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Temporal changes in cortisol secretion and their association with long-term outcomes in benign adrenal incidentalomas: a retrospective cohort study

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Summary

Background Mild autonomous cortisol secretion (MACS), the most common hormonal abnormality in adrenal incidentalomas, is associated with increased cardiometabolic risk. MACS is defined by cortisol concentrations higher than 50 nmol/L after a 1-mg overnight dexamethasone suppression test (1-mg DST). The prognostic value of a single test remains uncertain. We examined longitudinal changes in 1-mg DST results, cumulative cortisol exposure, and their associations with cardiometabolic outcomes and mortality.

Methods In this retrospective cohort study conducted in 25 adrenal centres that are part of the European Network for the Study of Adrenal Tumours consortium across 14 countries with adults (aged ≥18 years) with benign adrenal incidentalomas diagnosed from Jan 1, 2000, to Dec 1, 2020, two or more 1-mg DSTs, and follow-up of at least 36 months, we used multivariable Cox models to assess associations between longitudinal 1-mg DST results and all-cause mortality and cardiovascular and thrombotic events. Patients with Cushing's syndrome, primary aldosteronism, phaeochromocytoma, or androgen-secreting tumour at baseline; active malignancy within 36 months of incidentaloma diagnosis; or suspicion of unreliable 1-mg DST results were excluded, along with patients taking oral glucocorticoids, strong CYP3A4 modulators, adrenal enzyme inhibitors, oral oestrogen, or selective oestrogen receptor modulators. Cumulative cortisol exposure was estimated from serial 1-mg DSTs. Restricted mean survival time (RMST) quantified differences in event-free time. Data were collected from the electronic health records of all eligible patients at each participating centre.

Findings Among 2525 patients (median follow-up 80 months [IQR 49–122]), 563 (22·3%) had changes in 1-mg DST results leading to a change in diagnosis, most within 3 years of their baseline 1-mg DST. Patients with persistently abnormal 1-mg DST results (ie, MACS-to-MACS patients; n=839) were older and had a greater cardiometabolic burden than those with persistently normal results (ie, non-functioning adrenal tumours [NFAT]-to-NFAT patients; n=1103). MACS-to-MACS patients had a higher rate of worsening hypertension (adjusted hazard ratio 1·34 [95% CI 1·03–1·73]) and a shorter event-free time for worsening hypertension (10-year RMST 60·4 months [56·8–75·5] *vs* 86·1 months [79·1–93·4]) than NFAT-to-NFAT patients. In crude analyses, patients with higher baseline post-1-mg DST cortisol, patients with greater cumulative cortisol exposure, and MACS-to-MACS patients had shorter survival and event-free time; however, these associations were not independent of age and baseline cardiometabolic risk factors after multivariable adjustment.

Interpretation Longitudinal 1-mg DST changes are common. MACS-to-MACS patients had worsening hypertension, but rates of mortality and cardiovascular or thrombotic events were not significantly associated with 1-mg DST trajectories after adjustment for age and cardiovascular risk factors. These findings identify patients with persistently abnormal 1-mg DST results as a group with high cardiometabolic risk that warrants closer attention to modifiable risk factors. Prospective studies are needed to establish the clinical significance of repeated 1-mg DSTs for risk stratification.

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Research in context

Evidence before this study

The 2023 guideline from the European Society of Endocrinology (ESE) and the European Network for the Study of Adrenal Tumours (ENSAT) recommends that all patients with adrenal incidentalomas undergo clinical assessment and a 1-mg overnight dexamethasone suppression test (1-mg DST) to exclude a diagnosis of cortisol excess. On the basis of their 1-mg DST results, patients without clinical features of Cushing's syndrome and normal results on other relevant hormonal investigations are classified as having mild autonomous cortisol secretion (MACS, ie, 1-mg DST serum cortisol >50 nmol/L) or non-functioning adrenal tumours (NFAT, ie, 1-mg DST serum cortisol ≤50 nmol/L). MACS is the most common hormonal abnormality in adrenal incidentalomas, with a prevalence of up to 50%. Patients with MACS have a higher risk of cardiovascular comorbidities and mortality compared with patients with NFAT. The current guideline does not routinely recommend repeating the 1-mg DST in patients with adrenal incidentalomas; however, the evidence supporting this recommendation is insufficient, and whether a single 1-mg DST adequately reflects long-term risk is uncertain. We conducted a comprehensive literature search of PubMed and the Cochrane Database of Systematic Reviews for studies published between Jan 1, 2000, and Dec 31, 2023, focusing on incident changes in 1-mg DST results, with the keywords "adrenal incidentaloma" AND "dexamethasone suppression test" AND "mild autonomous cortisol secretion" OR "subclinical Cushing". We considered only English-language studies but did not place restrictions on study type.

A meta-analysis of 19 studies involving 2083 patients showed that only 4·3% of individuals initially classified as having NFAT later had disease progression to MACS. However, findings varied considerably among studies, probably due to heterogeneity in diagnostic criteria and small sample sizes in several cohorts. Four studies (involving 525 patients in total) published after this systematic review and relying on the 2023 ESE–ENSAT guideline diagnostic criteria reported that 10–28% of patients with NFAT at baseline developed MACS during follow-up.

Added value of this study

To our knowledge, this is the largest and most geographically diverse study to date examining longitudinal 1-mg DST changes in benign adrenal incidentalomas. By applying contemporary guideline-based diagnostic criteria consistently across all participants, we provide the most methodologically homogeneous estimates of 1-mg DST result trajectories available. To our knowledge, this study is the first to quantify cumulative cortisol exposure from serial 1-mg DST results and to link this directly to long-term clinical outcomes; it is also the first to characterise five distinct cortisol secretion trajectory groups—including a fluctuating phenotype—and to show, over a median follow-up exceeding 6 years, a cardiometabolic risk gradient across these groups, with persistent MACS independently associated with a higher rate of worsening hypertension. Patients with persistent MACS experienced 2er months free of hypertension worsening over 10 years compared with those with persistently normal 1-mg DST.

Implications of all the available evidence

Changes in 1-mg DST results over time are more common than previously recognised. We found that patients with persistent MACS carry the greatest cardiometabolic burden and are independently at risk of worsening hypertension, which is consistent with recent randomised trial evidence supporting adrenalectomy for blood pressure improvement in selected patients. Associations with all-cause mortality and cardiovascular and thrombotic events were not independent of age and baseline cardiometabolic risk factors, suggesting that these—rather than cortisol trajectory per se—are the primary determinants of long-term outcomes in this population. Collectively, these findings identify patients with persistently abnormal 1-mg DST as a group with enriched cardiometabolic risk, in whom optimisation of modifiable risk factors, including hypertension, dyslipidaemia, and smoking, warrants particular attention within standard care. Whether repeated hormonal assessment contributes meaningfully to risk stratification beyond these risk factors requires evaluation in prospective studies.

Introduction

Adrenal masses are found in 3–7% of adults and are usually benign adrenocortical tumours discovered incidentally (incidentalomas).[1–3] The 2023 European Society of Endocrinology (ESE)–European Network for the Study of Adrenal Tumours (ENSAT) adrenal incidentaloma guideline recommends clinical assessment and a 1-mg overnight dexamethasone suppression test (DST) to exclude cortisol excess.[4] In patients without features of Cushing's syndrome, tumours are classified as non-functioning adrenal tumours (NFATs; defined by a 1-mg DST cortisol concentration ≤50 nmol/L) or mild autonomous cortisol secretion (MACS; defined by a 1-mg DST concentration >50 nmol/L), with normal results on other hormonal investigations.

MACS, diagnosed in 19–50% of adrenal incidentalomas,[1,3,5–8] is associated with increased mortality risk[9] and a higher prevalence of cardiovascular comorbidities potentially attributable to cortisol excess, including hypertension, type 2 diabetes, and dyslipidaemia, compared with NFATs.[5,6,9]

A single 1-mg DST might not reflect long-term cortisol exposure or prognosis; the 2023 ESE–ENSAT guideline recommends repeating the test in patients with NFAT who develop new clinical signs of hormone excess or worsening comorbidities, and in patients with MACS when adrenalectomy is planned.[4] Evidence underpinning

repeat testing is scarce, and small-scale studies suggest 1-mg DST results vary over time:[10–12] up to 28% of patients with initially normal 1-mg DST results developed MACS during follow-up, and this progression was associated with worse cardiovascular outcomes.[10–13]

In this study, we examine longitudinal changes in 1-mg DST results, their clinical correlates, and the association between cumulative cortisol exposure and cardiovascular events and mortality.

Methods

Study design

We conducted an international, multicentre, retrospective cohort study across 24 adrenal specialist centres in 13 countries in Europe and one specialist centre in the USA, all of which are part of the ENSAT consortium (appendix pp 33–34). The ENSAT Scientific Board approved the study in February, 2023 (Project 2023–001). Data originally obtained from Jan 1, 2000, to Dec 31, 2023, were collected from the centres for the purposes of this analysis between March 1, 2023, and Dec 31, 2023. All centres obtained local ethical approval for the pseudonymised data collection and sharing. The study protocol can be found in the appendix (pp 3–10).

Participants

Detailed inclusion and exclusion criteria are provided in the appendix (pp 3–10). Inclusion criteria included being aged 18 years or older; a benign adrenal incidentaloma diagnosis between Jan 1, 2000 and Dec 1, 2020; availability of at least two 1-mg DST results (baseline and ≥6 months post-baseline, before adrenalectomy if surgery was performed); and at least 36 months of follow-up after diagnosis. Exclusion criteria included a diagnosis of Cushing's syndrome, primary aldosteronism, phaeochromocytoma, or androgen-secreting tumour at baseline; oral glucocorticoid, strong CYP3A4 modulator, or adrenal enzyme inhibitor intake at testing; oral oestrogen or selective oestrogen receptor modulator intake within 6 weeks of testing; suspicion of an unreliable 1-mg DST (eg, due to documented non-compliance or dexamethasone measurements below locally established cutoffs); and active malignancy within 36 months of incidentaloma diagnosis. If malignancy developed later, follow-up was censored at the malignancy diagnosis. Exclusion criteria for the reliability of 1-mg DSTs were applied both to baseline and follow-up tests. All patients in the databases of the included centres who met the inclusion criteria were included. Written informed consent was obtained unless waived by local committees.

Procedures

Age, sex (binary option of male or female), ethnicity, adrenal tumour characteristics, and smoking status at baseline were collected by all authors except MG and VA. We also obtained data for BMI, comorbidities (ie, hypertension, type 2 diabetes, lipid-lowering agent use, chronic kidney disease, osteoporosis, and cardiovascular or thrombotic events as detailed in the appendix p 10), and 1-mg DST results collected at baseline (ie, the time of the patient's first 1-mg DST) and at each follow-up 1-mg DST up until the last available clinical assessment. All data were collected from the electronic health records of the participating centres. Cardiovascular and thrombotic events were myocardial infarction, coronary revascularisation, heart failure hospitalisation, atrial fibrillation, stroke, and thromboembolic events. Information on Cushing's syndrome development, adrenalectomy, survival, and cause of death was also collected.

Post-1-mg DST cortisol concentrations that were measured at baseline and each follow-up assessment were obtained for our analysis. Adrenocorticotropic hormone (ACTH), dehydroepiandrosterone sulfate (DHEAS), and 24-h urinary free cortisol concentrations, measured before dexamethasone administration, were also recorded and obtained for analysis, as well as moderate CYP3A4 inducer or inhibitor or inhaled glucocorticoid use.

At baseline, patients were classified as having NFAT or MACS on the basis of 1-mg DST serum cortisol results (≤50 nmol/L—ie, normal, and >50 nmol/L—ie, abnormal, respectively).[4] The same cutoff was applied to follow-up 1-mg DST results, which resulted in five cortisol secretion groups: NFAT-to-NFAT patients (ie, patients with persistently normal 1-mg DST results), NFAT-to-MACS patients (ie, patients with an initial normal result followed by an abnormal 1-mg DST result), MACS-to-NFAT patients (ie, patients with an initial abnormal 1-mg DST result followed by a normal result), MACS-to-MACS patients (ie, patients with persistently abnormal 1-mg DST results), and patients with fluctuating cortisol secretion (ie, patients with 1-mg DST results that did not fit any of the other categories—eg, normal at baseline, then abnormal, then normal).

Outcomes

Our primary objective was to evaluate longitudinal changes in 1-mg DST results and cumulative cortisol exposure in relation to all-cause mortality and composite cardiovascular and thrombotic events. Secondary outcomes were cardiovascular comorbidity development and progression of hypertension and type 2 diabetes across cortisol secretion groups, as well as baseline prevalence of chronic kidney disease, dyslipidaemia, and osteoporosis across cortisol secretion groups (appendix p 7). We conducted subgroup analyses of mortality and cardiovascular and thrombotic outcomes by sex and age. Cox regression analyses were adjusted for baseline health characteristics.

Statistical analysis

We selected all ENSAT sites that were part of the non-aldosterone-producing adrenocortical adenoma working group and agreed to participate in the study. Categorical

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See Online for appendix

variables are presented as counts with percentages and continuous variables as medians with IQRs. We used χ^2, Fisher's, Student's *t*, Wilcoxon, ANOVA, or Kruskal–Wallis tests as appropriate, with Bonferroni-adjusted pairwise comparisons for multiple groups. Multivariable logistic regression assessed associations between MACS and comorbidities, adjusted for age, sex, and smoking status, with adjusted effect estimates reported as odds ratios with 95% CIs. A two-sided p-value of less than 0·05 was considered statistically significant.

Survival probabilities were estimated using Kaplan–Meier methods. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% CIs, before and after adjustment for prespecified covariates: age; sex; smoking status; BMI; tumour size and laterality; baseline hypertension, type 2 diabetes, and chronic kidney disease; lipid-lowering therapy; previous cardiovascular or thrombotic events; and baseline ACTH, DHEAS, and post-1-mg DST cortisol concentrations. Variables reaching statistical significance were retained in the final models. Patients with NFAT served as the reference group for baseline analyses, and NFAT-to-NFAT patients for analyses of cortisol secretion groups. Missing data were handled using multiple imputation combined with the Markov Chain Monte Carlo simulations for the survival analyses.[14] Separate analyses were conducted for the entire cohort and for a subcohort excluding patients who underwent adrenalectomy. Restricted mean survival times (RMSTs) were calculated as the area under the Kaplan–Meier curve from baseline up to prespecified truncation times (5, 7, and 10 years), providing assumption-free absolute measures of average event-free survival time. RMST analyses are reported as descriptive summaries of observed health trajectories within each group, reflecting the clinical profile of patients in those groups rather than the independent effect of cortisol status.

Time-to-event outcomes were defined as the interval from tumour diagnosis to the first documented event or death, censored at last follow-up before adrenalectomy for surgical patients. Patients diagnosed with adrenal Cushing's syndrome during follow-up were excluded from survival analyses.

Cumulative cortisol exposure was estimated as the area under the curve from serial 1-mg DST results and expressed as nmol/L×months, using a 24-month observation window; 1735 (68·7%) of 2525 patients had data for this timeframe and were included in this analysis. Restricted cubic splines were used to model associations between baseline post-1-mg DST cortisol and cumulative cortisol exposure—treated as continuous variables—and all-cause mortality and composite cardiovascular and thrombotic events.

Statistical analyses were performed using R (version 4.3.2) and SPSS (version 27.0). Sankey diagrams were created with SankeyMATIC.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Of 6683 consecutive patients with adrenal incidentalomas screened, 4040 were excluded, 1642 of whom only had a single 1-mg DST (appendix p 11). Of 2643 potentially eligible patients, 2525 met all the inclusion criteria and were included. Median follow-up was 80 months (IQR 49–122). During follow-up, 132 (5·2%) of the 2525 patients underwent adrenalectomy. The majority of patients (1775 [70·3%] of 2525) received their incidentaloma diagnosis before 2016, when annual hormonal reassessment was standard practice under earlier guidelines.

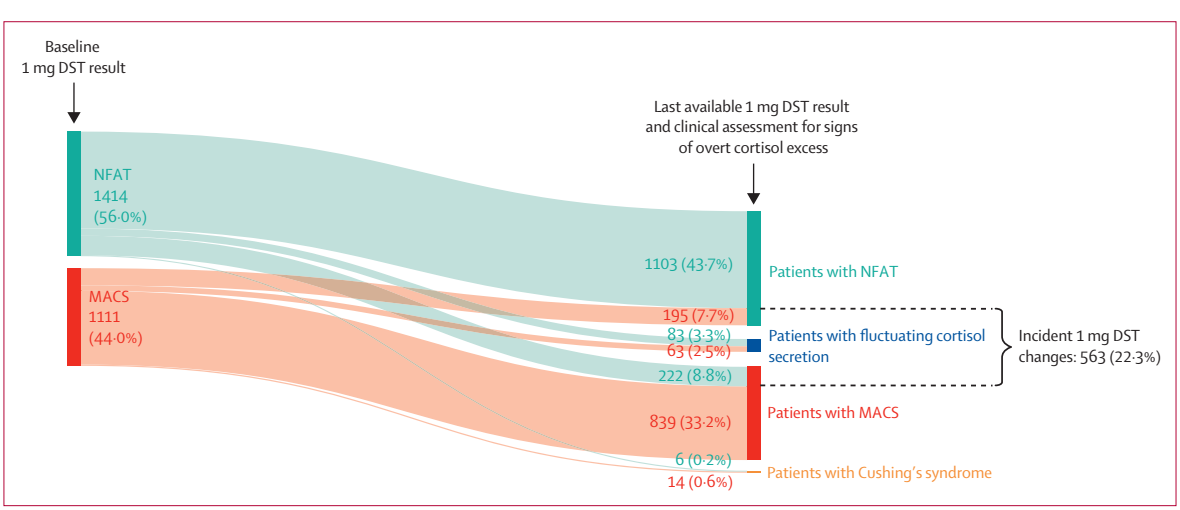

Figure 1: **Sankey diagram of longitudinal 1-mg DST results and incident changes in cortisol suppression status**
1 mg DST=1-mg overnight dexamethasone suppression test. MACS=mild autonomous cortisol secretion. NFAT=non-functioning adrenal tumour.

The median age at tumour diagnosis was 61 years (IQR 52–67). 1638 (64·9%) of the 2525 patients were female and 887 (35·1%) were male (appendix p 35). At baseline, MACS was diagnosed in 1111 (44·0%) patients and NFAT was diagnosed in 1414 (56·0%) patients. Compared with patients with NFAT, patients with MACS were more often female, older, more likely to be current smokers, had larger adrenal tumours, and were more than twice as likely to have bilateral tumours (appendix p 35). Patients with MACS also had a higher prevalence of cardiometabolic morbidity, osteoporosis, and cardiovascular or thrombotic events at baseline (appendix p 35). Increased hypertension, type 2 diabetes, and chronic kidney disease prevalence remained significantly higher in patients with MACS compared with patients with NFAT after adjustment for age, sex, and smoking status (appendix p 36). Increasing age was associated with higher cortisol concentrations after the first 1-mg DST, although this association was only significant in patients with NFAT (appendix pp 12–13). Among patients with NFAT at baseline, post-1-mg DST cortisol concentration increased by an estimated 0·25 nmol/L per year (95% CI 0·20–0·29; p<0·0001; appendix pp 12–13), whereas no significant age-related increase was observed in patients with MACS at baseline. Post-1-mg DST cortisol was also higher in patients with MACS who smoked compared with those who did not smoke (appendix pp 14–15), but no significant differences were observed in patients using inhaled glucocorticoids or moderate CYP3A4 inhibitors (appendix p 16). Baseline ACTH concentrations were available in 831 (74·8%) of 1111 patients with MACS.

1137 (45·0%) of 2525 patients had one repeat 1-mg DST, 1223 (48·4%) had two or three repeat tests, and 165 (6·5%) had four or more repeat tests; of the 4935 repeat tests, 3091 (62·6%) were done within 36 months of baseline (appendix p 17). Among all 2525 patients, changes in 1-mg DST results leading to a change in diagnosis were observed in 563 (22·3%) patients overall (figure 1), resulting in 222 (8·8%) NFAT-to-MACS patients, 195 (7·7%) MACS-to-NFAT patients, and 146 (5·8%) patients with fluctuating cortisol secretion. Diagnosis was unchanged in 1942 patients, resulting in 1103 (43·7%) NFAT-to-NFAT patients and 839 (33·2%) MACS-to-MACS patients. Adrenal Cushing's syndrome developed in 20 (0·8%) patients during a median follow-up of 40 months (IQR 15–65): six with baseline NFAT and 14 with baseline MACS. Among NFAT-to-MACS patients and MACS-to-NFAT patients, 272 (64·5%) of 422 incident 1-mg DST changes occurred within 3 years of baseline (figure 2), and a small but significant increase in post-1-mg DST cortisol was found in patients tested beyond 3 years (appendix p 18).

MACS-to-MACS patients were more frequently female than male, people who smoke than non-smokers, and patients with bilateral tumours than those with unilateral tumours (appendix pp 19–21). Compared with NFAT-to-NFAT patients, NFAT-to-MACS patients were older and had larger tumours, higher post-1-mg DST cortisol concentrations, lower DHEAS concentrations, and higher serum cortisol-to-DHEAS ratios at baseline (table). By contrast, MACS-to-NFAT patients presented with smaller tumours, less prevalent bilateral adrenal involvement, lower post-1-mg DST cortisol concentrations, higher ACTH and DHEAS concentrations, and a lower cortisol-to-DHEAS ratio compared with MACS-to-MACS patients (table).

At baseline and during follow-up, an increasing prevalence of cardiometabolic morbidity and cardiovascular and thrombotic events was observed across all cortisol secretion groups except for patients with fluctuating cortisol secretion, with MACS-to-MACS patients having the highest risk (figure 3), paralleled by cumulative cortisol exposure (appendix p 22). Patients

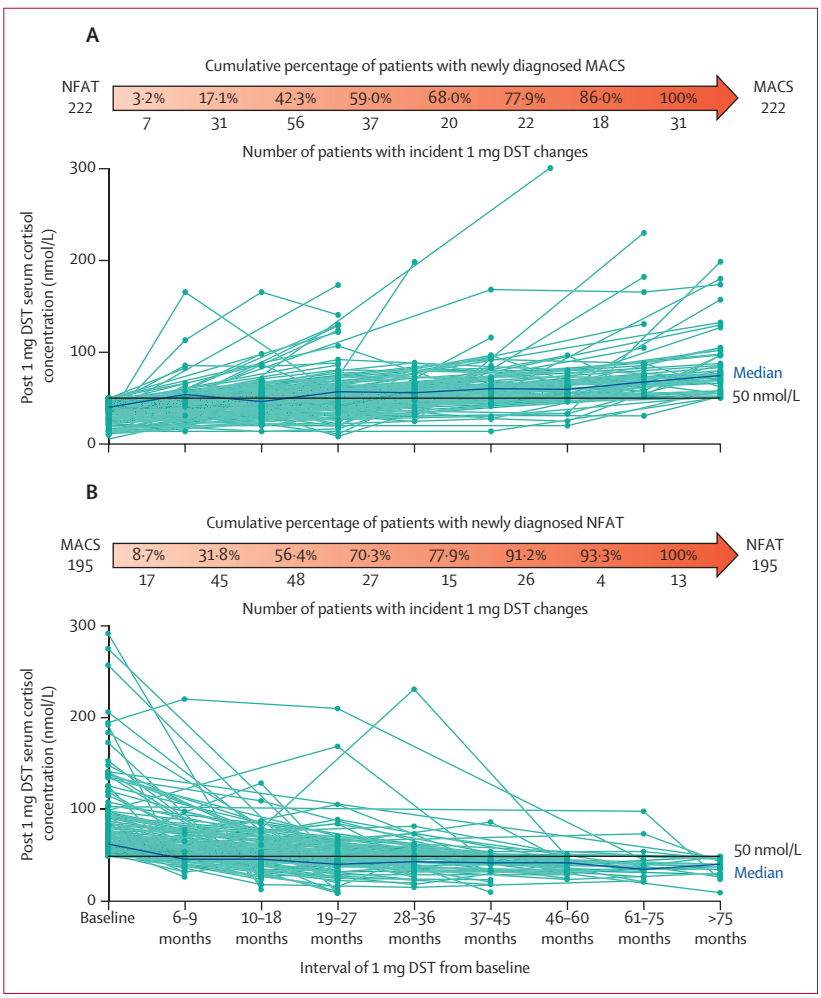

***Figure 2*: 1-mg DST serum cortisol concentrations during follow-up**
The horizontal black line represents the 50 nmol/L cutoff. Each green line represents an individual patient. The blue line represents the median. (A) Patients with an initial normal DST result followed by an abnormal 1-mg DST result (NFAT-to-MACS patients). (B) Patients with an initial abnormal 1 mg DST result followed by a normal test result (MACS-to-NFAT patients). 1 mg DST=1-mg overnight dexamethasone suppression test. MACS=mild autonomous cortisol secretion. NFAT=non-functioning adrenal tumour.

	NFAT-to-NFAT (n=1103)	NFAT-to-MACS (n=222)	MACS-to-NFAT (n=195)	MACS-to-MACS (n=839)	Fluctuating cortisol secretion (n=146)	p value for trend
Sex						
Female	682 (61·8%)	135 (60·8%)	111 (56·9%)	595 (70·9%)	102 (69·9%)	<0·0001
vs NFAT-to-NFAT	..	p=1·00	p=1·00	p=0·0004	p=0·72	..
vs NFAT-to-MACS	..	..	p=1·00	p=0·050	p=0·96	..
vs MACS-to-NFAT	..	..	..	p=0·0022	p=0·20	..
vs MACS-to-MACS	..	..	..	..	p=1·00	..
Male	421 (38·2%)	87 (39·2%)	84 (43·1%)	244 (29·1%)	44 (30·1%)	<0·0001
Ethnicity						
White	1001/1064 (94·1%)*	205/211 (97·2%)*	192/193 (99·5%)*	797/811 (98·3%)*	139/145 (95·9%)*	<0·0001
vs NFAT-to-NFAT	..	p=0·94	p=0·0050	p<0·0001	p=1·00	..
vs NFAT-to-MACS	..	..	p=1·00	p=1·00	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=1·00	p=0·45	..
vs MACS-to-MACS	..	..	..	..	p=1·00	..
Non-White	63/1064 (5·9%)*	6/211 (2·8%)*	1/193 (0·5%)*	14/811 (1·7%)*	6/145 (4·1%)*	<0·0001
Asian	27/1064 (2·5%)*	3/211 (1·4%)*	1/193 (0·5%)*	5/811 (0·6%)*	1/145 (0·7%)*	..
Latino	14/1064 (1·3%)*	0	0	3/811 (0·4%)*	1/145 (0·7%)*	..
Black	13/1064 (1·2%)*	1/211 (0·5%)*	0	3/811 (0·4%)*	0	..
Middle Eastern	5/1064 (0·5%)*	0	0	3/811 (0·4%)*	2/145 (1·4%)*	..
Other	4/1064 (0·4%)*	2/211 (0·9%)*	0	0	2/145 (1·4%)*	..
Age						
Median age, years (IQR)	57 (48–65)	61 (53–67)	61 (56–67)	64 (57–70)	58 (51–67)	<0·0001
vs NFAT-to-NFAT	..	p=0·0008	p<0·0001	p<0·0001	p=0·39	..
vs NFAT-to-MACS	..	..	p=1·00	p=0·0002	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=0·060	p=0·64	..
vs MACS-to-MACS	..	..	..	..	p<0·0001	..
Age ≥65 years	281 (25·5%)	70 (31·5%)	73 (37·4%)	394 (47·0%)	51 (34·9%)	<0·0001
vs NFAT-to-NFAT	..	p=0·75	p=0·0075	p<0·0001	p=0·20	..
vs NFAT-to-MACS	..	..	p=1·00	p=0·0005	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=0·20	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=0·092	..
Baseline health characteristics						
BMI, kg/m²*	27·5 (24·0–32·0)	29·0 (25·7–32·6)	28·2 (24·8–32·7)	27·9 (24·6–31·8)	27·6 (24·4–31·6)	0·086
Current smoking	202/681 (29·7%)*	67/164 (40·9%)*	51/146 (34·9%)*	291/662 (44·0%)*	37/113 (32·7%)*	<0·0001
vs NFAT-to-NFAT	..	p=0·076	p=1·00	p<0·0001	p=1·00	..
vs NFAT-to-MACS	..	..	p=1·00	p=1·00	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=0·57	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=0·33	..
Hypertension	542 (49·1%)	142 (64·0%)	132 (67·7%)	621 (74·0%)	82 (56·2%)	<0·0001
vs NFAT-to-NFAT	..	p=0·0008	p<0·0001	p<0·0001	p=1·00	..
vs NFAT-to-MACS	..	..	p=1·00	p=0·040	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=0·89	p=0·39	..
vs MACS-to-MACS	..	..	..	..	p=0·0002	..
Type 2 diabetes	146 (13·2%)	44 (19·8%)	38 (19·5%)	188 (22·4%)	30 (20·5%)	<0·0001
vs NFAT-to-NFAT	..	p=0·14	p=0·28	p<0·0001	p=0·24	..
vs NFAT-to-MACS	..	..	p=1·00	p=1·00	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=1·00	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=1·00	..

(Table continues on next page)

	NFAT-to-NFAT (n=1103)	NFAT-to-MACS (n=222)	MACS-to-NFAT (n=195)	MACS-to-MACS (n=839)	Fluctuating cortisol secretion (n=146)	p value for trend
(Continued from previous page)						
Dyslipidaemia	303 (27·5%)	72/221 (32·6%)*	72 (36·9%)	319/835 (38·2%)*	45 (30·8%)	<0·0001
vs NFAT-to-NFAT	..	p=1·00	p=0·096	p<0·0001	p=1·00	..
vs NFAT-to-MACS	..	..	p=1·00	p=1·00	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=1·00	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=1·00	..
Chronic kidney disease	61/1065 (5·7%)*	16/214 (7·5%)*	11/191 (5·8%)*	72/799 (9·0%)*	4/133 (3·0%)*	0·021
vs NFAT-to-NFAT	..	p=1·00	p=1·00	p=0·084	p=1·00	..
vs NFAT-to-MACS	..	..	p=1·00	p=1·00	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=1·00	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=0·30	..
Osteoporosis	81/959 (8·4%)*	28/171 (16·4%)*	20/163 (12·3%)*	147/692 (21·2%)*	38/127 (29·9%)*	<0·0001
vs NFAT-to-NFAT	..	p=0·020	p=1·00	p<0·0001	p<0·0001	..
vs NFAT-to-MACS	..	..	p=1·00	p=1·00	p=0·082	..
vs MACS-to-NFAT	..	..	..	p=0·13	p=0·0034	..
vs MACS-to-MACS	..	..	..	..	p=0·42	..
Dementia	4 (0·4%)	1 (0·5%)	1/174 (0·6%)*	5/734 (0·7%)*	0	0·89
Psychiatric diseases	86/985 (8·7%)*	28/194 (14·4%)*	20/178 (11·2%)*	89/741 (12·0%)*	12/127 (9·4%)*	0·076
Previous myocardial infarction or coronary intervention	41 (3·7%)	12 (5·4%)	12 (6·2%)	64 (7·6%)	10 (6·8%)	0·0056
vs NFAT-to-NFAT	..	p=1·00	p=1·00	p=0·0024	p=1·00	..
vs NFAT-to-MACS	..	..	p=1·00	p=1·00	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=1·00	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=1·00	..
Previous hospitalisation for heart failure	5 (0·5%)	2 (0·9%)	0	20 (2·4%)	2 (1·4%)	0·0012
vs NFAT-to-NFAT	..	p=1·00	p=1·00	p=0·0031	p=1·00	..
vs NFAT-to-MACS	..	..	p=1·00	p=1·00	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=0·21	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=1·00	..
Previous atrial fibrillation	39 (3·5%)	13 (5·9%)	9 (4·6%)	54 (6·4%)	5 (3·4%)	0·041
vs NFAT-to-NFAT	..	p=1·00	p=1·00	p=0·043	p=1·00	..
vs NFAT-to-MACS	..	..	p=1·00	p=1·00	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=1·00	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=1·00	..
Previous stroke	21 (1·9%)	5 (2·3%)	5 (2·6%)	31 (3·7%)	1 (0·7%)	0·088
Previous deep vein thrombosis or pulmonary embolism	18 (1·6%)	9 (4·1%)	5 (2·6%)	23 (2·7%)	1 (0·7%)	0·095
At least one previous cardiovascular or thrombosis event	109 (9·9%)	33 (14·9%)	34 (17·4%)	152 (18·1%)	16 (11·0%)	<0·0001
vs NFAT-to-NFAT	..	p=0·38	p=0·029	p<0·0001	p=1·00	..
vs NFAT-to-MACS	..	..	p=1·00	p=1·00	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=1·00	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=0·45	..
Maximum tumour diameter, mm†	20·0 (14·9–25·0)	25·0 (17·0–30·0)	23·0 (16·0–30·0)	27·0 (20·0–34·0)	25·0 (19·0–30·0)	<0·0001
vs NFAT-to-NFAT	..	p<0·0001	p<0·0001	p<0·0001	p<0·0001	..
vs NFAT-to-MACS	..	..	p=1·00	p<0·0001	p=1·00	..
vs MACS-to-NFAT	..	..	..	p<0·0001	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=0·010	..

(Table continues on next page)

	NFAT-to-NFAT (n=1103)	NFAT-to-MACS (n=222)	MACS-to-NFAT (n=195)	MACS-to-MACS (n=839)	Fluctuating cortisol secretion (n=146)	p value for trend
(Continued from previous page)						
Bilateral adrenal lesions	143/1101 (13·0%)*	41 (18·5%)	37 (19·0%)	290 (34·6%)	37 (25·3%)	<0·0001
vs NFAT-to-NFAT	..	p=0·41	p=0·34	p<0·0001	p=0·0011	..
vs NFAT-to-MACS	..	..	p=1·00	p<0·0001	p=1·00	..
vs MACS-to-NFAT	..	..	..	p=0·0004	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=0·37	..
1-mg DST serum cortisol, nmol/L	28 (22–36)	40 (30–45)	63 (55–83)	80 (65–114)	47 (39–60)	<0·0001
vs NFAT-to-NFAT	..	p<0·0001	p<0·0001	p<0·0001	p<0·0001	..
vs NFAT-to-MACS	..	..	p<0·0001	p<0·0001	p<0·0001	..
vs MACS-to-NFAT	..	..	..	p<0·0001	p<0·0001	..
vs MACS-to-MACS	..	..	..	..	p<0·0001	..
Plasma ACTH, ng/L*	15·7 (10·3–22·8); n=617	13·6 (8·4–21·3); n=143	13·8 (8·2–20·9); n=137	9·5 (5·8–14·6); n=629	11·0 (7·0–17·1); n=119	<0·0001
vs NFAT-to-NFAT	..	p=0·23	p=0·58	p<0·0001	p<0·0001	..
vs NFAT-to-MACS	..	..	p=1·00	p<0·0001	p=0·13	..
vs MACS-to-NFAT	..	..	..	p<0·0001	p=0·070	..
vs MACS-to-MACS	..	..	..	..	p=0·26	..
Serum DHEAS, µmol/L*	2·1 (1·2–3·8); n=370	1·4 (0·7–2·4); n=91	1·8 (1·1–2·9); n=82	1·1 (0·5–1·8); n=389	1·6 (0·6–2·7); n=68	<0·0001
vs NFAT-to-NFAT	..	p=0·0006	p=0·10	p<0·0001	p=0·010	..
vs NFAT-to-MACS	..	..	p=1·00	p=0·080	p=1·00	..
vs MACS-to-NFAT	..	..	..	p<0·0001	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p=0·15	..
Serum cortisol-to-DHEAS ratio*	14·2 (7·0–27·2); n=370	25·7 (15·2–57·3); n=91	45·3 (25·7–64·2); n=82	83·9 (44·9–160·6); n=389	36·5 (17·5–97·9); n=68	<0·0001
vs NFAT-to-NFAT	..	p<0·0001	p<0·0001	p<0·0001	p<0·0001	..
vs NFAT-to-MACS	..	..	p=0·040	p<0·0001	p=1·00	..
vs MACS-to-NFAT	..	..	..	p<0·0001	p=1·00	..
vs MACS-to-MACS	..	..	..	..	p<0·0001	..
24-h urinary free cortisol, nmol*	125·0 (72·5–204·6); n=427	125·5 (73·6–209·8); n=117	135·2 (77·3–249·0); n=97	120·0 (72·0–210·2); n=459	155·5 (93·0–250·8); n=90	0·25

Data are n (%) or median (IQR) unless otherwise indicated. Data from 20 patients who developed adrenal Cushing's syndrome during the study are not reported. p values represent post-hoc pairwise comparisons adjusted using the Bonferroni correction. NFAT-to-NFAT refers to patients with persistently normal 1-mg DST results, NFAT-to-MACS refers to patients with an initial normal result followed by an abnormal 1-mg DST result, MACS-to-NFAT refers to patients with an initial abnormal 1-mg DST result followed by a normal result, MACS-to-MACS refers to patients with persistently abnormal 1-mg DST results, and fluctuating cortisol secretion refers to patients with 1-mg DST results that did not fit any of the other categories (eg, normal at baseline, then abnormal, then normal). 1-mg DST=1-mg overnight dexamethasone suppression test. ACTH=adrenocorticotropic hormone. DHEAS=dehydroepiandrosterone sulfate. MACS=mild autonomous cortisol secretion. NFAT=non-functioning adrenal tumour. *Some data are missing; more information about missing data is reported in the appendix (p 44). †For bilateral adrenal tumours, the maximum diameter of the largest tumour was considered.

Table: **Baseline demographic, radiological, and biochemical characteristics of patients stratified by cortisol secretion group**

with fluctuating cortisol secretion had cardiometabolic profiles similar to those of NFAT-to-MACS patients and MACS-to-NFAT patients but displayed the highest osteoporosis rates (figure 3).

During follow-up, 84 (3·3%) of 2525 patients died; the leading causes of death were cancer (n=26), cardiovascular events (n=18), and infections (n=13). Patients with MACS at baseline had higher crude all-cause mortality than patients with NFAT at baseline (HR 2·08 [95% CI 1·34–3·22]; p=0·0012); however, this association was no longer significant after multivariable adjustment (adjusted HR [aHR] 1·44 [0·92–2·26]; p=0·11; appendix p 23). In subgroup analyses by sex and age (younger than 65 years *vs* 65 years or older), male patients younger than 65 years with MACS tended to have a higher risk of all-cause mortality than male patients younger than 65 years with NFAT (aHR 3·83 [0·99–14·89]; p=0·054), but differences between MACS and NFAT groups were not significant for the other age and sex combinations (appendix p 24).

Unadjusted RMST per cortisol secretion group at 7 and 10 years was shorter in MACS-to-MACS patients (77·2 months [71·9–79·5] and 98·4 [90·8–104·5] months, respectively) than in NFAT-to-NFAT patients (81·0 months [75·9–82·5] and 111·5 months [106·1–115·1]), reflecting the older age and greater

baseline cardiometabolic burden in this group (5-year differences are reported in the appendix pp 37–40). MACS-to-MACS patients also had the highest crude all-cause mortality rate compared with NFAT-to-NFAT patients (HR 2·47 [95% CI 1·49–4·10]; p=0·0005); however, this association was not statistically significant after multivariable adjustment (aHR 1·56 [0·92–2·63]; p=0·091; appendix p 25), which is consistent with the higher baseline burden of age and cardiometabolic risk factors in MACS-to-MACS patients being the primary driver of the observed crude mortality difference.

Factors independently associated with increased all-cause mortality were a history of cardiovascular and thrombotic events at baseline, chronic kidney disease, smoking, older age, and higher post-1-mg DST cortisol concentration at baseline. By contrast, bilateral adrenal tumours were associated with a reduced mortality rate (figure 4A, B).

Patients with MACS at baseline had a higher crude rate of developing cardiovascular or thrombotic events than patients with NFAT at baseline (HR 1·53 [95% CI 1·15–2·03]; p=0·0029), although this finding was no longer significant after multivariable adjustment (appendix p 26), and no significant results were seen by sex and age (appendix p 27). The highest rate of cardiovascular or thrombotic events was observed in MACS-to-MACS patients versus NFAT-to-NFAT patients (HR 1·59 [95% CI 1·15–2·21]; p=0·0053); however, this association was not statistically significant after multivariable adjustment (aHR 1·15 [0·82–1·61]; p=0·41; appendix p 28). Unadjusted restricted mean time to a cardiovascular or thrombotic event was shorter in MACS-to-MACS patients at 7 years (74·5 months [95% CI 66·4–77·1]) and 10 years (89·5 months [80·6–96·5]) than in NFAT-to-NFAT patients (80·4 months [72·2–81·7] and 106·4 months [100·5–110·1]; 5-year differences are reported in the appendix pp 37–40), consistent with the higher baseline risk in this group. Multivariable analysis showed that history of cardiovascular or thrombotic events, smoking, chronic kidney disease, and age were independently associated with higher rates of new-onset cardiovascular or thrombotic events (figure 4C, D).

A higher baseline post-1-mg DST cortisol concentration was associated with higher crude rates of mortality and cardiovascular or thrombotic events; each 10 nmol/L increase higher than 50 nmol/L was linked to a 6·4% increase in mortality and a 2·5% increase in cardiovascular or thrombotic events (appendix p 29). Similar associations were observed for cumulative cortisol exposure over 24 months (appendix p 29).

In multivariable models, a baseline diagnosis of MACS was independently associated with a higher rate of worsening hypertension during follow-up (figure 5A) compared with a baseline diagnosis of NFAT. A similarly higher rate was observed in MACS-to-MACS patients compared with NFAT-to-NFAT patients (figure 5B). Additionally, restricted mean time to worsening hypertension was shorter in MACS-to-MACS patients at 5 years (50·0 months [46·1–52·7]), 7 years (57·0 months [50·6–61·1]), and 10 years (60·4 months [56·8–75·5]) compared with NFAT-to-NFAT patients (54·0 months [50·6–55·6] at 5 years, 64·1 months [59·5–67·8] at 7 years, and 86·1 months [79·1–93·4] at 10 years; appendix pp 37–40). Rates of hypertension and type 2 diabetes development were not significantly different between patients with MACS and patients with NFAT at baseline (appendix p 31). Patients with fluctuating cortisol secretion had the lowest rate of hypertension development (aHR 0·22 [0·05–0·91]; p=0·039; appendix p 32).

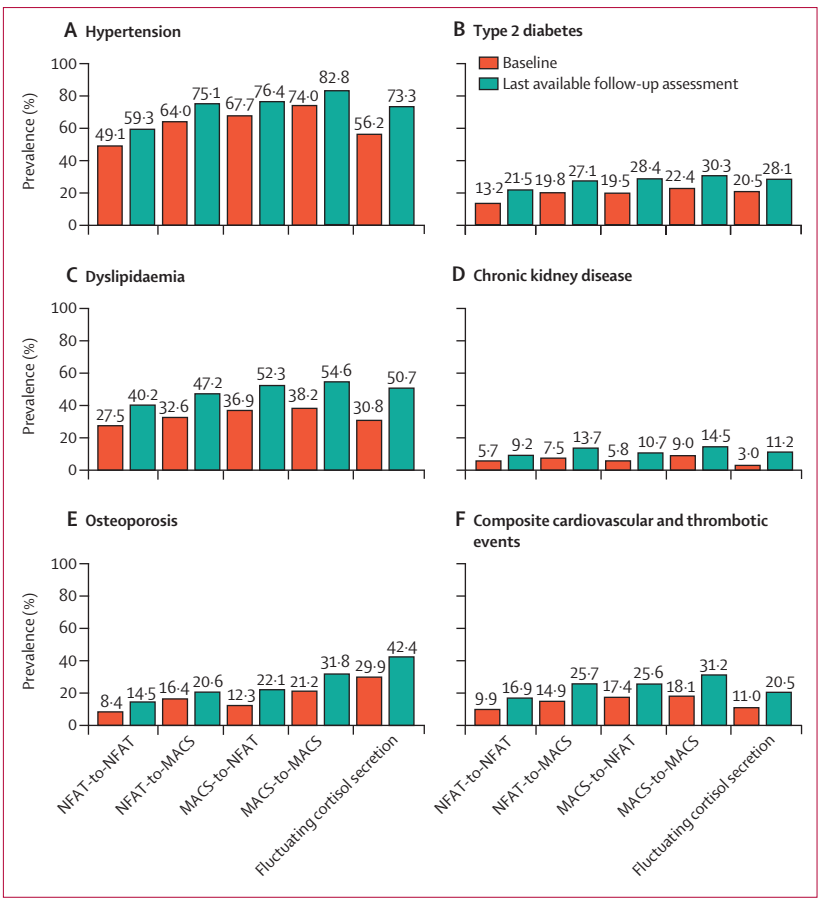

Figure 3: **Prevalence of cardiometabolic morbidity during the study**
Prevalence of hypertension (A), type 2 diabetes (B), dyslipidaemia (C), chronic kidney disease (D), osteoporosis (E), and composite cardiovascular and thrombotic events (F) at baseline (red) and last available follow-up assessment (green). In patients undergoing surgery, clinical information was recorded before adrenalectomy. NFAT-to-NFAT refers to patients with persistently normal 1-mg DST results, NFAT-to-MACS refers to patients with an initial normal result followed by an abnormal 1-mg DST result, MACS-to-NFAT refers to patients with an initial abnormal 1-mg DST result followed by a normal result, MACS-to-MACS refers to patients with persistently abnormal 1-mg DST results, and fluctuating cortisol secretion refers to patients with 1-mg DST results that did not fit any of the other categories (eg, normal at baseline, then abnormal, then normal). MACS=mild autonomous cortisol secretion. NFAT=non-functioning adrenal tumour.

Discussion

Approximately one in five patients with benign adrenal incidentalomas undergoing repeated 1-mg DSTs had changes in cortisol suppression status over time, most

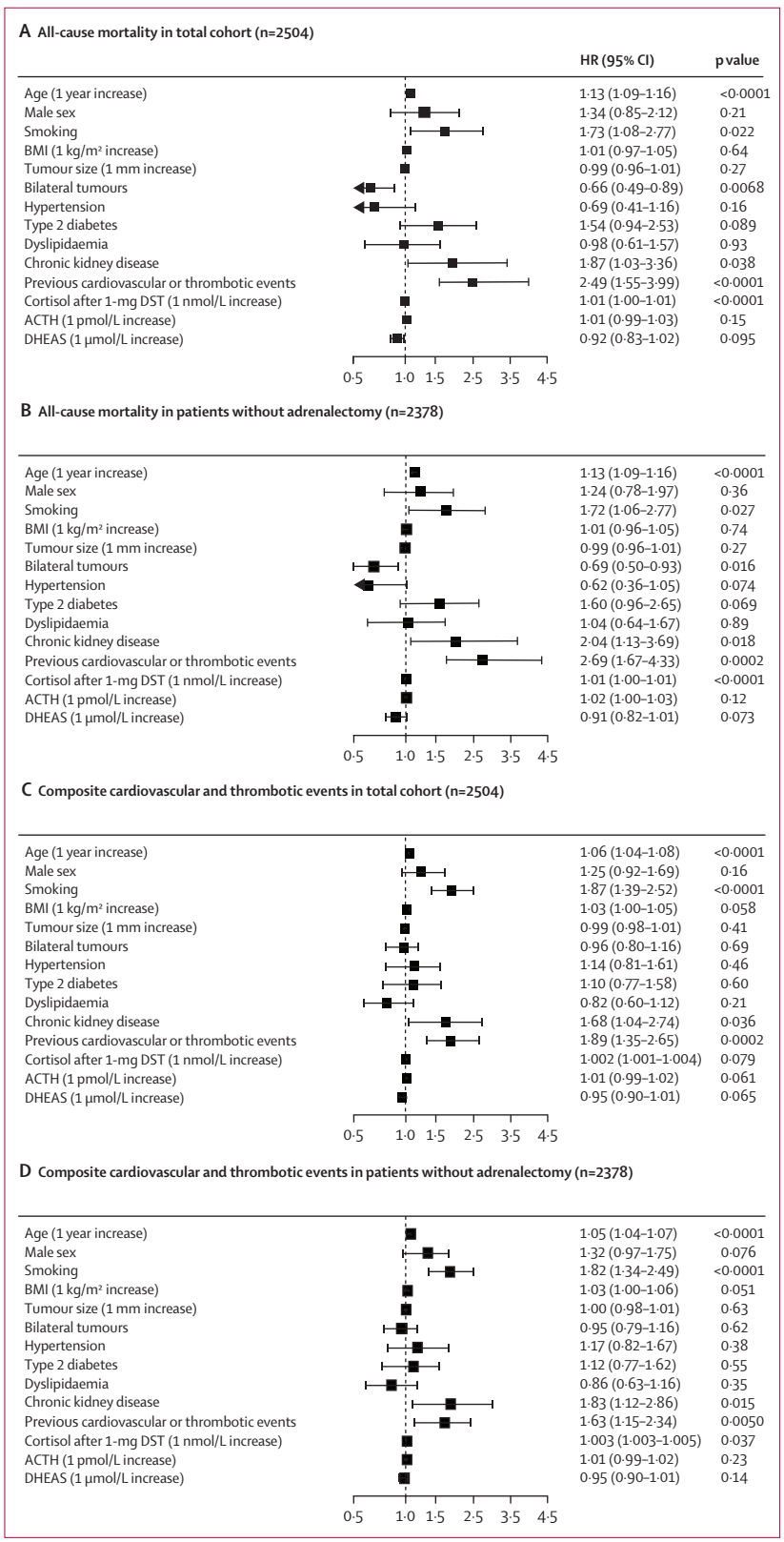

Figure 4: **Factors associated with all-cause mortality and composite cardiovascular and thrombotic events**

Forest plots from multivariable Cox proportional hazards models showing factors associated with all-cause mortality (A, B) and composite cardiovascular and thrombotic events (C, D). Results are reported for the entire cohort (A, C; n=2504) and after excluding patients who underwent adrenalectomy (B, D; n=2378). Arrows indicate 95% CIs falling outside the x-axis range. 1-mg DST=1-mg overnight dexamethasone suppression test. ACTH=adrenocorticotropic hormone. DHEAS=dehydroepiandrosterone sulfate.

frequently within the first 3 years following diagnosis. Patients with persistently abnormal 1-mg DST (MACS-to-MACS patients) were older, had the greatest cardiometabolic burden at baseline and during follow-up, and were most likely to have worsening hypertension compared with NFAT-to-NFAT patients. In time-to-event analyses, these patients had shorter event-free times than those with persistently normal 1-mg DST results (NFAT-to-NFAT), particularly for worsening hypertension; however, differences in survival between NFAT-to-NFAT and MACS-to-MACS patients were modest, and no significant associations with mortality or cardiovascular or thrombotic events were observed after multivariable adjustment. These findings are consistent with higher baseline cardiometabolic risk being the primary determinant of adverse outcomes in MACS-to-MACS patients, although the independent contribution of cortisol status to long-term prognosis remains to be established in prospective studies. Independent predictors of adverse outcomes included smoking, chronic kidney disease, and previous cardiovascular or thrombotic events, which underscores the importance of proactive management of modifiable risk factors in patients with these risk factors.

Our findings add to the existing literature on the natural history of adrenal incidentalomas. Previous smaller cohort studies and meta-analyses reported NFAT-to-MACS progression rates from 4% to 28%, with substantial heterogeneity attributable to differing diagnostic thresholds, follow-up duration, and sample sizes.[10–13,15] By applying contemporary guideline-based definitions, our study suggests 1-mg DST-based cortisol suppression categories could change over time in a substantial proportion of patients. Apparent transitions between categories are expected when classification relies on a fixed post-1-mg DST threshold, and most patients in these groups had cortisol concentrations close to 50 nmol/L, which suggests biological and analytical variability rather than true changes in cortisol autonomy (appendix p 43). By contrast, MACS-to-MACS patients had post-1-mg DST cortisol concentrations at baseline and follow-up that were substantially higher than the diagnostic threshold, which supports a more consistent biochemical phenotype over time.

Persistent MACS was more frequently observed in female patients, people who smoke, and patients with

bilateral tumours, which suggests that these factors might predispose individuals to sustained cortisol autonomy. We observed an age-related increase in post-1-mg DST cortisol concentrations, in line with previous reports,[9,16,17] but this increase was modest and confined to patients with NFAT at baseline. The estimated annual increase of 0·25 nmol/L (95% CI 0·20–0·29; p<0·0001) in this group is unlikely to explain the incident transitions to MACS, as patients with MACS had similarly consistent elevated cortisol concentrations irrespective of age. Our results differ from a South Korean study reporting increasing post-1-mg DST cortisol with age, in both patients with NFAT and patients with MACS,[18] which could reflect differences in study design and population characteristics.

Consistent with previous reports[6,9,12] and meta-analyses,[15,19] cardiometabolic burden was considerable and increased over a median follow-up exceeding 6 years. MACS-to-MACS patients had the highest prevalence and incidence of cardiometabolic disease, paralleling cumulative cortisol exposure. Baseline post-1-mg DST cortisol and cumulative cortisol exposure were associated with mortality and cardiovascular and thrombotic events in unadjusted analyses; however, these associations were not independent of established cardiovascular risk factors after multivariable adjustment.

MACS-to-NFAT patients had intermediate risk profiles, with higher cardiometabolic burden and cortisol exposure than NFAT-to-NFAT patients, but smaller tumours, lower post-1-mg DST cortisol concentrations, higher ACTH concentrations, and higher DHEAS concentrations than MACS-to-MACS patients. These features suggest a less clear-cut phenotype of ACTH-independent cortisol excess and highlight biological heterogeneity within patients with MACS. In this context, repeat 1-mg DSTs might be informative in patients with borderline biochemical evidence of autonomous cortisol secretion, particularly when adrenalectomy is being considered.

Although associations with all-cause mortality and cardiovascular or thrombotic events were not significant after adjustment for established cardiovascular risk factors (consistent with the baseline cardiometabolic burden being the primary determinant of adverse outcomes in this cohort), some of the covariates could themselves be affected by cortisol excess, and residual confounding in either direction cannot be excluded. RMST analyses showed similar short-term survival (5 years) across cortisol secretion groups but progressively shorter RMSTs in MACS-to-MACS patients over longer follow-up, corresponding to differences of 13·1 months for all-cause mortality and 16·9 months for cardiovascular and thrombotic events at 10 years compared with NFAT-to-NFAT patients. These differences describe the observed outcome trajectories of clinically distinct patient groups and do not imply an independent effect of cortisol status.

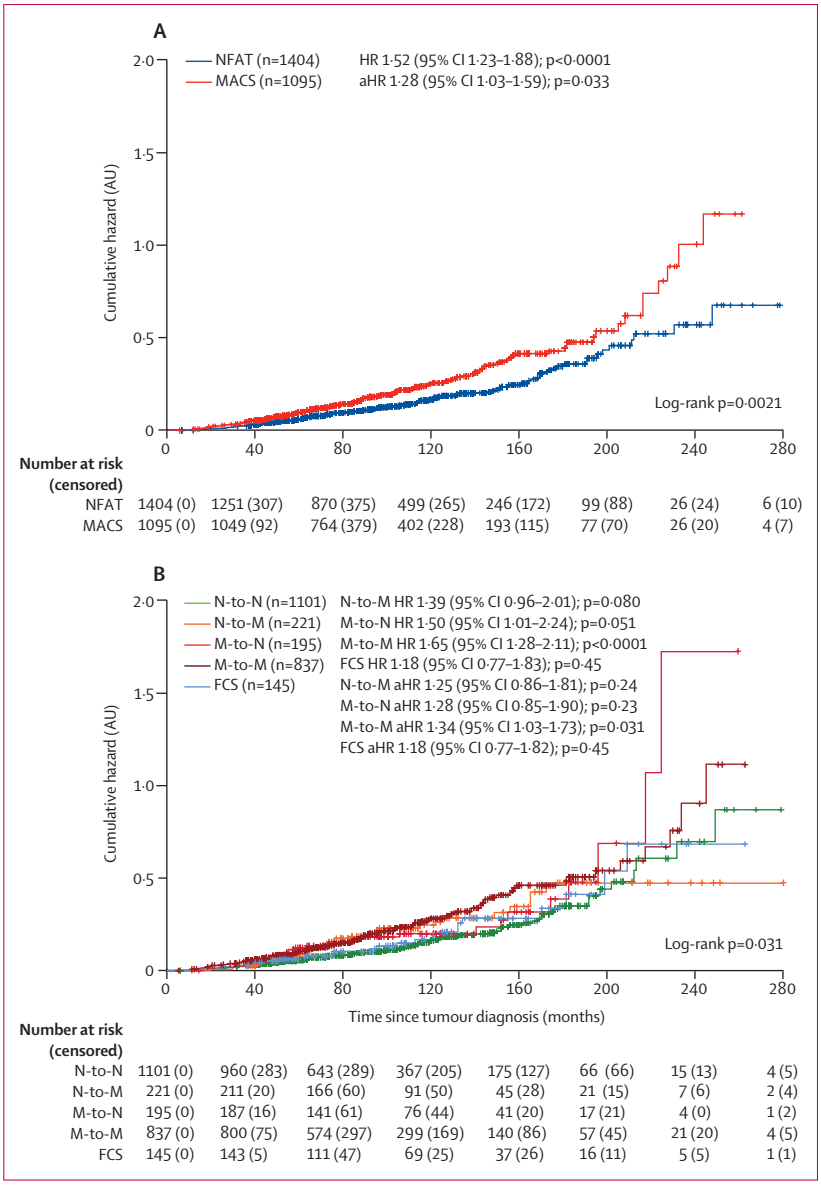

Figure 5: **Hypertension worsening according to baseline and incident 1-mg DST results**
Kaplan–Meier curves of hypertension worsening in the full cohort, where only patients with pre-existing hypertension at baseline contributed to the Kaplan–Meier curves and Cox regression models. (A) Hypertension worsening in patients with MACS compared with patients with NFAT (number of patients with pre-existing hypertension: NFAT n=731, MACS n=803; number of patients with worsening hypertension during follow-up: NFAT n=163, MACS n=184). (B) Hypertension worsening stratified by incident 1-mg DST results (N-to-N was used as the reference group for each comparison; number of patients with pre-existing hypertension: N-to-N n=542, N-to-M n=142, M-to-N n=132, M-to-M n=621; FCS n=82; number of patients with worsening hypertension during follow-up: N-to-N n=113, N-to-M n=38, M-to-N n=31, M-to-M n=140, FCS n=25). Numbers at risk represent the total study population at each timepoint (including patients without pre-existing hypertension, who did not contribute to events or censoring); only patients with pre-existing hypertension could experience the outcome of hypertension worsening, with each such patient contributing a maximum of one event. Censored counts represent cumulative censoring events across follow-up timepoints and might therefore exceed the number of unique patients censored. aHRs were calculated using multivariable Cox regression analysis adjusted for age, smoking status, chronic kidney disease, baseline history of cardiovascular or thrombotic events, baseline 1-mg DST serum cortisol, and baseline serum DHEAS. 1-mg DST=1-mg overnight dexamethasone suppression test. aHR=adjusted hazard ratio. AU=arbitrary units. FCS=fluctuating cortisol secretion patients. MACS=mild autonomous cortisol secretion. M-to-M=MACS-to-MACS patients. M-to-N=MACS-to-NFAT patients. NFAT=non-functioning adrenal tumour. N-to-M=NFAT-to-MACS patients. N-to-N=NFAT-to-NFAT patients.

The leading causes of death observed in this study—cancer, cardiovascular events, and infections—are consistent with previous studies,[8,9,17] and indicate that excess mortality in patients with MACS extends beyond cardiovascular disease. Emerging evidence suggests links between MACS and immune dysregulation, which might contribute to infection and cancer risk;[20] however, reverse causation, whereby severe illness activates the hypothalamic–pituitary–adrenal axis, cannot be excluded.

Age-specific and sex-specific analyses showed patterns that only partly align with previous reports. Whereas earlier studies described increased mortality predominantly among younger female patients with MACS,[9] we observed a higher mortality signal in male patients aged younger than 65 years with MACS versus those with NFAT (aHR 3·83 [95% CI 0·99–14·89]; p=0·054). This finding might reflect differences in cohort composition and cardiovascular risk profiles, as male patients in our cohort had a greater risk factor burden and more than twice the prevalence of previous cardiovascular and thrombotic events compared with female patients (appendix p 41). Previous studies found increased mortality in patients with NFAT aged younger than 65 years compared with control participants without adrenal adenoma, but no sex difference.[21]

Unexpectedly, patients with bilateral tumours had lower mortality rates (aHR 0·66 [95% CI 0·49–0·89]; p=0·0068), despite being older, more often smokers, and having higher rates of metabolic syndrome, larger adrenal tumours, and higher post-1-mg DST cortisol compared with patients with unilateral tumours (appendix p 42). This finding, which has not been observed in previous studies,[8,12] should be interpreted cautiously given the small number of deaths in this group (eight, including six patients with MACS at baseline) and the potential for immortal time bias. Enhanced longitudinal monitoring and earlier management of cardiometabolic risk factors in patients perceived to be at higher risk[6] could also have contributed to the lower mortality observed in this group.

Rates of newly diagnosed hypertension or type 2 diabetes did not differ significantly across 1-mg DST cortisol secretion groups; however, among patients with pre-existing hypertension, MACS-to-MACS patients were the most likely group to have worsening blood pressure control. These findings are consistent with recent randomised controlled trials showing that adrenalectomy can improve blood pressure control in patients with MACS,[22,23] and that support consideration of surgery in selected patients with poorly controlled hypertension.

Several limitations should be acknowledged. The retrospective design, non-standardised frequency and timing of hormonal and clinical assessments, and the lack of centralisation of biochemical tests could have introduced variability in the estimation of cumulative cortisol exposure, particularly if patients undergoing more frequent testing differed clinically from those with fewer assessments. Extended intervals between 1-mg DSTs could have reduced sensitivity for detecting longitudinal changes in autonomous cortisol secretion. Residual confounding related to comorbidity management cannot be excluded, and the predominantly White composition of the cohort (2354 [96·3%] of 2444 patients with available data; appendix p 33) limits generalisability. Prospective studies on ethnically diverse cohorts, with standardised testing intervals and rigorous sample handling, are needed to support our findings.

The minimum follow-up requirement of 36 months introduced immortal time bias, potentially attenuating observed mortality rates. Consistent with this bias, the overall mortality rate in our cohort (84 [3·3%] of 2525 patients) was lower than previously reported (352 [9·6%] of 3656 patients),[9] which could have limited statistical power. As MACS is a chronic condition, however, and treatment strategies aim to reduce long-term cardiometabolic risk, 1-mg DSTs are not routinely done in patients with short life expectancy;[4] therefore, our cohort probably reflects real-world practice.

All potential causes of false-positive 1-mg DST results cannot be excluded. Cortisol assay changes in seven centres during the study period (appendix p 45) and the low availability of serum dexamethasone concentrations (94 [3·7%] of 2525 patients) are acknowledged; however, baseline ACTH was available in 831 (74·8%) of 1111 patients with MACS supporting ACTH-independent cortisol excess. Inhaled glucocorticoids and moderate CYP3A4 inhibitors did not significantly affect post-1-mg DST cortisol concentrations (appendix p 16), supporting reliable test interpretation in patients receiving these medications.

The strict inclusion criteria could have introduced selection bias by favouring centres that routinely repeated 1-mg DSTs. Moreover, repeat testing might have been more likely in patients with worsening clinical status. Nevertheless, our cohort remained otherwise unselected, as repeating 1-mg DSTs reflected standard clinical practice under earlier guidelines. In 2009, guidelines recommended annual hormonal assessment for up to 5 years,[24] and recommendations against routine repeat testing were issued only in 2016 and 2019.[25,26] Most patients in our cohort (1775 [70·3%] of 2525) received their incidentaloma diagnosis before 2016, when having repeat 1-mg DSTs was standard practice.

Strengths of this study include its large sample size, international multicentre design, extended follow-up period, and serial hormonal measurements. To our knowledge, this is the first study to quantify cumulative cortisol exposure from serial 1-mg DSTs and to link this cortisol exposure directly to long-term clinical outcomes.

In conclusion, longitudinal changes in 1-mg DST results are frequent in benign adrenal incidentalomas.

Patients with persistent MACS had the greatest cardiometabolic burden and shorter event-free time, particularly for worsening hypertension. Rates of mortality and cardiovascular and thrombotic events were not significantly associated with 1-mg DST trajectories after multivariable adjustment, which is consistent with baseline cardiometabolic burden being the primary determinant of long-term outcomes in this study population. These findings identify patients with persistently abnormal 1-mg DST as a group with increased cardiometabolic risk, in whom optimisation of modifiable risk factors might be particularly relevant within standard care. The clinical significance of repeated hormonal assessment for risk stratification requires more evaluation in prospective studies.

Contributors

OS, GR, and APr designed the study. Except for MG and VA, all authors collected clinical data from patients. OS, MG, and VA had full access to all the data in the study and performed the statistical analyses. GR and APr directly accessed and verified the underlying data reported in the manuscript and supervised the statistical analyses. OS, GR, and APr wrote the first draft of the manuscript. All authors contributed to writing the manuscript, had full access to all the data in the study, and had final responsibility for the decision to submit for publication.

Declaration of interests

ML is currently an employee of Eli Lilly and Company; the research presented in this publication was conducted before the author's employment at Eli Lilly and Company. Eli Lilly and Company had no role in the study design, data collection, analysis, or interpretation.
FCe received consulting fees and honoraria from Recordati and Esteve. KIA holds research contracts with Recordati; received consulting fees, honoraria, and support for attending meetings from Recordati; and participated in advisory boards for Recordati. HF holds a higher clinical researcher position funded by Karolinska Institutet, Stockholm Läns Landsting. AMI holds research contracts with Crinetics, Neurocrine, Corcept, and Recordati; received consulting fees from Corcept and Recordati; and received honoraria from Corcept, Recordati, and Esteve. CLR holds research contracts with Esteve. IB holds research contracts with Recordati, Esteve, and the National Institute of Diabetes and Digestive and Kidney Diseases; holds royalties with Elsevier; served as a consultant for Recordati, Corcept, Novo Nordisk, Xesir, and Esteve; received support for attending meetings from Esteve; and submitted a patent in the field of mild autonomous cortisol secretion.
GM participated in advisory boards for Recordati. TD received honoraria and support for attending meetings from Recordati. MT received honoraria from Esteve and Recordati and participated in advisory boards for Esteve and Recordati. APr holds research contracts with Neurocrine, Recordati, and Esteve; served as a consultant for Neurocrine, Lundbeck, Corcept, and Recordati; and received honoraria from Neurocrine and Recordati. All other authors declare no competing interests.

Data sharing

We will consider sharing deidentified, individual participant-level data that underlie the results reported in this Article on receipt of a request detailing the study hypothesis and statistical analysis plan. All requests should be sent to the corresponding author. The corresponding author and lead investigators of this study will discuss all requests and make decisions about whether data sharing is appropriate based on the scientific rigour of the proposal. All applicants will be asked to sign a data access agreement.

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