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Age-dependent and sex-dependent disparity in mortality in patients with adrenal incidentalomas and autonomous cortisol secretion: an international, retrospective, cohort study

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Age-dependent and sex-dependent disparity in mortality in patients with adrenal incidentalomas and autonomous cortisol secretion: an international, retrospective, cohort study / Deutschbein T.; Reimondo G.; Di Dalmazi G.; Bancos I.; Patrova J.; Vassiliadi D.A.; Nekic A.B.; Debono M.; Lardo P.; Ceccato F.; Petramala L.; Prete A.; Chiodini I.; Ivovic M.; Pazaitou-Panayiotou K.; Alexandraki K.I.; Hanzu F.A.; Loli P.; Yener S.; Langton K.; Spyroglou A.; Kocjan T.; Zacharieva S.; Valdes N.; Ambroziak U.; Suzuki M.; Detomas A.; Pappas S.; Tucci L.; Delivanis D.A.; Margaritopoulos D.; Dusek T.; Maggio R.; Scaroni C.; Concistre A.; Vassiliadi D.A.; Mosconi G.; Diamantopoulos A.; Jisnez-Ariza N.M.; Vicennati V.; Pia A.; Kroiss M.; Kaltsas G.; Chrisoulidou A.; Marina L.V.; Morelli V.; Arlt W.; Letizia C.; Boscaro M.; Stigliano A.; Kastelan D.; Tsagarakis S.; Athimulam S.; Pagotto U.; Maeder U.; Falhammar H.; Newell-Price J.; Terzolo M.; Fassnacht M. In: THE LANCET DIABETES & ENDOCRINOLOGY. - ISSN 2213-8587. - ELETTRONICO. - 10:7(2022), pp. 499-508. [10.1016/S2213-8587(22)00100-0]

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Age-dependent and sex-dependent disparity in mortality in patients with adrenal incidentalomas and autonomous cortisol secretion: an international, retrospective, cohort study,

The Lancet Diabetes & Endocrinology,

Volume 10, Issue 7,

2022,

Pages 499-508,

ISSN 2213-8587

The final published version is available online at:

[https://doi.org/10.1016/S2213-8587\(22\)00100-0](https://doi.org/10.1016/S2213-8587(22)00100-0)

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Age- and sex-dependent disparity in mortality in patients with adrenal incidentalomas and autonomous cortisol secretion: an international cohort study

Authors:

Timo Deutschbein, MD (1, 2)*, Giuseppe Reimondo, MD (3)*, Guido Di Dalmazi, MD (4,5), Irina Bancos, MD (6), Jekaterina Patrova, MD (7), Dimitra Argyro Vassiliadi, MD (8), Anja Barač Nekić, MD (9), Miguel Debono, MD (10), Pina Lardo, MD (11), Filippo Ceccato, MD (12), Luigi Petramala, MD (13), Alessandro Prete, MD (14,15,16), Iacopo Chiodini, MD (17), Miomira Ivović, MD (18), Kalliopi Pazaitou-Panayiotou, MD (19), Krystallenia I Alexandraki, MD (20), Felicia Alexandra Hanzu, MD (21), Paola Loli, MD (22), Prof. Serkan Yener, MD (23), Katharina Langton, MD (24), Ariadni Spyroglou, MD (25,26), Tomaz Kocjan, MD (27,28), Prof. Sabina Zacharieva, MD (29), Nuria Valdés, MD (30,31,32), Urszula Ambroziak, MD (33), Mari Suzuki, MD (34), Mario Detomas, MD (1), Soraya Puglisi, MD (3), Lorenzo Tucci, MD (4,5), Danae Anastasia Delivanis, MD (6), Dimitris Margaritopoulos, MD (8), Tina Dusek, MD (9), Roberta Maggio, MD (11), Carla Scaroni, MD (12), Antonio Concistrè, MD (13), Cristina Lucia Ronchi, MD (1,14,15,16), Barbara Altieri, MD (1), Cristina Mosconi, MD (5,35), Aristidis Diamantopoulos, MD (8), Nicole Marie Iñiguez-Ariza, MD (6,36), Valentina Vicennati, MD (4,5), Anna Pia, MD (3), Prof. Matthias Kroiss, MD (1,26), Prof. Gregory Kaltsas, MD (20), Alexandra Chrisoulidou, MD (19), Ljiljana Marina, MD (18), Valentina Morelli, MD (37), Prof. Wiebke Arlt, MD (14,15,16,38), Claudio Letizia, MD (13), Prof. Marco Boscaro, MD (12), Antonio Stigliano, MD (11), Prof. Darko Kastelan, MD (9), Stylianos Tsagarakis, MD (8), Shobana Athimulam, MD (6,39), Prof. Uberto Pagotto, MD (4,5), Uwe Maeder, PhD (40), Henrik Falhammar, MD (41,42), Prof. John Newell-Price, MD (10), Prof. Massimo Terzolo, MD (3)[#], Prof. Martin Fassnacht, MD (1,40)[#] (* joint first author; [#] joint last author)

Author affiliations:

- (1) Department of Internal Medicine I, Division of Endocrinology and Diabetes, University Hospital, University of Würzburg, Würzburg, Germany
- (2) Medicover Oldenburg MVZ, Oldenburg, Germany
- (3) Department of Clinical and Biological Sciences, San Luigi Hospital, University of Turin, Turin, Italy
- (4) Unit of Endocrinology and Diabetes Prevention and Care, Division of Endocrinology, Department of Medical and Surgical Sciences, Alma Mater University of Bologna, S. Orsola-Malpighi Hospital, Bologna, Italy
- (5) IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
- (6) Division of Endocrinology, Diabetes and Metabolism, Mayo Clinic, Rochester, MN, USA
- (7) Department of Clinical Science and Education, Södersjukhuset AB, Karolinska Institutet, Stockholm, Sweden
- (8) Department of Endocrinology, Diabetes and Metabolism, National Expertise Centre for Rare Endocrine Diseases, Evangelismos Hospital, Athens, Greece
- (9) Department of Endocrinology, University Hospital Centre Zagreb, Zagreb, Croatia
- (10) Department of Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom
- (11) Endocrinology, Department of Clinical and Molecular Medicine, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy
- (12) Endocrinology Unit, Department of Medicine DIMED, University-Hospital of Padova, Padova, Italy
- (13) Second Hypertension Unit, Department of Translational and Precision Medicine, University Sapienza, Rome, Italy
- (14) Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, United Kingdom

- (15) Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham, United Kingdom
- (16) Department of Endocrinology, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom
- (17) Istituto Auxologico Italiano, IRCCS, Milan, Italy; University of Milan, Milan, Italy
- (18) Clinic for Endocrinology, Diabetes and Metabolic Diseases, University Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia
- (19) Department of Endocrinology, Theagenio Cancer Hospital, Thessaloniki, Greece
- (20) 1st Department of Propaedeutic and Internal Medicine, Endocrine Oncology Unit, Laiko Hospital, National and Kapodistrian University of Athens, Athens, Greece
- (21) Endocrinology and Nutrition, Hospital Clinic de Barcelona, Barcelona, Spain
- (22) Department of Endocrinology, Ospedale Niguarda Cà Granda, Milan, Italy
- (23) Department of Endocrinology, Dokuz Eylul University School of Medicine, Izmir, Turkey
- (24) Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Dresden, Dresden, Germany
- (25) Klinik für Endokrinologie, Diabetologie und Klinische Ernährung, Universitäts-Spital Zürich, Zürich, Switzerland
- (26) University Hospital Munich, Ludwig-Maximilians-Universität München, Munich, Germany
- (27) Department of Endocrinology, Diabetes and Metabolic Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia
- (28) Department of Internal Medicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
- (29) Department of Endocrinology, University Hospital of Endocrinology, Medical University, Sofia, Bulgaria
- (30) Hospital Universitario Central de Asturias, Oviedo, Spain
- (31) Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain
- (32) Hospital Universitario de Cabueñes, Gijón, Spain
- (33) Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Warsaw, Poland
- (34) Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD), NIH, Bethesda, MD, USA
- (35) Diagnostic and Interventional Radiology Unit, Department of Diagnostic and Preventive Medicine, Alma Mater Studiorum University of Bologna, Bologna, Italy
- (36) Department of Endocrinology and Metabolism, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
- (37) Unit of Endocrinology, Fondazione IRCCS Cà Granda-Ospedale Maggiore, Milan, Italy
- (38) NIHR Birmingham Biomedical Research Centre, University of Birmingham and University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom.
- (39) Division of Endocrinology, Diabetes, Bone and Mineral Disorders, Henry Ford Health System, Detroit, MI, USA
- (40) Comprehensive Cancer Center Mainfranken, University of Würzburg, Würzburg, Germany
- (41) Department of Endocrinology, Karolinska University Hospital, Stockholm, Sweden
- (42) Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

Corresponding author:

Martin Fassnacht, MD
Department of Internal Medicine I
Division of Endocrinology and Diabetes
University Hospital
University of Würzburg
Oberdürrbacherstr. 6
97080 Würzburg, Germany
e-mail: fassnacht_m@ukw.de

1 **Abstract**

2 **Background.** The association between cortisol secretion and mortality in patients with adrenal
3 incidentalomas is controversial. This study aimed to assess all-cause mortality, prevalence of
4 comorbidities, and occurrence of cardiovascular (CV) events in uniformly stratified patients with
5 cortisol autonomy.

6 **Methods.** The Non-Aldosterone-Producing Adrenocortical Adenoma (NAPACA) Outcome study is
7 an international retrospective multi-centre cohort study investigating the effects of cortisol autonomy
8 (defined as non-suppressible serum cortisol on dexamethasone-suppression testing) on mortality and
9 CV morbidity in patients with adrenal incidentalomas. Patients with clinically apparent hormone
10 excess, active malignancy, or follow-up <36 months were excluded. Patients were stratified according
11 to the 0800-0900h serum cortisol values after a 1 mg dexamethasone-suppression test (<50nmol/L,
12 non-functioning adenoma (NFA); 50-138nmol/L, possible Autonomous Cortisol Secretion (PACS);
13 >138nmol/L, ACS). The primary study endpoint was all-cause mortality. Secondary endpoints were
14 prevalence of cardiometabolic comorbidities, CV events, and cause-specific mortality.

15 **Findings.** 3656 patients (57% NFA, 36% PACS, 7% ACS) were included (64% women; median age
16 61 years; median follow-up 7.0 years). During follow-up, 352 patients (9.6%) died. All-cause
17 mortality (adjusted for age, sex, comorbidities, and former CV events) was significantly increased in
18 PACS (HR 1.52; 95%CI 1.19-1.94) and ACS (1.77; 1.20-2.62). In women <65 years, ACS was
19 associated with higher mortality compared to NFA (HR 4.37; 95%CI 1.93-9.91), while in men this
20 was not observed. Cardiometabolic comorbidities were significantly less frequent in NFA than in
21 PACS and ACS (hypertension: n=1186 (59%), n=944 (74%), n=179 (75%); dyslipidaemia: n=724
22 (36%), n=547 (44%), n=123 (52%); diabetes: n=365 (18%), n=288 (23%), n=62 (27%); always
23 p<0.001).

24 **Interpretation.** Cortisol autonomy is associated with increased all-cause mortality, especially in
25 women <65 years. However, until results from randomised interventional trials will be available, a
26 conservative therapeutic approach seems to be justified in most patients with adrenal incidentaloma.

27 **Funding.** Deutsche Forschungsgemeinschaft, Associazione Italiana per la Ricerca sul Cancro,
28 Università di Torino.

29 **Research in context**

30

31 Evidence before this study

32 Adrenal incidentalomas are found in at least 3% of adults. In up to 50% of these individuals, endocrine
33 investigation identifies evidence of biochemical hypercortisolism without clinically overt
34 glucocorticoid excess, a condition historically described as 'subclinical Cushing syndrome'. During
35 preparation of the European Society of Endocrinology / European Network for the Study of Adrenal
36 Tumours (ENSAT) Clinical Guidelines on Management of Adrenal Incidentalomas (2016), a
37 comprehensive literature search was performed, using three well established databases (i.e., Pubmed,
38 NHS Economic Evaluation Database (NHSEED), and Cochrane Database of Systematic Reviews and
39 Database of Abstracts of Reviews of Effects), from January 1, 2000, to November 30, 2014, to
40 identify all systematic reviews and studies that had assessed any association between autonomy of
41 cortisol secretion (defined as non-suppressible serum cortisol on dexamethasone-suppression testing)
42 with morbidity and mortality. This search revealed only two small studies, that together summarized
43 404 patients (including only 39 deaths), showing an increased mortality in patients with unsuppressed
44 cortisol after dexamethasone. To confirm or refute this association, we initiated the present study
45 under the auspices of ENSAT. Due to the lack of available multi-centre data for a sound power
46 calculation, we aimed initially at the collection of data from at least 2000 patients. In 2021, we
47 updated our previous literature search (now covering the period from December 1, 2014, to July 31,
48 2021), and identified a systematic review and a Swedish cohort study, published in 2020 and 2021,
49 respectively. The review based on 1356 patients from nine studies and could not confirm the claimed
50 association between cortisol autonomy and mortality, whereas the new cohort study with 1048 patients
51 found increased mortality in patients in whom serum cortisol after dexamethasone was >83 nmol/L. In
52 our current study, our pre-determined diagnostic criteria were those used in the above-mentioned
53 guideline. We stratified, therefore, the patients according to the serum cortisol value after the 1 mg
54 overnight dexamethasone-suppression test as having 'autonomous cortisol secretion' (ACS: >138
55 nmol/L), 'possible ACS' (PACS: 50-138 nmol/L), and 'non-functioning adenoma' (NFA: <50 nmol/L).

56 Added value of this study

57 Our large retrospective international cohort study with more than 3600 patients with adrenal adenomas
58 and a follow-up of at least three years (median 7 years) provides additional strong evidence for an
59 overall association between PACS and ACS with all-cause mortality. For the first time our study
60 indicates that this risk varies by age and sex. Women below the age of 65 years with ACS bear the
61 highest relative risk of death with an adjusted hazard ratio of 4.37 (95% CI 1.93-9.91), whereas men
62 older than 65 years do not appear to be at increased risk (hazard ratio of 1.09 (95% CI 0.55-2.16)). We
63 have also confirmed that the prevalence of cardiometabolic morbidity increases progressively with the
64 degree of cortisol autonomy, itself more frequently detected in women and in the presence of bilateral
65 tumours.

66

67 Implications of all the available evidence

68 Although our study confirms the association between cortisol autonomy, mortality and
69 cardiometabolic morbidity, it calls for caution regarding therapeutic interventions. Our data suggest
70 that women younger than 65 years of age could benefit most from normalizing cortisol secretion.
71 However, only randomised interventional trials will determine whether any intervention (either
72 medical treatment or surgery) is able to mitigate both cardiometabolic morbidity and mortality in
73 patients with adrenal adenomas. Our study clearly provides the rationale and the statistical basis for
74 such an outcome trial. Until these data are available, however, a conservative approach seems
75 reasonable, especially in men older than 65 years.

76 **Introduction**

77 Over the last decades, wider availability and use of cross-sectional imaging have resulted in an
78 increased incidental detection of clinically inapparent adrenal masses. Such adrenal 'incidentalomas'
79 have an increasing age-dependent prevalence, ranging from 3% in adults of 50 years of age to 10% in
80 those over 70 years.¹⁻³

81 The majority of these tumours are benign non-functioning adrenal adenomas (NFA).^{3,4} However,
82 endocrine workup may find biochemical evidence of hypercortisolism in 30-50% of patients without
83 clinically overt glucocorticoid excess, a condition historically described as 'subclinical Cushing
84 syndrome'. As only very few of these cases progress to overt Cushing syndrome,^{5,6} it is currently
85 recommended that patients be categorised by the serum cortisol value after the 1 mg overnight
86 dexamethasone-suppression test (DST) as having 'autonomous cortisol secretion' (ACS: >138 nmol/L),
87 'possible ACS' (PACS: 50-138 nmol/L), and NFA (<50 nmol/L).⁷

88 Recently, a cohort study reported a slightly elevated mortality in 969 patients with adrenal
89 incidentalomas compared to 2907 patients without.⁸ Furthermore, several studies have focused on the
90 association between ACTH-independent cortisol autonomy (defined as non-suppressible serum
91 cortisol after DST) and mortality in these patients, but results are conflicting. Three single centre
92 studies that included 198 to 365 patients⁹⁻¹¹ and one population-based study from Sweden (with 1048
93 patients)¹² reported an increased mortality in persons with elevated cortisol after the 1 mg DST. In
94 contrast, a systematic review (with 32 studies and 4121 patients) found cardiovascular (CV) and
95 metabolic risk factors (i.e., hypertension, diabetes mellitus, dyslipidaemia, and obesity) to be more
96 prevalent in the presence of what the authors termed 'mild autonomous cortisol excess'.⁶ However,
97 mortality was only studied in a subgroup of 1356 patients from nine studies and remained comparable
98 to patients with NFA. In line with this, a population-based study from Minnesota (USA) compared
99 1004 patients with adrenal incidentalomas to sex- and age-matched subjects without adrenal tumours
100 and found no difference in mortality.¹³ These discrepancies may be explained in part by the
101 heterogeneity of the criteria used for the definition of cortisol autonomy in these studies.

102 Taken together, although it is plausible that there is an association between low-grade cortisol excess
103 (as disclosed by DST), comorbidities (including CV events) and mortality, previously reported cohorts

104 were limited by low numbers and potential single-centre bias. Accordingly, we have performed a large
105 international multicentre cohort study to assess all-cause mortality, prevalence of comorbidities, and
106 occurrence of CV events in patients with adrenal incidentalomas, applying unified diagnostic criteria
107 to define cortisol autonomy.

108

109

110 **Methods**

111 Study design and setting

112 The Non-Aldosterone-Producing Adrenocortical Adenoma (NAPACA) Outcome study was approved
113 by the European Network for the Study of Adrenal Tumours (ENSAT) (www.ensat.org) in December
114 2014. Subsequently, a total of 30 centres from 16 countries agreed to participate. Each had local
115 ethical approval for pseudonymised, standardised phenotype recording. All patients provided written
116 informed consent (except for nine centres, where the Ethics Committees waived this requirement).
117 Centres were asked to report patients in a consecutive manner to minimize selection bias.
118 Retrospective data acquisition was carried out over a 56-month period (from January 2015 to August
119 2019).

120

121 Criteria for patient selection

122 Patients fulfilling the following inclusion criteria were considered eligible: age ≥ 18 years; adrenal
123 incidentaloma (uni- or bilateral with a diameter ≥ 1 cm) detected by cross-sectional imaging between
124 January 1, 1996 and December 31, 2015; diagnosis of an adrenal adenoma based on typical imaging
125 characteristics⁷ or follow-up imaging excluding malignancy; availability of a 1 mg DST result at the
126 time of the initial diagnosis; follow-up data on living status and occurrence of CV events; follow-up
127 duration ≥ 36 months. Exclusion criteria included a confirmed diagnosis of clinically overt Cushing
128 syndrome (defined according to an established clinical practice guideline¹⁴ as presence of
129 hypercortisolism along with specific clinical signs of cortisol excess (such as easy bruising, facial
130 plethora, and proximal myopathy), ACTH-dependent hypercortisolism, pheochromocytoma, primary
131 aldosteronism, surgery within 36 months after initial diagnosis, or any active malignancy (including

132 adrenocortical carcinoma) at the time of primary diagnosis of the adrenal mass. The considerable
133 variation in use of other diagnostic tests at different centres, including plasma ACTH and urinary free
134 cortisol, precluded formal analysis of other tests. Patients undergoing surgery after ≥ 36 months of
135 follow-up were censored, setting the date of surgery as the date of last follow-up. For sub-analyses,
136 patients were categorized according to their age at diagnosis (< 65 vs. ≥ 65 years, based on age-
137 dependent thresholds established to assess CV risk in patients with diabetes or hypertension^{15,16}), with
138 separate analyses based on sex.

139

140 Variables

141 Following the European guideline on the management of adrenal incidentalomas,⁷ patients were
142 categorised according to their first serum cortisol 1 mg DST result after initial diagnosis of the adrenal
143 incidentaloma: serum cortisol < 50 nmol/L, NFA; 50-138 nmol/L, PACS; > 138 nmol/L, ACS). The
144 conversion factor for serum cortisol is: nmol/L divided by $27.59 = \mu\text{g/dL}$ (hence, important cutoffs for
145 the 1 mg DST are $50 \text{ nmol/L} = 1.8 \mu\text{g/dL}$, and $138 \text{ nmol/L} = 5.0 \mu\text{g/dL}$).

146 The following clinical annotations were collected: age, sex, and body mass index (BMI) at the time of
147 the initial diagnosis of adrenal incidentaloma; tumour characteristics (i.e., size and side); medical
148 history (e.g., cardiometabolic risk factors and CV events) both at primary diagnosis and during follow-
149 up. Diagnosis of comorbidities was done according to the existing guidelines available at the time of
150 adrenal tumour diagnosis.

151

152 Outcomes

153 The primary endpoint of the NAPACA Outcome study was all-cause mortality. Pre-specified
154 secondary endpoints were: prevalence of cardiometabolic comorbidities (hypertension, diabetes
155 mellitus, and dyslipidaemia), occurrence of CV events, and cause-specific mortality. For CV
156 morbidity, we defined a composite endpoint of the following Major Adverse Cardiovascular Events
157 (MACE): myocardial infarction or coronary revascularization (either bypass surgery or percutaneous
158 intervention), stroke, or CV-related death. In addition, we collected data on venous thrombosis and
159 pulmonary embolism.

160 Statistical analysis

161 Absolute numbers and percentages were calculated for categorical data. Missing values were
162 discounted when calculating proportions. The results for continuous variables are expressed as
163 medians and quartiles. The intergroup differences between the different DST categories were analysed
164 via χ^2 -test. All-cause mortality was calculated as the time between the initial diagnosis of the adrenal
165 incidentaloma and death or last follow-up. A power analysis was performed based on the assumption
166 of a clinical meaningful hazard ratio (HR) of at least 1.5 for a two-group comparison and a mortality
167 rate of about 10%. Using a type 1 error alpha of 0.05 and a power of 80%, about 2000 patients with
168 191 deaths would have to be included. Survival curves were constructed using the Kaplan-Meier
169 method, and the log-rank test was used for subgroup analysis. Data were censored either at the date of
170 last follow up, adrenalectomy, or death. Relevant prognostic variables were identified by univariable
171 and multivariable analyses, using the Cox proportional hazards model. HR were provided along with
172 the corresponding 95% confidence intervals (CI). Multivariable Cox analyses included three different
173 post-DST groups (NFA, PACS, ACS) and the following known prognostic factors for all-cause
174 mortality and CV events as covariables: age, sex, diabetes mellitus, hypertension, dyslipidaemia, and
175 any former CV event. To study the functional forms of a relationship between cortisol after the 1 mg
176 DST as a continuous variable and all-cause mortality, we applied restricted cubic splines. In addition,
177 we categorised the cohort based on age and sex. For this analysis we used a formal 3-way interaction
178 test, using a Cox regression for age (<65, \geq 65 years), sex (male, female), and DST category (NFA,
179 PACS, ACS). Time to first MACE was defined as the time between the initial diagnosis of the adrenal
180 incidentaloma and first documentation of any MACE thereafter. As a quality check for data integrity,
181 a completeness index was calculated for each centre: patients with available follow-up data within the
182 last 12 months on December 31st, 2018 were counted as complete (i.e., centres with an index of \geq 90%
183 qualified for a sub-analysis, and the results were then compared to those derived from the whole study
184 group). Two-tailed p values of <0.05 were judged as significant. Statistical analysis was performed
185 using SPSS (version28.0, New York, USA) and R (version4.0.2) software using the packages
186 'survival' (version3.2-13) and 'smoothHR' (version1.0.3).

187 **Role of the funding sources**

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

190

191

192 **Results**

193 Out of the entire cohort of 4374 reported cases, 3656 patients from 28 centres and 15 countries were
194 eligible for the mortality analysis. As suggested (<http://www.strobe-statement.org/>), **Supplementary**
195 **Figure 1** provides the reasons for excluding patients. **Supplementary Table 1** depicts details on the
196 patients per centres. In 131 patients, adrenalectomy was performed later than 36 months after initial
197 diagnosis (details in **Supplementary Table 2**). These patients were censored at the time of surgery.

198 According to the result of the first DST, subjects were categorised as NFA (n=2089, 57.1%), PACS
199 (n=1320, 36.1%), and ACS (n=247, 6.8%). Median age at initial diagnosis was 61 years, and almost
200 two-third of patients were women. Bilateral tumours were most frequent in ACS, and this group also
201 had the largest median tumour diameter. Patient characteristics at initial diagnosis of the adrenal
202 incidentaloma are summarized in **Table 1**.

203 As shown in a scatter plot provided in **Supplementary Figure 2**, serum cortisol after the 1 mg DST
204 increased with age. None of the patients developed overt Cushing syndrome during follow-up.

205

206 During a median follow-up of 7.0 (4.7-10.2) years, 352 of 3656 patients (9.6%) died. **Figure 1A**
207 depicts the crude overall survival of the three study subgroups. Compared to the NFA group, the
208 proportion of deaths observed in PACS and ACS was higher: 143/2089 (6.8%) vs. 168/1320 (12.7%)
209 and 41/247 (16.6%). The hazard ratios for PACS and ACS remained significantly higher than the
210 NFA group after multivariable Cox analysis adjusting for age, sex, hypertension, diabetes mellitus,
211 dyslipidaemia, and former CV events (HR for death in PACS, 1.52 (95% CI 1.19-1.94; p=0.001) and
212 ACS, 1.77 (1.20-2.62; p=0.004; **Figure 1B**). Bilateral adenomas had a greater association with PACS
213 and ACS, but presence of bilateral adenomas was itself not an independent risk factor for death.

214 Following the cutoff criteria of a very recently published study,¹² we performed a post-hoc analysis of
215 our study. Here we divided our cohort in four subgroups (i.e., serum cortisol post-DST <50 nmol/L,
216 51-80 nmol/L, 81-138 nmol/L, and >138 nmol/L) and found that the mortality of the 766 patients with
217 a serum cortisol after the 1 mg DST between 51 and 80 nmol/L was not significantly higher than the
218 NFA group (HR 1.29, 95% CI 0.97-1.71; p=0.085); see also **Supplementary Table 3**. Furthermore,
219 we studied serum cortisol after the 1 mg DST as a continuous variable in relation to all-cause mortality
220 (**Supplementary Figure 3**). Whilst there was no significant linear relationship in the entire cohort, we
221 found a linear increase in the HR for death for serum cortisol after the 1 mg DST \leq 138 nmol/L.

222
223 Sensitivity analyses led to the following observations: (I) 10-year overall survival was heterogeneous
224 among centres (ranging from 69% to 100%). To reduce the risk that overall survival was
225 overestimated due to insufficient follow-up (leading to a lack of reported deaths), we performed an
226 additional analysis restricted to the 21 centres with more reliable follow-up (as illustrated by a
227 completeness index score \geq 90%). However, overall survival of this cohort of 2730 patients was not
228 changed in a relevant manner compared to the entire cohort (**Supplementary Table 4**). Accordingly,
229 we decided not to exclude any centre from the analysis. (II) The association between mortality and the
230 degree of cortisol autonomy was age-dependent: in patients <65 years, mortality was significantly
231 higher in ACS than in NFA (adjusted HR for death: 3.16, 95% CI 1.65-6.05), whereas this was not the
232 case for patients \geq 65 years (adjusted HR for death: 1.43, 95% CI 0.87-2.33). (III) The association
233 between mortality and serum cortisol after the 1 mg DST was much stronger in women than in men
234 (adjusted HR for death, ACS vs. NFA: 2.50 [95% CI 1.45-4.31] in women vs. 1.19 [95% CI 0.67-
235 2.10] in men). Consequently, we undertook a combined analysis of age- and sex- specific mortality,
236 which is presented in **Table 2** and **Figure 3**. This analysis revealed a significant interaction of age,
237 sex, and the DST category (p<0.01). It is important to note, however, that the number of patients in
238 each of these groups meant that a separate formal analysis group by group was underpowered.

239 Information on the individual causes of death was available in 306 of 352 deceased patients (87.4%)
240 (**Figure 2**). The two most frequent causes of death were cancer and CV-related events in 98 and 95
241 patients, respectively. **Supplementary Table 5** depicts the cause of death according to age and sex.

242
243 Data on cardiometabolic morbidity and CV events were available in 3484 of 3656 patients (95.3%;
244 2002 NFA, 1250 PACS, 232 ACS). Overall, hypertension was the most frequent comorbidity at initial
245 diagnosis (65.3%), followed by dyslipidaemia (40.0%), and diabetes mellitus (20.5%). As outlined in
246 **Table 1**, the prevalence increased as a continuum from NFA to PACS and ACS patients, and this was
247 true for each of these comorbid conditions.

248 For CV endpoints, 319 patients (9.3%) had experienced at least one CV event by the time of the initial
249 diagnosis of the adrenal incidentaloma (**Table 1**). During follow-up, a total of 476 non-fatal CV events
250 occurred in 375 patients with more CV events being found in patients with PACS and ACS: overall,
251 297 of 3484 patients with available data (8.5%) experienced a MACE (NFA, 7.3%; PACS, 10.3%;
252 ACS, 9.4%). A detailed overview of the reported CV events in the three subgroups is provided in
253 **Supplementary Table 6**. However, when adjusting for cardiometabolic comorbidities, time to the
254 first MACE was only significantly shorter in the women ≥ 65 years with ACS (**Table 3**).

255

256

257 **Discussion**

258 The NAPACA Outcome study is by far the largest retrospective analysis on mortality and CV
259 morbidity in patients with adrenal incidentalomas performed to date. In contrast to a meta-analysis
260 from 2019,⁶ but similar to a very recent study from Sweden,¹² we found overall an increased mortality
261 in patients with PACS and ACS. Due to our large sample size (>3600 patients) we were able to
262 reliably analyse effects of age and sex on mortality. Our data show that ACS in women <65 years of
263 age was associated with a 4-fold increase in adjusted mortality, whereas mortality in older women and
264 men <65 years was only moderately increased and not affected in older men.

265 We found that PACS and ACS were associated with an increased frequency of cardiometabolic
266 comorbidities. In particular, hypertension had a higher prevalence in both PACS and ACS compared

267 to NFA, while diabetes mellitus and dyslipidaemia showed a progressively increased frequency from
268 NFA to PACS and ACS, reflecting a continuum in metabolic disturbance, as shown previously.^{17,18}
269 Furthermore, CV events occurring either before or after the initial diagnosis of the adrenal tumour
270 were more frequently observed in patients with PACS and ACS than in NFA. However, when
271 adjusting for cardiometabolic comorbidities, a significant increase in MACE was only found in
272 women with ACS ≥ 65 years, suggesting that glucocorticoid-related CV events may not be the main
273 drivers of overall mortality in this cohort, as it has been suggested by others.^{9,10,12} This is in line with
274 the reported causes of death, which indicated only few CV-related deaths in women < 65 years with
275 cortisol autonomy. In our study, we found a relative increase in CV-related mortality that paralleled
276 that for other causes of death in patients with ACS. Another study pointed to cancer as the leading
277 cause of death in presence of ACS;¹¹ we could only partly confirm this observation in our large cohort
278 in which CV and cancer-related deaths were almost equal in patients with cortisol autonomy (n=58 vs.
279 n=56). In line with others, however, our study suggests that cortisol autonomy might have systemic
280 detrimental effects.¹⁸⁻²⁰ Nevertheless, we are well aware that a retrospective study can - by definition -
281 never prove any causal relationship.

282 The fact that the association between ACS and mortality appeared to be clinically relevant mostly in
283 younger women has not yet been described by others and may suggest that ACS is a prognostic factor
284 that has greater influence at younger ages when other age-related comorbidities are less prominent.
285 Although a different clinical presentation was observed for men and women with overt Cushing
286 disease,²¹ less is known on sex-specific organ effects by hypercortisolism. Recent studies on stress
287 associated with the COVID-19 pandemic showed that younger and middle-aged women were more
288 susceptible to stress than men, displaying an increased vascular reactivity to glucocorticoids.²²
289 Besides, it has been shown that women with diabetes or coronary heart disease were likely to receive
290 less aggressive medical management of their CV risk factors and this may have contributed to sex
291 differences in CV mortality.^{23,24} In the present study, however, we adjusted our survival analysis for
292 comorbidities to mitigate the risk of such a confounder. Interestingly, a very recently published large
293 prospective multi-centre study in 1305 patients with adrenal adenomas demonstrated an increased risk
294 and severity of hypertension and type 2 diabetes in patients with cortisol autonomy and, like us,

295 showed an increasing proportion of affected women with increasing cortisol after 1 mg DST.²⁵
296 Whereas it would be important to screen for (and treat) ACS in young, and presumably otherwise
297 more healthy patients, it is probably less relevant to do so in frail and elderly patients. However, only a
298 large randomised intervention trial would provide a definitive answer, and such a trial is not available.
299 Thus, for the time being, our study suggests that any decision on initiating cortisol-lowering treatment
300 or surgery has to be taken with care, and on an individual basis.

301 We also observed that serum cortisol after the 1 mg DST increases with age. A retrospective study,
302 however, cannot establish whether this association may also reflect chronic stress associated with age-
303 related illnesses. Future studies will have to confirm this finding and to clarify if this is a hallmark of
304 the brain aging process affecting the hypothalamic-pituitary-adrenal axis,²⁶ reduced cortisol
305 inactivation due to a reduced activity of 11 β -hydroxysteroid dehydrogenase type 2 consequent to a
306 lower nephron mass in ageing,²⁷ a matter of increasing adrenal tumour mass with age,¹⁸ or potentially
307 accelerated metabolism of dexamethasone (e.g., CYP3A4 induction due to polypharmacy in elderly
308 patients)²⁸. Overall, these data raise questions as to the significance of elevated serum cortisol after the
309 1 mg DST in the more elderly population. Besides, as recently reported,¹² we could not find any clear
310 relationship between cortisol after DST and all-cause mortality in the entire cohort. However, there
311 was a near linear relationship when serum cortisol was ≤ 138 nmol/L. For higher values, the accuracy
312 of the results are likely be limited by the low number of patients.

313 Our study has several limitations. First, a retrospective design is always prone to bias, including
314 heterogeneous or possibly inaccurate capture of relevant clinical information. Nevertheless, we tried to
315 minimize such an impact by requesting consecutively recruited patients, a minimum number of
316 included patients per centre, and a sensitivity analysis focusing on centres with a follow-up rate of
317 more than 90%. Second, the number of patients with the highest serum cortisol after the 1 mg DST
318 (i.e., the ACS group) was small compared to the other two subgroups PACS and NFA; this may have
319 weakened the statistical power of some analyses. However, the 247 ACS exceeded the total number of
320 patients included in all previous studies on this topic (n=154).⁹⁻¹² Third, we relied on a single 1 mg
321 DST only, with variability in the performance of the cortisol assays used between centres over time,
322 and without availability of dexamethasone serum concentrations²⁹. However, the biochemical tests

323 used to assess if there is cortisol autonomy have not been changed over the last 25 years. Fourth, it is
324 possible that the inclusion criteria '1 mg DST result' by itself leads to some bias, because some patients
325 with adrenal incidentaloma may not have undergone testing. However, this bias is not resolvable, as
326 shown by a recent population-based study in which only few patients with adrenal incidentalomas
327 underwent some type of endocrine screening.¹³ In addition, we acknowledge that all participating
328 institutions are tertiary care centres and our series might not be representative of cases seen in the
329 community. Finally, the diagnostic criteria of the comorbidities were not uniform across centres and
330 have obviously changed over the study period of 23 years.

331 In conclusion, our large retrospective international cohort study provides additional strong evidence
332 for an overall association between PACS and ACS with increased mortality (of note, causality cannot
333 be proven due to its retrospective nature). However, this risk is not equally distributed. Women <65
334 years with ACS bear the highest relative risk, whereas men ≥ 65 years do not appear to be at adverse
335 risk (irrespective of the degree of cortisol autonomy). Although several studies have claimed benefits
336 of adrenalectomy in patients with ACS, all of them were prone to bias and limited in numbers.³⁰
337 Randomised interventional trials are needed to determine whether intervention (either medical
338 treatment or surgery) is able to mitigate the cardiometabolic morbidity and mortality in patients with
339 adrenal adenomas. Based on our findings, and until results from such trials will be available, we
340 suggest that a conservative approach may be prudent, in particular in men with cortisol autonomy ≥ 65
341 years.

342

343

344 **Author contributions**

345 Timo Deutschbein, Giuseppe Reimondo, Massimo Terzolo, and Martin Fassnacht designed the study.
346 Except for Uwe Maeder, all authors collected samples and clinical data from patients. Uwe Maeder,
347 Timo Deutschbein, Giuseppe Reimondo, Massimo Terzolo, and Martin Fassnacht had full access to all
348 the data in the study and performed the statistical analyses. Timo Deutschbein, Giuseppe Reimondo,
349 Massimo Terzolo, and Martin Fassnacht drafted the manuscript and John Newell-Price conducted an

350 extensive content and language editing. All authors contributed to writing the manuscript and
351 approved the final version to be published.

352 **Acknowledgements**

353 This project has been supported by the European Network for the Study of Adrenal Tumours, the
354 Deutsche Forschungsgemeinschaft (DFG) project number 314061271 (CRC/Transregio 205/1 'The
355 Adrenal: Central relay of health and disease', grant to Martin Fassnacht), the Associazione Italiana per
356 la Ricerca sul Cancro (AIRC, grant number IG2019-23069 to Massimo Terzolo), and the Ricerca
357 Locale Università di Torino 2020 (RILO 2020, grant to Giuseppe Reimondo). Irina Bancos is the
358 recipient of a NIDDK/NIH K23 Award (grant number K23DK121888). Alessandro Prete is the
359 recipient of a Diabetes UK Sir George Alberti Research Training Fellowship (grant number
360 18/0005782). We are grateful to Yvonne Möhres (University Hospital Würzburg) for her help in data
361 management. We also thank the staff of the participating centres for their commitment to the
362 NAPACA Outcome study. Mari Suzuki works in the meantime for the U.S. Federal government,
363 however, the presented views are not necessarily those of the U.S. Federal government.

364

365

366 **Disclosure Summary**

367 Irina Bancos served as consultant for Corcept Therapeutics, Sparrow Pharmaceuticals, and Spruce
368 Biosciences, and was as member of advisory or data safety monitoring boards for Adrenas
369 Therapeutics, Recordati and Strongbridge Biopharma (in all cases, institution fees were provided); in
370 addition, personal honoraria were received from Elsevier ClinicalKey. Iacopo Chiodini received
371 consulting fees and honoraria from HRA Pharma Rare Diseases and Recordati, was a member of
372 advisory or data safety monitoring boards for HRA Pharma Rare Diseases and Recordati, and
373 participated in clinical studies from Corcept Therapeutics. Alexandra Chrisoulidou received personal
374 support for attending meetings and/or travel from Sanofi, and was a member of advisory or data safety
375 monitoring boards for Ipsen; in addition, personal honoraria were received from Ipsen. Timo
376 Deutschbein received personal consulting fees (for being a member of advisory or data safety
377 monitoring boards for HRA Pharma Rare Diseases and Recordati), and personal honoraria from
378 Novartis; in addition, he participated in clinical studies from Corcept Therapeutics and HRA Pharma
379 Rare Diseases (for these, institution fees were provided). Martin Fassnacht participated in clinical

380 studies from Corcept Therapeutics and HRA Pharma Rare Diseases (for these, institution fees were
381 provided). Ljiljana Marina was a member of the expert panel 'Focus Area Adrenal and Cardiovascular
382 Endocrinology' from the European Society of Endocrinology, and led the working group 5 of the
383 project 'CA20122 - Harmonizing clinical care and research on adrenal tumours in European countries'
384 from the European Cooperation in Science in Technology. John Newell-Price served as consultant for
385 and received honoraria from HRA Pharma Rare Diseases and Recordati (in all cases, institution fees
386 were provided). Carla Scaroni received consulting fees and honoraria from HRA Pharma Rare
387 Diseases and Recordati, was a member of advisory or data safety monitoring boards for HRA Pharma
388 Rare Diseases and Recordati, and served as coordinator of the Pituitary Club of the Italian Society of
389 Endocrinology. Massimo Terzolo received personal consulting fees (for being a member of advisory
390 or data safety monitoring boards for Corcept Therapeutics and HRA Pharma Rare Diseases), and
391 participated in clinical studies from HRA Pharma Rare Diseases (for the latter, institution fees were
392 provided). Stylianos Tsagarakis received personal support for attending meetings and/or travel from
393 Ipsen, Pfizer, and Recordati, and participated in clinical studies from Crinetics Pharmaceuticals,
394 Novartis, and Strongbridge Biopharma; in addition, personal honoraria were received from Recordati.
395 The other authors declare that there is no conflict of interest that could be perceived as prejudicing the
396 impartiality of the research reported.

397

398

399 **Funding**

400 This study was primarily supported by the Deutsche Forschungsgemeinschaft (DFG) project number
401 314061271 (CRC/Transregio 205/1 'The Adrenal: Central relay of health and disease', grant to Martin
402 Fassnacht), the Associazione Italiana per la Ricerca sul Cancro (AIRC, grant number IG2019-23069 to
403 Massimo Terzolo), and the Ricerca Locale Università di Torino 2020 (RILO 2020, grant to Giuseppe
404 Reimondo). Irina Bancos is the recipient of a NIDDK/NIH K23 Award (grant number
405 K23DK121888). Alessandro Prete is the recipient of a Diabetes UK Sir George Alberti Research
406 Training Fellowship (grant number 18/0005782).

407 **Data sharing**

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

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505 **Figure legends**

506

507 **Figure 1.** Overall survival of the entire cohort.

508 Results are presented as (A) Kaplan-Meier curve and (B) multivariable Cox regression analysis. (A) The Kaplan-Meier
509 analysis included all 3656 patients. Median survival was not reached in NFA, was 246 months in PACS, and 206 months
510 (95% CI 187-209) in ACS. Overall log-rank was $p < 0.001$ (NFA vs. PACS, $p < 0.001$; NFA vs. ACS, $p < 0.001$; PACS vs.
511 ACS, $p = 0.102$). (B) Multivariable Cox regression analysis (including $n = 3379$ cases; adjusted for sex, age, hypertension,
512 dyslipidaemia, diabetes mellitus, and former CV events). Patients with missing variables were excluded from the analysis.
513 Abbreviations: ACS, autonomous cortisol secretion; HR, hazard ratio; NFA, non-functioning adenoma; PACS, possible
514 autonomous cortisol secretion.
515

516 **Figure 2.** Mortality in patients with adrenal incidentalomas

517 Abbreviations: ACS, autonomous cortisol secretion; HR, hazard ratio; NFA, non-functioning adenoma; PACS, possible
518 autonomous cortisol secretion.
519

520 **Figure 3.** Overall survival according to sex and age.

521 Multivariable Cox regression analysis adjusted for hypertension, dyslipidaemia, diabetes mellitus, and former CV event.
522 Patients with missing variables were excluded from the analysis. Abbreviations: ACS, autonomous cortisol secretion; HR,
523 hazard ratio; NFA, non-functioning adenoma; PACS, possible autonomous cortisol secretion.