Clinical and pharmacological determinants of serum dexamethasone concentrations during dexamethasone suppression test

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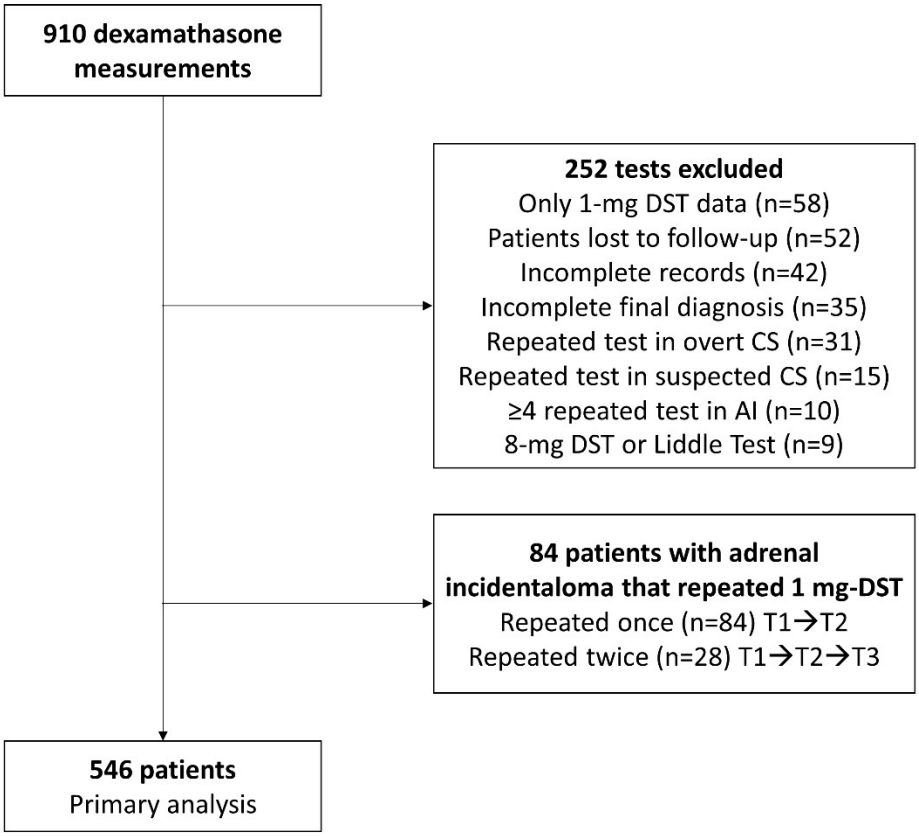
Supplementary material

Supplementary Figure 1. Flow-chart of the study cohort, depicting those patients excluded according to selection criteria and those selected for the primary analysis, as well as the sub-cohort of patients with adrenal incidentaloma with repeated paired tests.



Supplementary Table 1. Pearson correlation between dexamethasone and cortisol levels after 1 mg-DST (tertiles' distribution in patients <65 years).

Diagnostic group	Number of tests	χ^2 (df)	P value
MACS	37	6.35 (4)	0.175
NF-AI	68	0.41 (2)	0.815
Suspected CS	159	1.51 (4)	0.825

Supplementary Table 2. Dexamethasone and cortisol levels after 1-mg DST according to age, BMI and eGFR (MACS excluded).

	Number of tests	Cortisol after 1-mg DST (nmol/L)	*p* value	Dexamethasone (nmol/L)	*p* value
Age					
<65 years	222	29.5 (20-42)	0.695	11.2 (7.8-20.8)	0.653
≥65 years	160	29 (21.2-42)		11.7 (7.8-15.5)	
BMI					
<25 kg/m²	72	32 (24-43)[a]	0.006	9.3 (6.4-14.2)[a]	0.035
25-29 kg/m²	92	31 (22.2-47)[b]		11.8 (8.4–15)	
≥30 kg/m²	134	25 (16-38)		12.3 (7.8-17.6)	
eGFR					
>90 mL/min	140	34 (26.5-42.7)	0.06	10.5 (7.2–14.5)	0.009
60-89 mL/min	78	31 (23-44)		12.4 (8.9–16.9)	
≤59 mL/min	14	27 (16-40)		15.4 (10.6-19.9)	

Data are presented as median and IQR (in brackets). Significance values have been adjusted by the Bonferroni correction for multiple tests. Significance: [a]: p <0.05 BMI <25 vs ≥30 kg/m²; [b]: p <0.05 BMI 25-29 vs ≥30 kg/m².

Supplementary Table 3. Dexamethasone and cortisol levels after 1-mg DST by gender and diagnostic category.

	Number of tests	Cortisol after 1-mg DST (nmol/L)	*p* value	Dexamethasone (nmol/L)	*p* value
All cohort					
Males	202	40 (23-63.2)	0.582	12.2 (8.6-16.4)	0.329
Females	344	35 (23.2-66)		11.6 (8.1-15.7)	
MACS					
Males	55	71 (52-109)	0.398	13.2 (9.6-19.2)	0.125
Females	75	73 (52-127)		12.4 (8.4-16.4)	
NF-AI					
Males	61	34 (26-42.5)	0.175	12.2 (9.3-15.8)	0.745
Females	97	31 (24-40)		11.7 (8.3-15.9)	
Suspected CS					
Males	58	22 (13-33)	0.131	11.8 (8.3-15.6)	0.754
Females	146	26 (16.7-34.2)		11.6 (6.9-15.9)	

Data are presented as median and IQR (in brackets).

Supplementary Table 4. Serum dexamethasone and cortisol levels after 1-mg DST according to concomitant medication use (MACS excluded).

		Use of medication	No medication	*p* value
Statins (n=88)	**Dexamethasone (nmol/L)**	12.9 (8.3-16.2)	11.05 (7.7-15.1)	0.081
	Cortisol after 1-mg DST (nmol/L)	30.5 (23.3-43.7)	28 (20-42)	0.145
Protein pump inhibitors (n=81)	**Dexamethasone (nmol/L)**	12.7 (8.6-18.7)	11.2 (7.7-14.9)	0.071
	Cortisol after 1-mg DST (nmol/L)	37 (26-48)	28 (19-40)	<0.001
Calcium Channel Blockers (n=59)	**Dexamethasone (nmol/L)**	11.8 (7.8-15.8)	11.4 (7.8-15.6)	0.766
	Cortisol after 1-mg DST (nmol/L)	29 (21-42)	29 (20-41)	0.603
SSRI/SNRI (n=7)	**Dexamethasone (nmol/L)**	9.5 (6.7-12.7)	11.6 (7.9-15.6)	0.256
	Cortisol after 1-mg DST (nmol/L)	26 (21-42)	29 (23-36)	0.735

Data are presented as median and IQR (in brackets).

Supplementary Table 5. Patients with suboptimal dexamethasone levels and unsuppressed cortisol levels after 1-mg DST.

	Final Diagnosis	Serum cortisol after 1-mg DST (nmol/L)	Dexamethasone (nmol/L)	BMI (kg/m^2)	eGFR (ml/min)	Pharmacological treatment
Patient 1	Suspected CS	477	<1	37.8	61	Quetiapine
Patient 2	Suspected CS	345	2.9	18.6	99	Quercetin
Patient 3	Adrenal incidentaloma	51	3.3	28.2	88	Lercanidipine
Patient 4	Suspected CS	89	3.7	16.7	107	-
Patient 5	CS follow up	59	2.7	24.9	99	Pantoprazole, rosuvastatin
Patient 6	Adrenal incidentaloma	237	2.1	n.a.	n.a	Lercanidipine
Patient 7	Adrenal incidentaloma	210	2.5	36.3	119	-
Patient 8	Adrenal incidentaloma	66	3.7	30.9	n.a.	Carbamazepine
Patient 9	Adrenal incidentaloma	444	<1	25.8	102	Carbamazepine, phenobarbital
Patient 10	Adrenal incidentaloma	60	<1	30	77	Amlodipine, mirtazapine
Patient 11	Active CS	504	4.5	26.6	51	Simvastatin
Patient 12	Active CS	455	4.2	20.3	115	Ketoconazole, atorvastatin

Supplementary Table 6. Proportion of patients in our cohort who would be classified as having inadequate dexamethasone levels when applying different cut-offs (as reported in earlier studies).

Study and number of cases (reference in brackets)	**Dexamethasone cut-off (nmol/L)**	**% of patient with inadequate dexamethasone exposure within the cited study**	**% of patient with inadequate dexamethasone exposure within our primary cohort (n=546)**
Ueland GA *et al.*, Eur J Endocrinol 2017, 302 cases (1)	<3.3	20%	1.6%
Hawley JM *et al.*, Ann Clin Biochem 2018, 95 cases (2)	<3.3	5%	1.6%
De Graaf AJ *et al.*, Ann Clin Biochem 2019, 1901 cases (3)	<3.9	10%	2.2%
Ceccato F *et al.*, J Endocrinol Invest 2020, 200 cases (4)	<4.5	6%	3.3%
Roper SM *et al.*, J Appl Lab Med 2021, 63 cases (5)	<3.3	14%	1.6%
Genere N *et al.*, J Clin Endocrinol Metab 2022, 196 cases (6)	<2.8	3%	1.2%
Vogg N *et al.*, Clin Chem 2021, 400 cases (7)	<4.6	7%	3.2%
Gao Y *et al.*, Endocrine Practice 2026, 221 cases (8)	<4.3	9.5%	2.9%
Parasiliti-Caprino M *et al.*, Eur J Endocrinol 2026, cases (9)	<3.1	2.5%	1.5%
This study	**<4.5**	**-**	**3.3%**

Supplementary Table 7. Comparison of ACTH levels between Test 1 and Test 2 in patients with MACS.

	MACS at Test 1 (n=51)	**MACS at Test 2 (n=47)**	***p* value**
ACTH (ng/L)	8 (6-14)	8.9 (5-13)	0.754
ACTH <10 ng/L, n (%)	25 (58%)	20 (54%)	0.888

Data are presented as median and IQR (in brackets).

Supplementary Table 8. Cortisol levels after 1-mg DST according to diagnostic stability between Test 1 and Test 2. Δ cortisol (T1−T2): difference between cortisol levels after 1-mg DST between T1 and T2.

Status at Test 2	**n**	**Cortisol after 1-mg DST Test 1 (nmol/L)**	**Cortisol after 1-mg DST Test 2 (nmol/L)**	**Δ cortisol (T1−T2, nmol/L)**	***p* value**
Remained MACS	42	108 (83-156)	106 (72–168)	2.5 (−14.5, 30.2)	0.334
Reclassified as NF-AI	9	65 (56-100)	38 (33–44)	29 (18-66)	0.004

Data are presented as median and IQR (in brackets).

Abbreviations: 1-mg DST, 1-mg overnight dexamethasone suppression test; AI: adrenal incidentaloma; BMI, body mass index; eGFR, estimated glomerular filtration rate; CS, Cushing's syndrome; IQR, interquartile range; MACS, mild autonomous cortisol secretion; NF-AI, non-functioning adrenal incidentalomas; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin–norepinephrine reuptake inhibitor.

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