	**UFC**	**LNSC**	**DST**
n	94	96	98	59	71
r (95% CI)	0·26 (0·05-0·44)	0·27 (0·08-0·45)	0·27 (0·06-0·45)	0·49 (0·26-0·67)	0·25 (0·01-0·46)
p-value	0·013	0·0072	0·0083	<0·0001	0·035

Supplemental Table S9. Clinical signs and symptoms in patients with cyclic Cushing's syndrome.

	Available data	Always present	Only present during peaks (i.e., worsening during peaks and improving during troughs)	Never present
Round face, n (%)	110	49 (45%)	39 (35%)	22 (20%)
Plethora, n (%)	110	31 (28%)	47 (43%)	32 (29%)
Hair loss, n (%)	103	18 (17%)	21 (20%)	63 (61%)
Hirsutism*, n (%)	83	23 (28%)	19 (23%)	41 (49%)
Acne, n (%)	107	14 (13%)	14 (13%)	79 (74%)
Dorsocervical fat pad, n (%)	107	42 (39%)	28 (26%)	37 (35%)
Central obesity, n (%)	109	68 (62%)	23 (21%)	18 (17%)
Weight gain, n (%)	109	39 (36%)	56 (51%)	14 (13%)
Striae, n (%)	109	29 (27%)	15 (14%)	65 (60%)
Easy bruising, n (%)	107	34 (32%)	34 (32%)	39 (36%)
Muscle weakness, n (%)	107	37 (35%)	47 (44%)	23 (21%)
Fragile skin, n (%)	105	32 (30%)	25 (24%)	48 (46%)
Peripheral oedema, n (%)	109	15 (14%)	39 (36%)	55 (50%)
Menstrual irregularities/ amenorrhea, n (%)**	48	15 (31%)	15 (31%)	18 (38%)
Headache, n (%)	102	14 (14%)	14 (14%)	74 (73%)
Hypokalemia, n (%)	105	13 (12%)	38 (36%)	54 (51%)

Besides hair loss in one patient (1/103, 1%), none of the investigated clinical signs and symptoms were only present during troughs.
*Only in female patients (n=84). **Only in premenopausal females (defined as age ≤50 years or earlier if menopause had already occurred, n=51) with available data.

Supplemental Table S10. Therapeutical intervention and clinical and biochemical outcomes at the latest available follow-up.

Definitions: Overall follow-up duration, follow-up duration in months from initial evaluation until last available follow-up; Follow-up after complete remission, follow-up duration in months from complete remission following successful tumor surgery until last available follow-up; Time until complete remission, duration from initial evaluation until complete remission following successful tumor surgery; Complete biochemical remission, (secondary) adrenal insufficiency or physiological response to 1 mg DST following successful tumor surgery; Complete clinical remission, full resolution of all clinical signs of cortisol excess following successful tumor surgery; Partial biochemical remission, improvement of biochemical parameters following targeted tumor therapy (surgery, radiation, medical therapy); Partial clinical remission, improvement of clinical signs of cortisol excess following targeted tumor therapy (surgery, radiation, medical therapy); Spontaneous biochemical remission, (secondary) adrenal insufficiency or physiological response to 1 mg DST without concurrent therapy; Spontaneous clinical remission, resolution of clinical signs of cortisol excess without concurrent therapy; Biochemically controlled under steroidogenesis inhibitors, 24h UFC < ULN during use of steroidogenesis inhibitors. Abbreviations: BADX, bilateral adrenalectomy; BMI, body mass index; cCS, cyclic Cushing's syndrome; IQR, interquartile range.

	All patients	**Pituitary cCS**	**Ectopic cCS**	**Adrenal cCS**	**Occult cCS**
	110	**70**	**25**	**3**	**12**
Therapy					
Pituitary surgery/available data, n/n (%)	71/110 (65%)	63/70 (90%)	5/25 (20%)	0/3 (0%)	3/12 (250%)
Ectopic tumor surgery/available data, n/n (%)	14/110 (13%)	0/70 (0%)	14/25 (56%)	0/3 (0%)	0/12 (0%)
BADX ever/available data, n/n (%)	13/110 (12%)	4/70 (6%)	5/25 (20%)	0/3 (0%)	4/12 (33%)
Unilateral adrenalectomy ever/available data, n/n (%)	1/110 (1%)	0/70 (0%)	0/25 (0%)	1/3 (33%)	0/12 (0%)
Steroidogenesis inhibitors (ever)/available data, n/n (%)	60/110 (55%)	29/70 (40%)	19/25 (76%)	1/3 (33%)	11/12 (92%)
Use of titration approach only, n/n (%)	38/60 (63%)	22/29 (76%)	8/19 (42%)	1/1 (100%)	7/11 (64%)
Use of block-and-replace approach only, n/n (%)	9/60 (15%)	1/29 (3%)	7/19 (37%)	0/1 (0%)	1/11 (9%)
Use of titration and block and replace approach, n/n (%)	13/60 (22%)	6/29 (21%)	4/19 (21%)	0/1 (0%)	3/11 (27%)
Tumor radiation/available data, n/n (%)	20/110 (18%)	14/70 (19%)	6/25 (24%)	0/3 (0%)	0/12 (0%)

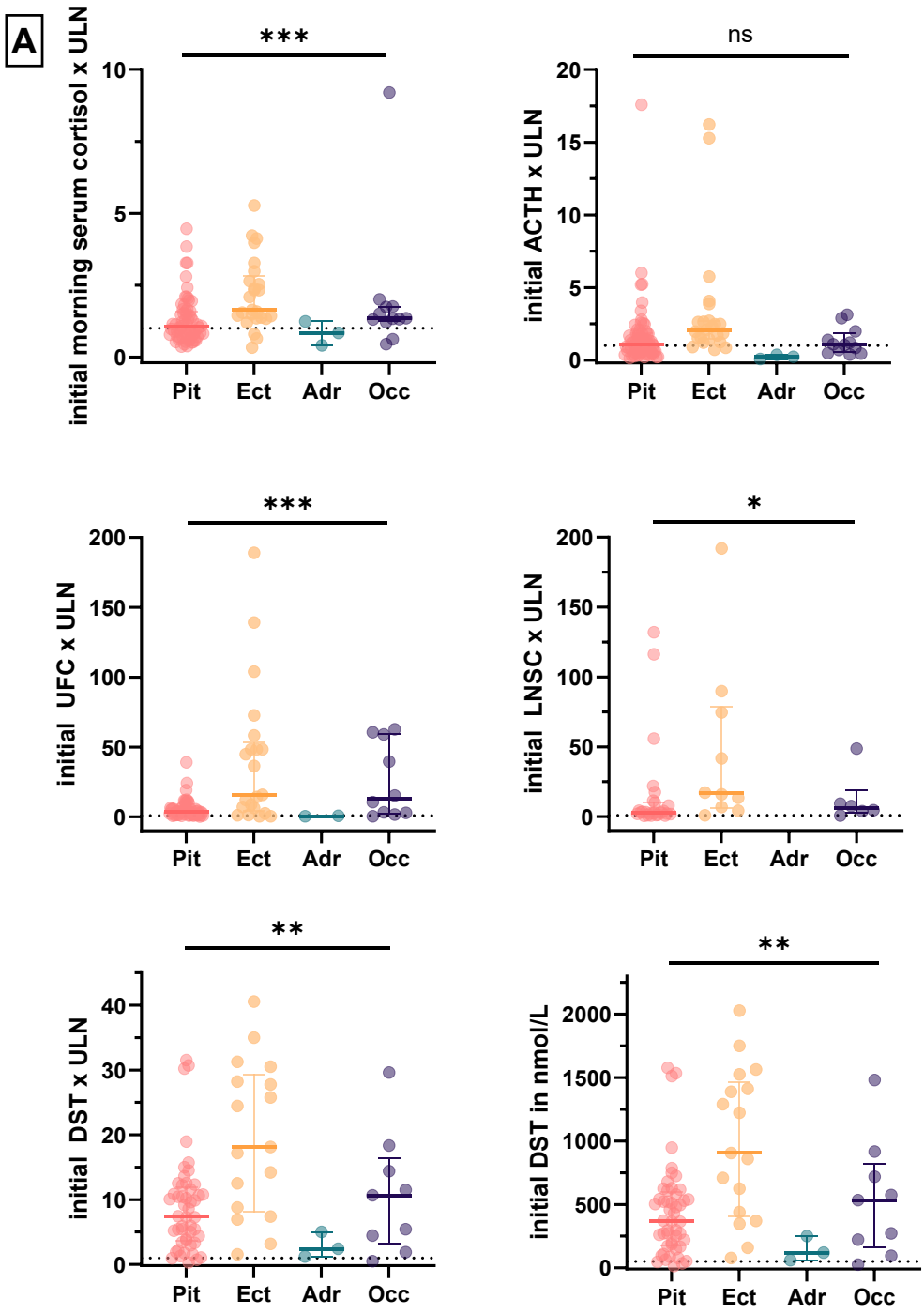
Outcome at the latest available follow-up					
Overall follow-up duration from diagnosis until last visit in months, median (IQR)	70·0 (31·0-126)	96·0 (49·5-150)	32·0 (15·5-89·5)	125 (70·0-137)	41·0 (24·0-57·0)
Clinical outcomes					
Overt, i.e. uncontrolled/available data, n/n (%)	11/110 (10%)	7/70 (10%)	2/25 (8%)	0/3 (0%)	2/12 (17%)
Complete therapy-induced remission/available data, n/n (%)	50/110 (45%)	35/70 (50%)	11/25 (44%)	0/3 (0%)	4/12 (33%)
Follow-up after complete therapy-induced remission, median (IQR)	31·0 (4·00-72·0)	36·0 (5·00-84·0)	29·5 (2·50-65·0)	NA	6·50 (1·00-36·8)
Time until complete therapy-induced remission, median (IQR)	23·0 (10·0-58·0)	31·0 (12·0-76·0)	10·0 (1·25-20·8)	NA	22·0 (8·25-68·8)
Partial remission/available data, n/n (%)	10/110 (9%)	8/70 (11%)	1/25 (4%)	0/3 (0%)	1/12 (8%)
Spontaneous remission/available data, n/n (%)	6/110 (5%)	1/70 (1%)	3/25 (12%)	1/3 (33%)	1/12 (8%)
Controlled under steroidogenesis inhibitors/available data, n/n (%)	21/110 (19%)	13/70 (19%)	5/25 (20%)	1 (33%)	2/12 (17%)
Patient’s death/available data, n/n (%)	3/110 (3%)	0/70 (0%)	3/25 (12%)	0/3 (0%)	0/12 (0%)
Unknown or lost to follow-up/available data, n/n (%)	9/110 (8%)	6/70 (9%)	0/25 (0%)	1 (33%)	2/12 (17%)
Biochemical outcomes					
Overt, i.e. uncontrolled/available data, n/n (%)	11/110 (10%)	7/70 (10%)	3/25 (12%)	0/3 (0%)	1/12 (8%)
Complete therapy-induced remission/available data, n/n (%)	55/110 (50%)	39/70 (56%)	12/25 (48%)	0/3 (0%)	4/12 (33%)
Partial remission/available data, n/n (%)	6/110 (5%)	4/70 (6%)	1/25 (4%)	0/3 (0%)	1/12 (8%)

Spontaneous remission/available data, n/n (%)	7/110 (6%)	2/70 (3%)	3/25 (12%)	1/3 (33%)	1/12 (8%)
Controlled under steroidogenesis inhibitors/available data, n/n (%)	22/110 (20%)	13/70 (19%)	5/25 (20%)	1/3 (33%)	3/12 (25%)
Unknown or lost to follow-up/available data, n/n (%)	9/110 (8%)	5/70 (7%)	1/25 (4%)	1/3 (33%)	2/12 (17%)
Complications					
Delayed diagnosis/available data, n/n (%)*	45/110 (41%)	27/70 (39%)	12/25 (48%)	0/3 (0%)	6/12 (50%)
Delayed therapy/available data, n/n (%)*	47/110 (43%)	31/70 (44%)	9/25 (36%)	0/3 (0%)	7/12 (58%)
Inappropriate surgery at the wrong anatomical site/available data, n/n (%)	9/110 (8%)	1/70 (1%)	5/25 (20%)	0/3 (0%)	3/12 (25%)
Spontaneous adrenal insufficiency/available data, n/n (%)	29/110 (26%)	18/70 (26%)	6/25 (24%)	0/3 (0%)	5/12 (52%)

*"Delayed diagnosis" and "delayed therapy" refer to self-assessments provided by the individual centers, based on their experience and in relation to potential diagnostic delays in Cushing's syndrome in general.

Supplementary Figure S1A-B. Biochemical assessment of cortisol concentrations at the initial evaluation.

All biochemical results are expressed as x-times upper limit of normal (ULN). Response to 1 mg 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 (*$p<0.05$, **$p<0.01$, ***$p<0.001$, 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. The graphs illustrate that not all patients with cCS present during a biochemically active phase at the initial investigation. A: overview of all available data, B: Zoom for differentiation of serum cortisol, ACTH, UFC, and LNSC concentrations in the lower range. Abbreviations: ACTH, adrenocorticotropic hormone; cCS, cyclic Cushing's syndrome; DST, dexamethasone suppression test; IQR, interquartile range; LNSC, late night salivary cortisol; UFC, urinary free cortisol; ULN, upper limit of normal.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initial DST x ULN
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initial DST in nmol/L
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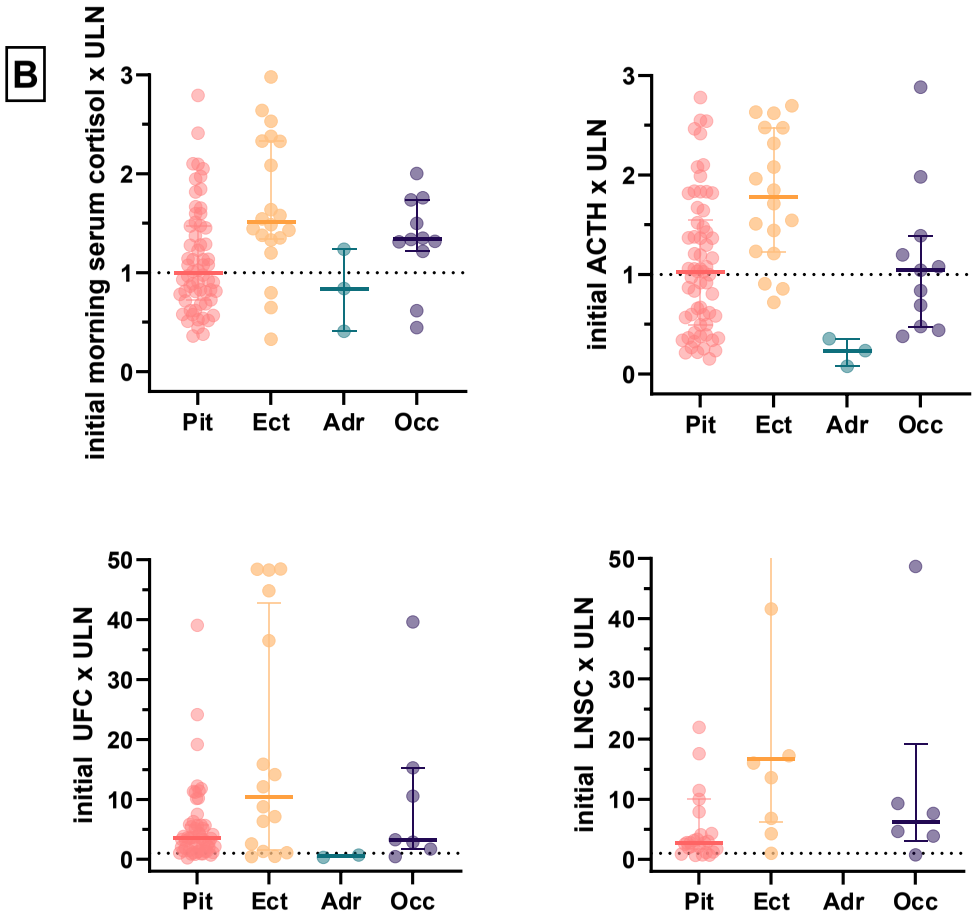
1500
1000
500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Study Protocol

A multicenter case series on cyclic Cushing's syndrome

Study protocol

Responsible project leaders:

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1. Scientific background of the study

Cushing's syndrome (CS) is a rare but severe endocrine disorder caused by an endogenous hypercortisolism, typically due to a benign endocrine tumor. It is associated with a range of metabolic and cardiovascular comorbidities and can be fatal if left untreated.[1-4] Cyclic Cushing syndrome (cCS) is a specific subtype of Cushing's syndrome in which phases of glucocorticoid excess alternate with phases of low or normal cortisol levels. This switch between hyper- and eucortisolism appears to occur spontaneously and without external influences. Due to the significant cortisol fluctuations, diagnosing cyclic Cushing's syndrome is challenging, and the time until diagnosis and initiation of treatment is significantly prolonged compared to non-cyclic Cushing's syndrome. Depending on the definition, up to 20% of all patients with CS may suffer from cCS. Additionally, patients with the cyclic form seem to undergo unnecessary surgeries more frequently and have an overall poorer clinical outcome.[5-9] The underlying mechanisms of these pronounced cortisol fluctuations are still not understood.

2. Aim of the study

The aim of the current study is to capture additional previously unpublished cases of patients with cyclic Cushing's syndrome. Due to the rarity of the condition, we intend to collect at least thirty cases. For this purpose, patients with clinically and biochemically confirmed Cushing's syndrome, who have experienced at least two spontaneous phases of cortisol excess and one spontaneous phase of low or normal cortisol levels, will be included. The gathered information is intended to help better understand the clinical, biochemical, and histopathological characteristics of the disease. Detecting common features has the potential to significantly contribute to the understanding of both cyclic and non-cyclic Cushing's syndrome.

3. Study design

This study is a multicenter international retrospective case series. The case series will be created using the CARE (CAse REport) guidelines for clinical case reports and case series.[10]

- Study population: Patients with confirmed cyclic Cushing's syndrome
- Data sources: Electronic medical records
- Data collection period: 3-6 months
- Planned total number of patients: At least thirty patients

4. Statistics and sample size estimation

For the retrospective case series, a minimum of thirty suitable cases shall be collected. The results will be presented as the median of individual values. Comparisons of examination results or different treatment modalities will be calculated using Wilcoxon tests for paired values or Mann-Whitney U-test for unpaired values. A p-value < 0.05 will be considered significant.

5. Risks and ethical aspects

The study will be conducted upon receiving a positive vote from the relevant ethics committees.

5.1 Informed consent

Patients with cyclic Cushing's syndrome who are still under endocrinological care at the respective study centers will receive detailed information from their treating study physician about the planned study. Before being included in the case series, patients will be informed that their participation is voluntary and that they can withdraw from the research project at any time without giving reasons and without suffering any disadvantages as a result. The patient will receive this information in a comprehensible written form. They will be given sufficient time and opportunity to ask questions and clarify any concerns. Written consent must be obtained before inclusion in the case series. By signing the consent form, the patient declares their voluntary participation in the research project. They also agree to the collection of data within the scope of the research project and to their inspection by the responsible study centers and ethics committee during inspections. The principal investigator confirms through countersigning of the consent form that an individual informed consent discussion has taken place and that a signed consent form has been obtained from the patient.

Patients with cyclic Cushing's syndrome who are no longer under endocrinological care at the participating study centers will be contacted by the respective study team by mail and/or by phone. If the patients cannot be reached (e.g., due to relocation or death), the data can still be used for the planned case series in a doubly pseudonymized form (see point 6.3) without a written consent form. This applies only if it complies with the local ethical requirements.

5.2 Early termination of the study by the patient

The patient can withdraw their consent to participate at any time without giving reasons and without any disadvantage for their further medical care. However, this is only applicable before the collected data has been analyzed or published.

5.3 Assessment of the balance between benefit and risk

Since this is a multicenter, retrospective observational study (case series), the risk for study participants to suffer harm from study-related measures is very low and ethically justifiable from the applicants' perspective. Since no new data will be collected and no new investigations will be conducted, the current study is not associated with any additional health risks for the participants.

6. Data protection

The legal data protection regulations are complied with. All collected data is subject to medical confidentiality.

6.1 Data collection

The study data is electronically recorded and stored by trained staff at each center. Subsequently, the data compilation and analysis will be conducted centrally at LMU hospital Munich, Germany.

6.2 Overview of the collected data

For the case series, data from patients with cyclic Cushing's syndrome will be collected. To ensure better comparability of the data, data collection will be based on pre-defined and clearly defined criteria. The following data will be collected:

- General information about patients (e.g., age, sex)
- Clinical characteristics (e.g., typical skin changes)
- Comorbidities (e.g., diabetes mellitus, arterial hypertension)
- Biochemical data (e.g., hormone levels from blood, urine, saliva)
- Diagnostic measures performed (e.g., imaging, stimulation tests)
- Therapeutic measures performed (e.g., medication therapy, surgery, radiation)
- Tumor characterization (e.g., macroscopic, microscopic)
- Disease progression (e.g., remission, recurrence, death)
- Complications (e.g., infections, cardiovascular events)
- Follow-up examinations (e.g., timing of the last examination to assess treatment success/remission duration)

6.3 Data security and data sharing

The data will be stored for a period of ten years initially. If the ethics committee approves the continuation of the study after ten years, the storage period can be extended for another ten years. All data that identifies the study participants (name, date of birth, address, etc.) will be stored exclusively at the local study centers and will not be shared with other study centers. The corresponding data will be immediately replaced with an identification code (pseudonymized) at the local level after collection. Access to the pseudonymization list by a treatment team is limited to patient data from their own center. Data exchange will only occur with a positive ethical approval from each participating study center. The data will be exchanged exclusively in pseudonymized form between the study centers. Data exchange is secured through access permissions. There will be no sharing of data with non-participating institutions.

Data exchange with participating study centers outside of the European Union (EU) will only occur if one of the following conditions is met:

- The European Commission has determined that the country has an adequate level of legal data protection, or if this has not been done:
- The LMU hospital agrees with the research partners on contractual data protection clauses that have been adopted or approved by the European Commission or the relevant supervisory authority. Study participants can obtain a copy of these data protection clauses from the LMU hospital.

6.4 Analysis and publication

The analysis of medical data will be conducted exclusively in a pseudonymized manner. Scientific publications of results will only be done in an anonymized and aggregated form. It will not be possible to draw conclusions about individual patients.

6.5 Right to withdraw and data deletion

Included patients have the right to withdraw their consent for the use of their data at any time. However, the legality of data usage prior to the withdrawal will not be affected. In case of withdrawal, the data will be deleted. Data deletion can only be carried out to the extent that it is technically feasible with reasonable effort. Additionally, data cannot be removed from already conducted analyses or after the publication of results.

7. Insurance

Due to the study design, an additional insurance is not considered necessary.

8. Signatures of project leaders

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Dr. med. Elisabeth Nowak

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Prof. Dr. med. Martin Reincke

9. Signature of participating centers

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Sources

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Data collection spreadsheet Term	Definition
Demographics	
Patient ID and Centre	-
Year of birth	YYYY
Age at diagnosis	In years
Year of diagnosis	Year of birth - age at diagnosis
Sex (assigned at birth)	Male/Female/Diverse
BMI at diagnosis	Kg/sqm
Ethnicity	African American or Black; Asian; European American or White; Other (please describe)
Tumor origin and histopathology	
ACTH-dependency	Yes/No
Tumor localization	Adrenal/Ectopic/Occult/Pituitary
Ectopic tumor localization	Specify if applicable
Tumor histopathologically proven	Yes/No
Histopathological proof of infarction/hemorrhage/necrosis	Yes/No
Tumor tissue available for further analyses	Yes/No
Comorbidities at baseline (i.e. at first presentation)	
Diabetes mellitus (type 2)	HbA1c ≥6.5%
Arterial hypertension	RR ≥140/90 mmHg and/or use of antihypertensive drugs
Osteoporosis	Mineral bone density T-score <2.5
Pathological fractures	Fractures with minimal or no trauma
Severe or opportunistic infections	Requiring hospitalization
Thromboembolic events	Deep vein thrombosis, pulmonary embolism, stroke, myocardial infarction, and peripheral arterial embolism
Depression	Physician's diagnosis (i.e., no self-reported diagnosis)
Clinical signs and symptoms	
Round ("moon") face	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Plethora	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Hair loss	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Hirsutism	Never/Always/During peaks only/During troughs only/Unknown/Not applicable

Acne	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Dorsocervical fat pad (“bullalo hump”)	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Central obesity	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Weight gain	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Striae	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Easy brusing	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Fragile skin	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Muscle weakness	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Peripheral oedema	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Menstrual irregularity/amenorrhea	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Headache	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Hypokalemia	Never/Always/During peaks only/During troughs only/Unknown/Not applicable
Other symptoms	Open comment field
Biochemical values (documented at first evaluation as well as the maximum documented biochemical values (during peak phase) and the minimum documented biochemical values (during trough phase)	
Serum cortisol	Unit of measure and reference ranges
Plasma ACTH	Unit of measure and reference ranges
24h urinary free cortisol	Unit of measure and reference ranges
Late night salivary cortisol	Unit of measure and reference ranges
Morning serum cortisol upon 1 mg dexamethasone suppression test	Unit of measure and reference ranges
Other biochemical tests	Open comment field
Bilateral inferior petrosal sinus sampling	
Perfomed	Yes, during peak phase; Yes, during trough phase; Not performed
Result	True pituitary; False pituitary; True ectopic; False ectopic; Not applicable (i.e. not performed)
Comment	Open comment field
Cycle characteristics	
Main biochemical test to screen for biochemical activity during trough phase	Open comment field
Number of peaks	N
Numer of troughs	N

Interval between peaks	Regular/Irregular
Time between peaks (i.e. duration of trough phases)	Days/Weeks/Months/Years
Worsening of symptoms during peak phases	Yes/No/Unknown
Improvement of symptoms during trough phases	Yes/No/Unknown
Spontaneous phases of adrenal insufficiency [Note: this does not refer to therapy induced adrenal insufficiency]	Yes/No/Unknown (Defined as low morning cortisol and/or need for glucocorticoid supplementation without prior intervention)
Spontaneous adrenal crisis	Yes/No/Unknown (As diagnosed per Kienitz 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)
Cyclic Cushing's syndrome first diagnosed after pituitary surgery	Yes/No/Not applicable (i.e. no pituitary surgery performed)
Imaging studies	
Imaging suggestive/diagnostic	Yes/No/not performed
Pituitary tumour	Yes/No/Unknown/imaging not performed
Ectopic tumour	Yes/No/Unknown/imaging not performed
Tumour size	In mm
Adrenal description	Bilaterally normal; Bilaterally abnormal/enlarged; Unilateral abnormal/enlarged; Unkown
Therapy	
Tumor surgery	Yes, successful; Yes, failed; No
Pituitary surgery	Yes, successful; Yes, failed; No
Bilateral adrenalectomy	Yes/No
Tumour radiation	Yes, successful; Yes, failed; No
Use of adrenostatic therapy	Yes/No
Adrenostatic therapy regimen	Titration regimen/block and replace regimen/both
Type of adrenostatic (or any other tumour targeted therapy)	Ketoconazole, Metyrapone, Osilodrostat, Other (please describe)
Complications	Open comment field
Outcome	
Clinical outcome	No remission, i.e. still overt; Complete remission; Partial remission; Controlled AND under adrenostatic therapy; Unkown/lost to follow up

Biochemical outcome	No remission, i.e. still overt; Complete remission; Partial remission; Controlled AND under adrenostatic therapy; Unkown/lost to follow up
Diagnostic errors	
Inappropriate surgery at the wrong anatomical site	Yes/No
Delayed diagnosis due to misleading findings	Yes/No
Delayed therapy due to misleading findings	Yes/No
Follow-up evaluation (most recent available)	
Date of last evaluation	DD/MM/YYYY
Total follow up duration from first to last visit	Months
Follow up after initiation of complete remission	Months (if applicable)
Further comments	Open comment field