Age-dependent and sex-dependent disparity in mortality in patients with adrenal incidentalomas and autonomous cortisol secretion

Deutschbein, Timo; Reimondo, Giuseppe; Di Dalmazi, Guido; Bancos, Irina; Patrova, Jekaterina; Vassiliadi, Dimitra Argyro; Nekić, Anja Barač; Debono, Miguel; Lardo, Pina; Ceccato, Filippo; Petramala, Luigi; Prete, Alessandro; Chiodini, Iacopo; Ivović, Miomira; Pazaitou-panayiotou, Kalliopi; Alexandraki, Krystallenia I; Hanzu, Felicia Alexandra; Loli, Paola; Yener, Serkan; Langton, Katharina

DOI:
10.1016/S2213-8587(22)00100-0

Document Version
Peer reviewed version

Citation for published version (Harvard):
Deutschbein, T, Reimondo, G, Di Dalmazi, G, Bancos, I, Patrova, J, Vassiliadi, DA, Nekić, AB, Debono, M, Lardo, P, Ceccato, F, Petramala, L, Prete, A, Chiodini, I, Ivović, M, Pazaitou-panayiotou, K, Alexandraki, KI, Hanzu, FA, Loli, P, Yener, S, Langton, K, Spyroglou, A, Kocjan, T, Zacharieva, S, Valdés, N, Ambroziak, U, Suzuki, M, Detomas, M, Puglisi, S, Tucci, L, Delivanis, DA, Margaritopoulos, D, Dusek, T, Maggio, R, Scaroni, C, Concistrè, A, Ronchi, CL, Altieri, B, Mosconi, C, Diamantopoulos, A, Iñiguez-ariza, NM, Vicennati, V, Pia, A, Kroiss, M, Kaltsas, G, Chrisoulidou, A, Marina, LV, 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 2022, '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 and Endocrinology*, vol. 10, no. 7, pp. 499-508. https://doi.org/10.1016/S2213-8587(22)00100-0

The Lancet Diabetes & Endocrinology

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

--Manuscript Draft--

Manuscript Number:	THELANCETDE-D-22-00031R2
Article Type:	Article (Original Research)
Keywords:	ACS; adrenal mass; adrenal tumor; adrenocortical; all-cause mortality; cardiometabolic; cardiovascular; cortisol autonomy; death; diabetes; dyslipidaemia; hypertension; MACE; morbidity; NAPACA; NFA; overall survival; PACS; subclinical

Corresponding Author:	Martin Fassnacht, MD University Hospital Wurzburg: Universitatsklinikum Wurzburg Würzburg, GERMANY
First Author:	Timo Deutschbein, MD
Order of Authors:	Timo Deutschbein, MD
	Giuseppe Reimondo
	Guido Di Dalmazi
	Irina Bancos
	Jekaterina Patrova
	Dimitra A Vassiliadi
	Anja Barač Nekić
	Miguel Debono
	Pina Lardo
	Filippo Ceccato
	Luigi Petramala
	Alessandro Prete
	Iacopo Chiodini
	Miomira Ivović, MD
	Kalliopi Pazaitou-Panayiotou
	Krystallenia I Alexandraki
	Felicia A Hanzu
	Paola Loli
	Serkan Yener
	Katharina Langton
	Ariadni Spyroglou
	Tomaz Kocjan
	Sabina Zacharieva
	Nuria Valdés
	Urszula Ambroziak
	Mari Suzuki
	Mario Detomas
	Soraya Puglisi

	Lorenzo Tucci
	Danae A Delivanis
	Dimitris Margaritopoulos
	Tina Dusek
	Roberta Maggio
	Carla Scaroni
	Antonio Concistrè
	Cristina L Ronchi
	Barbara Altieri
	Cristina Mosconi
	Aristidis Diamantopoulos
	Nicole M Iniguez Ariza
	Valentina Vicennati
	Anna Pia
	Matthias Kroiss
	Gregory Kaltsas
	Alexandra Chrisoulidou
	Ljiljana Marina
	Valentina Morelli
	Wiebke Arlt
	Claudio Letizia
	Marco Boscaro
	Antonio Stigliano
	Darko Kastelan
	Stylianos Tsagarakis
	Shobana Athimulam
	Uberto Pagotto
	Uwe Maeder
	Henrik Falhammar
	John Newell-Price
	Massimo Terzolo
	Martin Fassnacht
Manuscript Region of Origin:	GERMANY
Abstract:	Background. The association between cortisol secretion and mortality in patients with adrenal incidentalomas is controversial. This study aimed to assess all-cause mortality, prevalence of comorbidities, and occurrence of cardiovascular (CV) events in uniformly stratified patients with cortisol autonomy. Methods. The Non-Aldosterone-Producing AdrenoCortical Adenoma (NAPACA) Outcome study is an international retrospective multi-centre cohort study investigating the effects of cortisol autonomy (defined as non-suppressible serum cortisol on dexamethasone-suppression testing) on mortality and CV morbidity in patients with adrenal incidentalomas. Patients with clinically apparent hormone excess, active malignancy, or follow-up <36 months were excluded. Patients were stratified according

	to the 0800-0900h serum cortisol values after a 1 mg dexamethasone-suppression test (<50nmol/L, non-functioning adenoma (NFA); 50-138nmol/L, possible Autonomous Cortisol Secretion (PACS); >138nmol/L, ACS). The primary study endpoint was all-cause mortality. Secondary endpoints were prevalence of cardiometabolic comorbidities, CV events, and cause-specific mortality. Findings. 3656 patients (57% NFA, 36% PACS, 7% ACS) were included (64% women; median age 61 years; median follow-up 7·0 years). During follow-up, 352 patients (9·6%) died. All-cause mortality (adjusted for age, sex, comorbidities, and former CV events) was significantly increased in PACS (HR 1·52; 95%CI 1·19-1·94) and ACS (1·77; 1·20-2·62). In women <65 years, ACS was associated with higher mortality compared to NFA (HR 4·37; 95%CI 1·93-9·91), while in men this was not observed. Cardiometabolic comorbidities were significantly less frequent in NFA than in PACS and ACS (hypertension: n=1186 (59%), n=944 (74%), n=179 (75%); dyslipidaemia: n=724 (36%), n=547 (44%), n=123 (52%); diabetes: n=365 (18%), n=288 (23%), n=62 (27%); always p<0.001). Interpretation. Cortisol autonomy is associated with increased all-cause mortality, especially in women <65 years. However, until results from randomised interventional trials will be available, a conservative therapeutic approach seems to be justified in most patients with adrenal incidentaloma. Funding. Deutsche Forschungsgemeinschaft, Associazione Italiana per la Ricerca sul Cancro, Università di Torino. Background. The association between cortisol secretion and mortality in patients with adrenal incidentalomas is controversial. This study aimed to assess all-cause mortality, prevalence of comorbidities, and occurrence of cardiovascular (CV) events in uniformly stratified patients with cortisol autonomy. Methods. The Non-Aldosterone-Producing AdrenoCortical Adenoma (NAPACA) Outcome study is an international retrospective multi-centre cohort study investigating the effects of cortisol autonomy (defined as non-suppressible serum cortisol on dexamethasone-suppression testing) on mortality and CV morbidity in patients with adrenal incidentalomas. Patients with clinically apparent hormone excess, active malignancy, or follow-up <36 months were excluded. Patients were stratified according to the 0800-0900h serum cortisol values after a 1 mg dexamethasone-suppression test (<50nmol/L, non-functioning adenoma (NFA); 50-138nmol/L, possible Autonomous Cortisol Secretion (PACS); >138nmol/L, ACS). The primary study endpoint was all-cause mortality. Secondary endpoints were prevalence of cardiometabolic comorbidities, CV events, and cause-specific mortality. Findings. 3656 patients (57% NFA, 36% PACS, 7% ACS) were included (64% women; median age 61 years; median follow-up 7·0 years). During follow-up, 352 patients (9·6%) died. All-cause mortality (adjusted for age, sex, comorbidities, and former CV events) was significantly increased in PACS (HR 1·52; 95%CI 1·19-1·94) and ACS (1·77; 1·20-2·62). In women <65 years, ACS was associated with higher mortality compared to NFA (HR 4·37; 95%CI 1·93-9·91), while in men this was not observed. Cardiometabolic comorbidities were significantly less frequent in NFA than in PACS and ACS (hypertension: n=1186 (59%), n=944 (74%), n=179 (75%); dyslipidaemia: n=724 (36%), n=547 (44%), n=123 (52%); diabetes: n=365 (18%), n=288 (23%), n=62 (27%); always p<0.001). Interpretation. Cortisol autonomy is associated with increased all-cause mortality, especially in women <65 years. However, until results from randomised interventional trials will be available, a conservative therapeutic approach seems to be justified in most patients with adrenal incidentaloma. Funding. Deutsche Forschungsgemeinschaft, Associazione Italiana per la Ricerca sul Cancro, Università di Torino.

EDITORS' SPECIFIC POINTS:

1. Please move the Funding statement at the end of the article to the Acknowledgments section, as we do not have a separate Funding section.
RESPONSE: Done as suggested.

2. The Disclosure Summary in the article needs to match exactly the disclosures on the individual ICMJE forms. That is to say, anything declared on any individual ICMJE form needs to be declared in the disclosures section in the article. Please update this with the full declarations for each author.
RESPONSE: Done as suggested.

3. With regard to the table provided in your rebuttal (Clinical characteristics of patients with and without adrenalectomy, Reviewer 3 suggests you include this information about the 131 patients in the appendix. In confidential comments to the Editor, the statistical reviewer also suggested that this would be worthwhile. Please consider including this table in the appendix and briefly referring to it in the article, citing the appropriate appendix page number.
RESPONSE: We now provide the table in the appendix and cite this new Supplementary Table 2 in lines 230)

4. Unfortunately, none of the figures supplied with the revised submission are editable. Please supply each figure in editable format. If you are unsure, please just email me (david.holmes@lancet.com) and I will send you some more guidance. There are also some instructions on preparing editable figures under the Editors General Points below.
RESPONSE: Done as suggested. Thanks a lot for your support.

5. When you submit your revision please upload all files.
RESPONSE: Done as suggested.

REVIEWERS' COMMENTS:

Reviewer #1: All criticism are appropriately addressed by the authors in a very comprehensive way. The additionally provided analysis and supplementary data are useful and have strengthened the manuscript.

RESPONSE: We are grateful for this very positive feedback and thank the reviewer once more for his/her help in improving our manuscript.

Reviewer #2: The Reviewer acknowledges the responses of the authors to the previous comments. The article has been revised as suggested, however there are still certain outstanding comments and/or questions.

1. Obtaining accurate and meaningful data from a retrospective study (e.g. related to CV events, mortality, etc.) can be challenging, comparatively biased, and/or incomplete in comparison to a prospective study where there are well-defined end-points and design. This

is even more evident when a retrospective study is performed across several international institutes. An excellent and well-designed study published by Prete et al. in Annals of Internal Medicine was a prospective study with many key authors from this study, prompting a reasonable inquiry of whether nearly 300 patients were used from this previous study. For this reason, the present study should not be deemed an exclusively retrospective study. Please reconsider this with your experts and statisticians to best describe this study design.
RESPONSE: **We have considered carefully the important comments of the Reviewer and have sought further expert statistical advice. Although patients were followed prospectively at the different centers, the process of data capture was retrospective. In light of this the clear advice from our statistical experts was to keep the nomenclature as it is. We would like to stress also that the paper by Prete et al. was mainly a diagnostic study that did not report any outcome data. Therefore, the limited cohort that is shared by both studies has been used in the present paper for different purposes.**

2. No further comments/suggestions.
3. No further comments/suggestions.

4. The secretory status of some adrenal incidentalomas may have been misdiagnosed since certain biochemical markers for these tumors were not available before 2010. These tumors were classified as "NFA" when that is not a certainty, without complete biochemical evaluation. The resulting biases regarding these tumors not being NFAs would contribute towards morbidity and mortality... Furthermore, the Reviewer is convinced that a value of 4.5% (a conversion rate from NFA to (P)ACS) impacts some results and additional information about this issue should be mentioned in the revised manuscript.
RESPONSE: With regards to the first point raised above by the reviewer, we respectfully disagree. All biochemical tools available for assessment in 2010, were also available in 1996, the date when the first patients were included. Thus, the suggested bias does not exist. We have now added the following statement in the Discussion (line 382-85): “However, the biochemical tests used to assess if there is cortisol autonomy have not been changed over the last 25 years.”
Regarding the second point, we acknowledge that the presumed conversion rate could influence the results. From our clinical experience, however, this appears very unlikely. Assuming that a second DST would indeed have led to a pathological result in 4.3% of the patients with a non-functioning adenoma, this would have affected 89 of the 2089 NFA patients in our study. Even if we presume a mortality rate of 15% (corresponding to the ACS group), this would have resulted in re-classification of only 13 deaths and would therefore have only limited effect on the overall result. However, we mention the fact that our results rely on only one single 1mg DST in the Discussion as limitation (see line 380-82): “Third, we relied on a single 1 mg DST only, with variability in the performance of the cortisol assays used between centres over time, and without availability of dexamethasone serum concentrations.”

5. Almost every country performs yearly statistics related to morbidity and mortality within its population, which should include all the countries where this study took place. The Reviewer would like to learn, which country/ies could not provide such data (this data may be outdated

by a few years). It would be valuable for this information to be included in the revised manuscript.
RESPONSE: We agree that this would be an interesting analysis, but the data available were not sufficient to allow this to be done, as reliable survival statistics are not available in all countries (e.g. Serbia, Turkey). Furthermore, importantly, most of these population-based databases do not provide information on comorbidities, which need to be accounted for in our study.

6. How did you conclude that major CV events are less prone to adjudication bias? Please provide data/explanation/publications.
RESPONSE: The authors of this manuscript are convinced that major CV events that can be easily identified (like myocardial infarction, stroke, pulmonary embolism, or death) are less prone to adjunction/ascertainment bias than events where objective criteria are less well defined (like transitory ischemic attacks). Moreover, most patients and physicians recall and document relevant single-time events like myocardial infarction and stroke adequately (e.g. due to relevant subsequent health impairments or changes in life style). However, a transient episode without any long-term complaints may missed in the long-term (and this is particularly relevant for a retrospective study like ours that covers a time span of approximately two decades).

7. No further comments/suggestions.
8. No further comments/suggestions.

9. The Reviewer could not deduce from the manuscript, why mortality from cancer in ACS is not viewed as a confounding variable. Please explain.
RESPONSE: This is an interesting issue that the Reviewer raised in the first round of revision (#9). Here, we can only reiterate our previous reply commenting that autonomous cortisol secretion may be linked to increased cancer incidence. This hypothesis is sound since there is some evidence that cortisol may affect cancer incidence and progression. Apart from immunosuppressive effects of glucocorticoids, it is well known that cancer mortality is higher among patients with obesity and diabetes, or both, and the increase of these comorbidities in patients with ACS may provide a possible link. For these reasons, mortality from cancer in ACS should not be viewed just as a confounding issue.

10. No further comments/suggestions
11. The Reviewer congratulates the authors in incorporating important views and thoughts relevant to women and steroids, CV events, and cancer in the revised discussion.
RESPONSE: We appreciate the positive feedback.

12. No further comments/suggestions.
13. No further comments/suggestions.

Reviewer #3: Thank you for addressing my comments.
Comment 1. Nothing further
Comment 2. Nothing further
Comment 3. Thank you for answering the question and providing the table of summary information. I think the data are interesting even with limitations as authors suggested. I think it would be important to include these data whether in the main manuscript or in a supplemental Table.
RESPONSE: As indicated in our first rebuttal, we are happy to follow the reviewers' and editors' advice. Thus, we have now included this table in the Appendix and cite this new Supplementary Table 2 in line 230)

Reviewer #4: THELANCETDE-D-22-00031R1 Age- and sex-dependent disparity in mortality in patients with adrenal incidentalomas and autonomous cortisol secretion: an international cohort study

Statistical re-review
Comments for the Authors
The authors have responded fully and convincingly to the statistical queries of this is statistical reviewer, and likewise to the statistical queries from the other, clinical reviewers. They have made appropriate changes where necessary and the revised paper is clearer as a result.
There are no additional statistical queries on the revised paper.

RESPONSE: We are happy for the statement that the points raised by this reviewer were addressed adequately. We are convinced that the new aspects and analyses strengthen the manuscript.

Reviewer #5: I am happy with the changes that have been made

RESPONSE: We would like to thank the reviewer for his/her advice to improve our manuscript.

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

Gelöscht: .

(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

Abstract

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

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

Findings. 3656 patients (57% NFA, 36% PACS, 7% ACS) were included (64% women; median age 61 years; median follow-up 7·0 years). During follow-up, 352 patients (9·6%) died. All-cause mortality (adjusted for age, sex, comorbidities, and former CV events) was significantly increased in PACS (HR 1·52; 95%CI 1·19-1·94) and ACS (1·77; 1·20-2·62). In women <65 years, ACS was associated with higher mortality compared to NFA (HR 4·37; 95%CI 1·93-9·91), while in men this was not observed. Cardiometabolic comorbidities were significantly less frequent in NFA than in PACS and ACS (hypertension: n=1186 (59%), n=944 (74%), n=179 (75%); dyslipidaemia: n=724 (36%), n=547 (44%), n=123 (52%); diabetes: n=365 (18%), n=288 (23%), n=62 (27%); always p<0.001).

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

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

Research in context

Evidence before this study

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

Added value of this study

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

Implications of all the available evidence

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

Introduction

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

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

Recently, a cohort study reported a slightly elevated mortality in 969 patients with adrenal incidentalomas compared to 2907 patients without.[8] Furthermore, several studies have focused on the association between ACTH-independent cortisol autonomy (defined as non-suppressible serum cortisol after DST) and mortality in these patients, but results are conflicting. Three single centre studies that included 198 to 365 patients[9-11] and one population-based study from Sweden (with 1048 patients)[12] reported an increased mortality in persons with elevated cortisol after the 1 mg DST. In contrast, a systematic review (with 32 studies and 4121 patients) found cardiovascular (CV) and metabolic risk factors (i.e., hypertension, diabetes mellitus, dyslipidaemia, and obesity) to be more prevalent in the presence of what the authors termed 'mild autonomous cortisol excess'.[6] However, mortality was only studied in a subgroup of 1356 patients from nine studies and remained comparable to patients with NFA. In line with this, a population-based study from Minnesota (USA) compared 1004 patients with adrenal incidentalomas to sex- and age-matched subjects without adrenal tumours and found no difference in mortality.[13] These discrepancies may be explained in part by the heterogeneity of the criteria used for the definition of cortisol autonomy in these studies.

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

Gelöscht: benign

Gelöscht: 'non-functioning adenoma'

Gelöscht: NFA:

Gelöscht: from New York

Gelöscht: adrenal nodules

Gelöscht: on dexamethasone-suppression testing

Gelöscht: southern

Gelöscht: dexamethasone testing

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

Methods

Study design and setting

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

Criteria for patient selection

Patients fulfilling the following inclusion criteria were considered eligible: age ≥18 years; adrenal incidentaloma (uni- or bilateral with a diameter ≥1cm) detected by cross-sectional imaging between January 1, 1996 and December 31, 2015; diagnosis of an adrenal adenoma based on typical imaging characteristics[7] or follow-up imaging excluding malignancy; availability of a 1 mg DST result at the time of the initial diagnosis; follow-up data on living status and occurrence of CV events; follow-up duration ≥36 months. Exclusion criteria included a confirmed diagnosis of clinically overt Cushing syndrome (defined according to an established clinical practice guideline[14] as presence of hypercortisolism along with specific clinical signs of cortisol excess (such as easy bruising, facial plethora, and proximal myopathy), ACTH-dependent hypercortisolism, phaeochromocytoma, primary aldosteronism, surgery within 36 months after initial diagnosis, or any active malignancy (including

Gelöscht: To address these issues

Gelöscht: adrenal tumours

Gelöscht: community

Gelöscht: (including detailed description of clinical, biochemical, and imaging characteristics)

Gelöscht: / Institutional Review Boards

Gelöscht: During this time, data from 4374 patients were reported to the study coordinators.

Gelöscht: of at least

Gelöscht: of at least

Gelöscht:

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

Gelöscht: at least

Variables

Following the European guideline on the management of adrenal incidentalomas[7] patients were categorised according to their first serum cortisol 1 mg DST result after initial diagnosis of the adrenal incidentaloma: serum cortisol <50 nmol/L, NFA; 50-138 nmol/L, PACS; >138 nmol/L, ACS). The conversion factor for serum cortisol is: nmol/L divided by 27·59 = µg/dL (hence, important cutoffs for the 1 mg DST are 50 nmol/L = 1·8 µg/dL, and 138 nmol/L = 5·0 µg/dL).

Gelöscht: published jointly by the European Society of Endocrinology and ENSAT

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

Outcomes

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

Statistical analysis

Absolute numbers and percentages were calculated for categorical data. Missing values were discounted when calculating proportions. The results for continuous variables are expressed as medians and quartiles. The intergroup differences between the different DST categories were analysed via χ^2-test. All-cause mortality was calculated as the time between the initial diagnosis of the adrenal incidentaloma and death or last follow-up. A power analysis was performed based on the assumption of a clinical meaningful hazard ratio (HR) of at least 1.5 for a two-group comparison and a mortality rate of about 10%. Using a type 1 error alpha of 0.05 and a power of 80%, about 2000 patients with 191 deaths would have to be included. Survival curves were constructed using the Kaplan-Meier method, and the log-rank test was used for subgroup analysis. Data were censored either at the date of last follow up, adrenalectomy, or death. Relevant prognostic variables were identified by univariable and multivariable analyses, using the Cox proportional hazards model. HR were provided along with the corresponding 95% confidence intervals (CI). Multivariable Cox analyses included three different post-DST groups (NFA, PACS, ACS) and the following known prognostic factors for all-cause mortality and CV events as covariables: age, sex, diabetes mellitus, hypertension, dyslipidaemia, and any former CV event. To study the functional forms of a relationship between cortisol after the 1 mg DST as a continuous variable and all-cause mortality, we applied restricted cubic splines. In addition, we categorised the cohort based on age and sex. For this analysis we used a formal 3-way interaction test, using a Cox regression for age (<65, ≥65 years), sex (male, female), and DST category (NFA, PACS, ACS). Time to first MACE was defined as the time between the initial diagnosis of the adrenal incidentaloma and first documentation of any MACE thereafter. As a quality check for data integrity, a completeness index was calculated for each centre: patients with available follow-up data within the last 12 months on December 31st, 2018 were counted as complete (i.e., centres with an index of ≥90% qualified for a sub-analysis, and the results were then compared to those derived from the whole study group). Two-tailed p values of <0·05 were judged as significant. Statistical analysis was performed using SPSS (version28·0, New York, USA) and R (version4.0.2) software using the packages 'survival' (version3.2-13) and 'smoothHR' (version1.0.3).

Gelöscht: ¶
¶

Formatiert: Block, Abstand Nach: 0 pt, Zeilenabstand: Doppelt

Gelöscht: and

Gelöscht: the Statistical Package for Social Sciences (

Gelöscht: ,

Role of the funding sources

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

Results

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

According to the result of the first DST, subjects were categorised as NFA (n=2089, 57·1%), PACS (n=1320, 36·1%), and ACS (n=247, 6·8%). Median age at initial diagnosis was 61 years, and almost two-third of patients were women. Bilateral tumours were most frequent in ACS, and this group also had the largest median tumour diameter. Patient characteristics at initial diagnosis of the adrenal incidentaloma are summarized in **Table 1**.

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

During a median follow-up of 7·0 (4·7-10·2) years, 352 of 3656 patients (9·6%) died. **Figure 1A** depicts the crude overall survival of the three study subgroups. Compared to the NFA group, the proportion of deaths observed in PACS and ACS was higher: 143/2089 (6·8%) vs. 168/1320 (12·7%) and 41/247 (16·6%). The hazard ratios for PACS and ACS remained significantly higher than the NFA group after multivariable Cox analysis adjusting for age, sex, hypertension, diabetes mellitus, dyslipidaemia, and former CV events (HR for death in PACS, 1·52 (95% CI 1·19-1·94; p=0·001) and ACS, 1·77 (1·20-2·62; p=0·004; **Figure 1B**). Bilateral adenomas had a greater association with PACS and ACS, but presence of bilateral adenomas was itself not an independent risk factor for death.

Gelöscht: ¶ ¶

Gelöscht: Study population¶

Gelöscht: in the STROBE statement

Gelöscht: provides

Gelöscht: All-cause mortality¶

Gelöscht: out

Following the cutoff criteria of a very recently published study,[12] we performed a post-hoc analysis of our study. Here we divided our cohort in four subgroups (i.e., serum cortisol post-DST <50 nmol/L, 51-80 nmol/L, 81-138 nmol/L, and >138 nmol/L) and found that the mortality of the 766 patients with a serum cortisol after the 1 mg DST between 51 and 80 nmol/L was not significantly higher than the NFA group (HR 1·29, 95% CI 0·97-1·71; p=0·085); see also **Supplementary Table 3**. Furthermore, we studied serum cortisol after the 1 mg DST as a continuous variable in relation to all-cause mortality (**Supplementary Figure 3**). Whilst there was no significant linear relationship in the entire cohort, we found a linear increase in the HR for death for serum cortisol after the 1 mg DST ≤138 nmol/L.

Sensitivity analyses led to the following observations: (I) 10-year overall survival was heterogeneous among centres (ranging from 69% to 100%). To reduce the risk that overall survival was overestimated due to insufficient follow-up (leading to a lack of reported deaths), we performed an additional analysis restricted to the 21 centres with more reliable follow-up (as illustrated by a completeness index score ≥90%). However, overall survival of this cohort of 2730 patients was not changed in a relevant manner compared to the entire cohort (**Supplementary Table 4**). Accordingly, we decided not to exclude any centre from the analysis. (II) The association between mortality and the degree of cortisol autonomy was age-dependent: in patients <65 years, mortality was significantly higher in ACS than in NFA (adjusted HR for death: 3·16, 95% CI 1·65-6·05), whereas this was not the case for patients ≥65 years (adjusted HR for death: 1·43, 95% CI 0·87-2·33). (III) The association between mortality and serum cortisol after the 1 mg DST was much stronger in women than in men (adjusted HR for death, ACS vs. NFA: 2·50 [95% CI 1·45-4·31] in women vs. 1·19 [95% CI 0·67-2·10] in men). Consequently, we undertook a combined analysis of age- and sex- specific mortality, which is presented in **Table 2** and **Figure 3**. This analysis revealed a significant interaction of age, sex, and the DST category (p<0.01). It is important to note, however, that the number of patients in each of these groups meant that a separate formal analysis group by group was underpowered.

Gelöscht: 2

Formatiert: Block, Abstand Nach: 0 pt, Zeilenabstand: Doppelt

Gelöscht: 3

Information on the individual causes of death was available in 306 of 352 deceased patients (87·4%) (**Figure 2**). The two most frequent causes of death were cancer and CV-related events in 98 and 95 patients, respectively. **Supplementary Table 5** depicts the cause of death according to age and sex.

Data on cardiometabolic morbidity and CV events were available in 3484 of 3656 patients (95·3%; 2002 NFA, 1250 PACS, 232 ACS). Overall, hypertension was the most frequent comorbidity at initial diagnosis (65·3%), followed by dyslipidaemia (40·0%), and diabetes mellitus (20·5%). As outlined in **Table 1**, the prevalence increased as a continuum from NFA to PACS and ACS patients, and this was true for each of these comorbid conditions.

For CV endpoints, 319 patients (9·3%) had experienced at least one CV event by the time of the initial diagnosis of the adrenal incidentaloma (**Table 1**). During follow-up, a total of 476 non-fatal CV events occurred in 375 patients with more CV events being found in patients with PACS and ACS: overall, 297 of 3484 patients with available data (8·5%) experienced a MACE (NFA, 7·3%; PACS, 10·3%; ACS, 9·4%). A detailed overview of the reported CV events in the three subgroups is provided in **Supplementary Table 6**. However, when adjusting for cardiometabolic comorbidities, time to the first MACE was only significantly shorter in the women ≥65 years with ACS (**Table 3**).

Discussion

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

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

Gelöscht: ¶
¶
Cause-specific mortality¶

Gelöscht: the

Gelöscht: 4

Gelöscht: Cardiometabolic morbidity and cardiovascular events¶

Gelöscht: 5

Gelöscht: with 1048 patients

Gelöscht: , however, for the first time

Gelöscht: younger than

Gelöscht:

Gelöscht: , compared to NFA

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

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

Gelöscht: compared to patients with

Gelöscht: older than

Gelöscht: e

Gelöscht: cardiovascular

Gelöscht: cardiovascular

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

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

Our study has several limitations. First, a retrospective design is always prone to bias, including heterogeneous or possibly inaccurate capture of relevant clinical information. Nevertheless, we tried to minimize such an impact by requesting consecutively recruited patients, a minimum number of included patients per centre, and a sensitivity analysis focusing on centres with a follow-up rate of more than 90%. Second, the number of patients with the highest serum cortisol after the 1 mg DST (i.e., the ACS group) was small compared to the other two subgroups PACS and NFA; this may have weakened the statistical power of some analyses. However, the 247 ACS exceeded the total number of patients included in all previous studies on this topic (n=154).[9-12] Third, we relied on a single 1 mg DST only, with variability in the performance of the cortisol assays used between centres over time, and without availability of dexamethasone serum concentrations[29]. However, the biochemical tests

Gelöscht: values

Gelöscht: values were

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

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

Author contributions

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

Gelöscht: measurement of dexamethasone concentrations was not part of routine clinical assessment at the time the study was conducted.

Gelöscht: a minority of

Gelöscht: in their country

Gelöscht: below the age of

Gelöscht: older than

Gelöscht: randomised interventional

Gelöscht: who are older than

Gelöscht: ----------Seitenumbruch---------- ¶

Formatiert: Block, Abstand Nach: 0 pt, Zeilenabstand: Doppelt

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

Acknowledgements

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

Gelöscht: ¶
¶
¶

Gelöscht: (ENSAT). This study was primarily supported by

Disclosure Summary

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

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

Funding

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

Formatiert: Block, Abstand Nach: 0 pt, Zeilenabstand: Doppelt

Gelöscht: ¶
¶
¶

Data sharing

We will consider sharing de-identified, 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.

References

1. Reimondo G, Castellano E, Grosso M, Priotto R, Puglisi S, Pia A, et al. Adrenal Incidentalomas are Tied to Increased Risk of Diabetes: Findings from a Prospective Study. The Journal of clinical endocrinology and metabolism. 2020; **105**(4).
2. Ebbehoj A, Li D, Kaur RJ, Zhang C, Singh S, Li T, et al. Epidemiology of adrenal tumours in Olmsted County, Minnesota, USA: a population-based cohort study. The lancet Diabetes & endocrinology. 2020; **8**(11): 894-902.
3. Sherlock M, Scarsbrook A, Abbas A, Fraser S, Limumpornpetch P, Dineen R, et al. Adrenal Incidentaloma. Endocrine reviews. 2020; **41**(6): 775-820.
4. Mantero F, Terzolo M, Arnaldi G, Osella G, Masini AM, Ali A, et al. A survey on adrenal incidentaloma in Italy. Study Group on Adrenal Tumors of the Italian Society of Endocrinology. The Journal of clinical endocrinology and metabolism. 2000; **85**(2): 637-44.
5. Cawood TJ, Hunt PJ, O'Shea D, Cole D, Soule S. Recommended evaluation of adrenal incidentalomas is costly, has high false-positive rates and confers a risk of fatal cancer that is similar to the risk of the adrenal lesion becoming malignant; time for a rethink? European journal of endocrinology / European Federation of Endocrine Societies. 2009; **161**(4): 513-27.
6. Elhassan YS, Alahdab F, Prete A, Delivanis DA, Khanna A, Prokop L, et al. Natural History of Adrenal Incidentalomas With and Without Mild Autonomous Cortisol Excess: A Systematic Review and Meta-analysis. Annals of internal medicine. 2019; **171**(2): 107-16.
7. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, et al. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. European journal of endocrinology / European Federation of Endocrine Societies. 2016; **175**(2): G1-G34.
8. Taya M, Paroder V, Bellin E, Haramati LB. The relationship between adrenal incidentalomas and mortality risk. European radiology. 2019; **29**(11): 6245-55.
9. Debono M, Bradburn M, Bull M, Harrison B, Ross RJ, Newell-Price J. Cortisol as a marker for increased mortality in patients with incidental adrenocortical adenomas. J Clin Endocrinol Metab. 2014; **99**(12): 4462-70.
10. Di Dalmazi G, Vicennati V, Garelli S, Casadio E, Rinaldi E, Giampalma E, et al. Cardiovascular events and mortality in patients with adrenal incidentalomas that are either non-secreting or associated with intermediate phenotype or subclinical Cushing's syndrome: a 15-year retrospective study. The lancet Diabetes & endocrinology. 2014; **2**(5): 396-405.
11. Patrova J, Kjellman M, Wahrenberg H, Falhammar H. Increased mortality in patients with adrenal incidentalomas and autonomous cortisol secretion: a 13-year retrospective study from one center. Endocrine. 2017; **58**(2): 267-75.
12. Kjellbom A, Lindgren O, Puvaneswaralingam S, Londahl M, Olsen H. Association Between Mortality and Levels of Autonomous Cortisol Secretion by Adrenal Incidentalomas : A Cohort Study. Annals of internal medicine. 2021; **174**(8): 1041-9.
13. Zhang CD, Li D, Kaur RJ, Ebbehoj A, Singh S, Atkinson EJ, et al. Cardiometabolic Outcomes and Mortality in Patients with Adrenal Adenomas in a Population-based Setting. The Journal of clinical endocrinology and metabolism. 2021; **106**(11): 3320-30.
14. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, et al. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. The Journal of clinical endocrinology and metabolism. 2008; **93**(5): 1526-40.
15. Selvin E, Coresh J, Brancati FL. The burden and treatment of diabetes in elderly individuals in the u.s. Diabetes care. 2006; **29**(11): 2415-9.
16. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. European heart journal. 2018; **39**(33): 3021-104.
17. Lopez D, Luque-Fernandez MA, Steele A, Adler GK, Turchin A, Vaidya A. "Nonfunctional" Adrenal Tumors and the Risk for Incident Diabetes and Cardiovascular Outcomes: A Cohort Study. Annals of internal medicine. 2016; **165**(8): 533-42.

18. Prete A, Subramanian A, Bancos I, Chortis V, Tsagarakis S, Lang K, et al. Cardiometabolic Disease Burden and Steroid Excretion in Benign Adrenal Tumors : A Cross-Sectional Multicenter Study. Annals of internal medicine. 2022: epub 2022/01/04.
19. Whitworth JA, Williamson PM, Mangos G, Kelly JJ. Cardiovascular consequences of cortisol excess. Vascular health and risk management. 2005; **1**(4): 291-9.
20. Mohd Azmi NAS, Juliana N, Azmani S, Mohd Effendy N, Abu IF, Mohd Fahmi Teng NI, et al. Cortisol on Circadian Rhythm and Its Effect on Cardiovascular System. International journal of environmental research and public health. 2021; **18**(2).
21. Pecori Giraldi F, Moro M, Cavagnini F. Gender-related differences in the presentation and course of Cushing's disease. The Journal of clinical endocrinology and metabolism. 2003; **88**(4): 1554-8.
22. Dhaibar HA, Cruz-Topete D. Predisposition of Women to Cardiovascular Diseases: A Side-Effect of Increased Glucocorticoid Signaling During the COVID-19 Pandemic? Frontiers in global women's health. 2021; **2**: 606833.
23. Wang H, Ba Y, Cai RC, Xing Q. Association between diabetes mellitus and the risk for major cardiovascular outcomes and all-cause mortality in women compared with men: a meta-analysis of prospective cohort studies. BMJ open. 2019; **9**(7): e024935.
24. Mansour O, Golden SH, Yeh HC. Disparities in mortality among adults with and without diabetes by sex and race. Journal of diabetes and its complications. 2020; **34**(3): 107496.
25. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BM, Colao A. Complications of Cushing's syndrome: state of the art. The lancet Diabetes & endocrinology. 2016; **4**(7): 611-29.
26. Yiallouris A, Tsioutis C, Agapidaki E, Zafeiri M, Agouridis AP, Ntourakis D, et al. Adrenal Aging and Its Implications on Stress Responsiveness in Humans. Front Endocrinol (Lausanne). 2019; **10**: 54.
27. Quinkler M, Zehnder D, Lepenies J, Petrelli MD, Moore JS, Hughes SV, et al. Expression of renal 11beta-hydroxysteroid dehydrogenase type 2 is decreased in patients with impaired renal function. European journal of endocrinology / European Federation of Endocrine Societies. 2005; **153**(2): 291-9.
28. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013; **138**(1): 103-41.
29. Vogg N, Kurlbaum M, Deutschbein T, Grasl B, Fassnacht M, Kroiss M. Method-Specific Cortisol and Dexamethasone Thresholds Increase Clinical Specificity of the Dexamethasone Suppression Test for Cushing Syndrome. Clin Chem. 2021; **67**(7): 998-1007.
30. Terzolo M, Reimondo G. Insights on the Natural History of Adrenal Incidentalomas. Annals of internal medicine. 2019; **171**(2): 135-6.

Figure legends

Figure 1. Overall survival of the entire cohort.

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

Figure 2. Mortality in patients with adrenal incidentalomas

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

Figure 3. Overall survival according to sex and age.

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

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

Abstract

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

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

Findings. 3656 patients (57% NFA, 36% PACS, 7% ACS) were included (64% women; median age 61 years; median follow-up 7·0 years). During follow-up, 352 patients (9·6%) died. All-cause mortality (adjusted for age, sex, comorbidities, and former CV events) was significantly increased in PACS (HR 1·52; 95%CI 1·19-1·94) and ACS (1·77; 1·20-2·62). In women <65 years, ACS was associated with higher mortality compared to NFA (HR 4·37; 95%CI 1·93-9·91), while in men this was not observed. Cardiometabolic comorbidities were significantly less frequent in NFA than in PACS and ACS (hypertension: n=1186 (59%), n=944 (74%), n=179 (75%); dyslipidaemia: n=724 (36%), n=547 (44%), n=123 (52%); diabetes: n=365 (18%), n=288 (23%), n=62 (27%); always $p<0.001$).

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

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

Research in context

Evidence before this study

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

Added value of this study

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

Implications of all the available evidence

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

Introduction

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

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

Recently, a cohort study reported a slightly elevated mortality in 969 patients with adrenal incidentalomas compared to 2907 patients without.[8] Furthermore, several studies have focused on the association between ACTH-independent cortisol autonomy (defined as non-suppressible serum cortisol after DST) and mortality in these patients, but results are conflicting. Three single centre studies that included 198 to 365 patients[9-11] and one population-based study from Sweden (with 1048 patients)[12] reported an increased mortality in persons with elevated cortisol after the 1 mg DST. In contrast, a systematic review (with 32 studies and 4121 patients) found cardiovascular (CV) and metabolic risk factors (i.e., hypertension, diabetes mellitus, dyslipidaemia, and obesity) to be more prevalent in the presence of what the authors termed 'mild autonomous cortisol excess'.[6] However, mortality was only studied in a subgroup of 1356 patients from nine studies and remained comparable to patients with NFA. In line with this, a population-based study from Minnesota (USA) compared 1004 patients with adrenal incidentalomas to sex- and age-matched subjects without adrenal tumours and found no difference in mortality.[13] These discrepancies may be explained in part by the heterogeneity of the criteria used for the definition of cortisol autonomy in these studies.

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

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

Methods

Study design and setting

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

Criteria for patient selection

Patients fulfilling the following inclusion criteria were considered eligible: age ≥18 years; adrenal incidentaloma (uni- or bilateral with a diameter ≥1cm) detected by cross-sectional imaging between January 1, 1996 and December 31, 2015; diagnosis of an adrenal adenoma based on typical imaging characteristics[7] or follow-up imaging excluding malignancy; availability of a 1 mg DST result at the time of the initial diagnosis; follow-up data on living status and occurrence of CV events; follow-up duration ≥36 months. Exclusion criteria included a confirmed diagnosis of clinically overt Cushing syndrome (defined according to an established clinical practice guideline[14] as presence of hypercortisolism along with specific clinical signs of cortisol excess (such as easy bruising, facial plethora, and proximal myopathy), ACTH-dependent hypercortisolism, phaeochromocytoma, primary aldosteronism, surgery within 36 months after initial diagnosis, or any active malignancy (including

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

Variables

Following the European guideline on the management of adrenal incidentalomas,[7] patients were categorised according to their first serum cortisol 1 mg DST result after initial diagnosis of the adrenal incidentaloma: serum cortisol <50 nmol/L, NFA; 50-138 nmol/L, PACS; >138 nmol/L, ACS). The conversion factor for serum cortisol is: nmol/L divided by 27·59 = µg/dL (hence, important cutoffs for the 1 mg DST are 50 nmol/L = 1·8 µg/dL, and 138 nmol/L = 5·0 µg/dL).

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

Outcomes

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

Statistical analysis

Absolute numbers and percentages were calculated for categorical data. Missing values were discounted when calculating proportions. The results for continuous variables are expressed as medians and quartiles. The intergroup differences between the different DST categories were analysed via χ^2-test. All-cause mortality was calculated as the time between the initial diagnosis of the adrenal incidentaloma and death or last follow-up. A power analysis was performed based on the assumption of a clinical meaningful hazard ratio (HR) of at least 1.5 for a two-group comparison and a mortality rate of about 10%. Using a type 1 error alpha of 0.05 and a power of 80%, about 2000 patients with 191 deaths would have to be included. Survival curves were constructed using the Kaplan-Meier method, and the log-rank test was used for subgroup analysis. Data were censored either at the date of last follow up, adrenalectomy, or death. Relevant prognostic variables were identified by univariable and multivariable analyses, using the Cox proportional hazards model. HR were provided along with the corresponding 95% confidence intervals (CI). Multivariable Cox analyses included three different post-DST groups (NFA, PACS, ACS) and the following known prognostic factors for all-cause mortality and CV events as covariables: age, sex, diabetes mellitus, hypertension, dyslipidaemia, and any former CV event. To study the functional forms of a relationship between cortisol after the 1 mg DST as a continuous variable and all-cause mortality, we applied restricted cubic splines. In addition, we categorised the cohort based on age and sex. For this analysis we used a formal 3-way interaction test, using a Cox regression for age (<65, ≥65 years), sex (male, female), and DST category (NFA, PACS, ACS). Time to first MACE was defined as the time between the initial diagnosis of the adrenal incidentaloma and first documentation of any MACE thereafter. As a quality check for data integrity, a completeness index was calculated for each centre: patients with available follow-up data within the last 12 months on December 31st, 2018 were counted as complete (i.e., centres with an index of ≥90% qualified for a sub-analysis, and the results were then compared to those derived from the whole study group). Two-tailed p values of <0·05 were judged as significant. Statistical analysis was performed using SPSS (version28·0, New York, USA) and R (version4.0.2) software using the packages 'survival' (version3.2-13) and 'smoothHR' (version1.0.3).

Role of the funding sources

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

Results

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

According to the result of the first DST, subjects were categorised as NFA (n=2089, 57·1%), PACS (n=1320, 36·1%), and ACS (n=247, 6·8%). Median age at initial diagnosis was 61 years, and almost two-third of patients were women. Bilateral tumours were most frequent in ACS, and this group also had the largest median tumour diameter. Patient characteristics at initial diagnosis of the adrenal incidentaloma are summarized in **Table 1**.

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

During a median follow-up of 7·0 (4·7-10·2) years, 352 of 3656 patients (9·6%) died. **Figure 1A** depicts the crude overall survival of the three study subgroups. Compared to the NFA group, the proportion of deaths observed in PACS and ACS was higher: 143/2089 (6·8%) vs. 168/1320 (12·7%) and 41/247 (16·6%). The hazard ratios for PACS and ACS remained significantly higher than the NFA group after multivariable Cox analysis adjusting for age, sex, hypertension, diabetes mellitus, dyslipidaemia, and former CV events (HR for death in PACS, 1·52 (95% CI 1·19-1·94; p=0·001) and ACS, 1·77 (1·20-2·62; p=0·004; **Figure 1B**). Bilateral adenomas had a greater association with PACS and ACS, but presence of bilateral adenomas was itself not an independent risk factor for death.

Following the cutoff criteria of a very recently published study,[12] we performed a post-hoc analysis of our study. Here we divided our cohort in four subgroups (i.e., serum cortisol post-DST <50 nmol/L, 51-80 nmol/L, 81-138 nmol/L, and >138 nmol/L) and found that the mortality of the 766 patients with a serum cortisol after the 1 mg DST between 51 and 80 nmol/L was not significantly higher than the NFA group (HR 1·29, 95% CI 0·97-1·71; p=0·085); see also **Supplementary Table 3**. Furthermore, we studied serum cortisol after the 1 mg DST as a continuous variable in relation to all-cause mortality (**Supplementary Figure 3**). Whilst there was no significant linear relationship in the entire cohort, we found a linear increase in the HR for death for serum cortisol after the 1 mg DST ≤138 nmol/L.

Sensitivity analyses led to the following observations: (I) 10-year overall survival was heterogeneous among centres (ranging from 69% to 100%). To reduce the risk that overall survival was overestimated due to insufficient follow-up (leading to a lack of reported deaths), we performed an additional analysis restricted to the 21 centres with more reliable follow-up (as illustrated by a completeness index score ≥90%). However, overall survival of this cohort of 2730 patients was not changed in a relevant manner compared to the entire cohort (**Supplementary Table 4**). Accordingly, we decided not to exclude any centre from the analysis. (II) The association between mortality and the degree of cortisol autonomy was age-dependent: in patients <65 years, mortality was significantly higher in ACS than in NFA (adjusted HR for death: 3·16, 95% CI 1·65-6·05), whereas this was not the case for patients ≥65 years (adjusted HR for death: 1·43, 95% CI 0·87-2·33). (III) The association between mortality and serum cortisol after the 1 mg DST was much stronger in women than in men (adjusted HR for death, ACS vs. NFA: 2·50 [95% CI 1·45-4·31] in women vs. 1·19 [95% CI 0·67-2·10] in men). Consequently, we undertook a combined analysis of age- and sex- specific mortality, which is presented in **Table 2** and **Figure 3**. This analysis revealed a significant interaction of age, sex, and the DST category (p<0.01). It is important to note, however, that the number of patients in each of these groups meant that a separate formal analysis group by group was underpowered.

Information on the individual causes of death was available in 306 of 352 deceased patients (87·4%) (**Figure 2**). The two most frequent causes of death were cancer and CV-related events in 98 and 95 patients, respectively. **Supplementary Table 5** depicts the cause of death according to age and sex.

Data on cardiometabolic morbidity and CV events were available in 3484 of 3656 patients (95·3%; 2002 NFA, 1250 PACS, 232 ACS). Overall, hypertension was the most frequent comorbidity at initial diagnosis (65·3%), followed by dyslipidaemia (40·0%), and diabetes mellitus (20·5%). As outlined in **Table 1**, the prevalence increased as a continuum from NFA to PACS and ACS patients, and this was true for each of these comorbid conditions.

For CV endpoints, 319 patients (9·3%) had experienced at least one CV event by the time of the initial diagnosis of the adrenal incidentaloma (**Table 1**). During follow-up, a total of 476 non-fatal CV events occurred in 375 patients with more CV events being found in patients with PACS and ACS: overall, 297 of 3484 patients with available data (8·5%) experienced a MACE (NFA, 7·3%; PACS, 10·3%; ACS, 9·4%). A detailed overview of the reported CV events in the three subgroups is provided in **Supplementary Table 6**. However, when adjusting for cardiometabolic comorbidities, time to the first MACE was only significantly shorter in the women ≥65 years with ACS (**Table 3**).

Discussion

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

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

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

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

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

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

Our study has several limitations. First, a retrospective design is always prone to bias, including heterogeneous or possibly inaccurate capture of relevant clinical information. Nevertheless, we tried to minimize such an impact by requesting consecutively recruited patients, a minimum number of included patients per centre, and a sensitivity analysis focusing on centres with a follow-up rate of more than 90%. Second, the number of patients with the highest serum cortisol after the 1 mg DST (i.e., the ACS group) was small compared to the other two subgroups PACS and NFA; this may have weakened the statistical power of some analyses. However, the 247 ACS exceeded the total number of patients included in all previous studies on this topic (n=154).[9-12] Third, we relied on a single 1 mg DST only, with variability in the performance of the cortisol assays used between centres over time, and without availability of dexamethasone serum concentrations[29]. However, the biochemical tests

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

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

Author contributions

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

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

Acknowledgements

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

Disclosure Summary

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

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

Funding

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

Data sharing

We will consider sharing de-identified, 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.

References

1. Reimondo G, Castellano E, Grosso M, Priotto R, Puglisi S, Pia A, et al. Adrenal Incidentalomas are Tied to Increased Risk of Diabetes: Findings from a Prospective Study. The Journal of clinical endocrinology and metabolism. 2020; **105**(4).
2. Ebbehoj A, Li D, Kaur RJ, Zhang C, Singh S, Li T, et al. Epidemiology of adrenal tumours in Olmsted County, Minnesota, USA: a population-based cohort study. The lancet Diabetes & endocrinology. 2020; **8**(11): 894-902.
3. Sherlock M, Scarsbrook A, Abbas A, Fraser S, Limumpornpetch P, Dineen R, et al. Adrenal Incidentaloma. Endocrine reviews. 2020; **41**(6): 775-820.
4. Mantero F, Terzolo M, Arnaldi G, Osella G, Masini AM, Ali A, et al. A survey on adrenal incidentaloma in Italy. Study Group on Adrenal Tumors of the Italian Society of Endocrinology. The Journal of clinical endocrinology and metabolism. 2000; **85**(2): 637-44.
5. Cawood TJ, Hunt PJ, O'Shea D, Cole D, Soule S. Recommended evaluation of adrenal incidentalomas is costly, has high false-positive rates and confers a risk of fatal cancer that is similar to the risk of the adrenal lesion becoming malignant; time for a rethink? European journal of endocrinology / European Federation of Endocrine Societies. 2009; **161**(4): 513-27.
6. Elhassan YS, Alahdab F, Prete A, Delivanis DA, Khanna A, Prokop L, et al. Natural History of Adrenal Incidentalomas With and Without Mild Autonomous Cortisol Excess: A Systematic Review and Meta-analysis. Annals of internal medicine. 2019; **171**(2): 107-16.
7. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, et al. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. European journal of endocrinology / European Federation of Endocrine Societies. 2016; **175**(2): G1-G34.
8. Taya M, Paroder V, Bellin E, Haramati LB. The relationship between adrenal incidentalomas and mortality risk. European radiology. 2019; **29**(11): 6245-55.
9. Debono M, Bradburn M, Bull M, Harrison B, Ross RJ, Newell-Price J. Cortisol as a marker for increased mortality in patients with incidental adrenocortical adenomas. J Clin Endocrinol Metab. 2014; **99**(12): 4462-70.
10. Di Dalmazi G, Vicennati V, Garelli S, Casadio E, Rinaldi E, Giampalma E, et al. Cardiovascular events and mortality in patients with adrenal incidentalomas that are either non-secreting or associated with intermediate phenotype or subclinical Cushing's syndrome: a 15-year retrospective study. The lancet Diabetes & endocrinology. 2014; **2**(5): 396-405.
11. Patrova J, Kjellman M, Wahrenberg H, Falhammar H. Increased mortality in patients with adrenal incidentalomas and autonomous cortisol secretion: a 13-year retrospective study from one center. Endocrine. 2017; **58**(2): 267-75.
12. Kjellbom A, Lindgren O, Puvaneswaralingam S, Londahl M, Olsen H. Association Between Mortality and Levels of Autonomous Cortisol Secretion by Adrenal Incidentalomas : A Cohort Study. Annals of internal medicine. 2021; **174**(8): 1041-9.
13. Zhang CD, Li D, Kaur RJ, Ebbehoj A, Singh S, Atkinson EJ, et al. Cardiometabolic Outcomes and Mortality in Patients with Adrenal Adenomas in a Population-based Setting. The Journal of clinical endocrinology and metabolism. 2021; **106**(11): 3320-30.
14. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, et al. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. The Journal of clinical endocrinology and metabolism. 2008; **93**(5): 1526-40.
15. Selvin E, Coresh J, Brancati FL. The burden and treatment of diabetes in elderly individuals in the u.s. Diabetes care. 2006; **29**(11): 2415-9.
16. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. European heart journal. 2018; **39**(33): 3021-104.
17. Lopez D, Luque-Fernandez MA, Steele A, Adler GK, Turchin A, Vaidya A. "Nonfunctional" Adrenal Tumors and the Risk for Incident Diabetes and Cardiovascular Outcomes: A Cohort Study. Annals of internal medicine. 2016; **165**(8): 533-42.

18. Prete A, Subramanian A, Bancos I, Chortis V, Tsagarakis S, Lang K, et al. Cardiometabolic Disease Burden and Steroid Excretion in Benign Adrenal Tumors : A Cross-Sectional Multicenter Study. Annals of internal medicine. 2022: epub 2022/01/04.
19. Whitworth JA, Williamson PM, Mangos G, Kelly JJ. Cardiovascular consequences of cortisol excess. Vascular health and risk management. 2005; **1**(4): 291-9.
20. Mohd Azmi NAS, Juliana N, Azmani S, Mohd Effendy N, Abu IF, Mohd Fahmi Teng NI, et al. Cortisol on Circadian Rhythm and Its Effect on Cardiovascular System. International journal of environmental research and public health. 2021; **18**(2).
21. Pecori Giraldi F, Moro M, Cavagnini F. Gender-related differences in the presentation and course of Cushing's disease. The Journal of clinical endocrinology and metabolism. 2003; **88**(4): 1554-8.
22. Dhaibar HA, Cruz-Topete D. Predisposition of Women to Cardiovascular Diseases: A Side-Effect of Increased Glucocorticoid Signaling During the COVID-19 Pandemic? Frontiers in global women's health. 2021; **2**: 606833.
23. Wang H, Ba Y, Cai RC, Xing Q. Association between diabetes mellitus and the risk for major cardiovascular outcomes and all-cause mortality in women compared with men: a meta-analysis of prospective cohort studies. BMJ open. 2019; **9**(7): e024935.
24. Mansour O, Golden SH, Yeh HC. Disparities in mortality among adults with and without diabetes by sex and race. Journal of diabetes and its complications. 2020; **34**(3): 107496.
25. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BM, Colao A. Complications of Cushing's syndrome: state of the art. The lancet Diabetes & endocrinology. 2016; **4**(7): 611-29.
26. Yiallouris A, Tsioutis C, Agapidaki E, Zafeiri M, Agouridis AP, Ntourakis D, et al. Adrenal Aging and Its Implications on Stress Responsiveness in Humans. Front Endocrinol (Lausanne). 2019; **10**: 54.
27. Quinkler M, Zehnder D, Lepenies J, Petrelli MD, Moore JS, Hughes SV, et al. Expression of renal 11beta-hydroxysteroid dehydrogenase type 2 is decreased in patients with impaired renal function. European journal of endocrinology / European Federation of Endocrine Societies. 2005; **153**(2): 291-9.
28. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013; **138**(1): 103-41.
29. Vogg N, Kurlbaum M, Deutschbein T, Grasl B, Fassnacht M, Kroiss M. Method-Specific Cortisol and Dexamethasone Thresholds Increase Clinical Specificity of the Dexamethasone Suppression Test for Cushing Syndrome. Clin Chem. 2021; **67**(7): 998-1007.
30. Terzolo M, Reimondo G. Insights on the Natural History of Adrenal Incidentalomas. Annals of internal medicine. 2019; **171**(2): 135-6.

Figure legends

Figure 1. Overall survival of the entire cohort.

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

Figure 2. Mortality in patients with adrenal incidentalomas

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

Figure 3. Overall survival according to sex and age.

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

Table 1. Patient characteristics at initial diagnosis of the adrenal incidentaloma.

Characteristics	All patients (n=3656)	NFA (n=2089)	PACS (n=1320)	ACS (n=247)
Demographics [A]				
Women (n, %)	2350 (64·3%)	1321 (63·2%)	860 (65·2%)	169 (68·4%)
Men (n, %)	1306 (35·7%)	768 (36·8%)	460 (34·8%)	78 (31·6%)
Age, years	61 (53-68)	60 (52-67)	63 (56-70)	63 (55-70)
Age < 65 years (n, %)	2264 (61·9%)	1404 (67·2%)	726 (55·0%)	134 (54·3%)
Follow-up, years	7·0 (4·7-10·2)	7·2 (4·8-10·5)	6·9 (4·7-10·0)	6·9 (4·5-10·0)
Clinical characteristics [B]				
Body mass index, kg/m^2	28·1 (25·0-32·3)	28·6 (25·4-32·6)	27·8 (24·6-31·9)	27·7 (24·3-31·9)
Tumour characteristics [C]				
- Left (n, %)	1497 (44·6%)	946 (49·8%)	468 (38·1%)	83 (36·2%)
- Right (n, %)	1093 (32·6%)	646 (34·0%)	385 (31·4%)	62 (27·1%)
- Bilateral (n, %)	764 (22·8%)	306 (16·1%)	374 (30·5%)	84 (36·7%)
Maximum tumour diameter, mm	22 (15-30)	20 (15-25)	26 (19-33)	29 (20-37)
Biochemistry [D]				
1-mg DST serum cortisol, nmol/L	47 (30-72)	33 (28-50)	72 (61-94)	190 (157-253)
Comorbidities				
Hypertension (n, %) [E]	2309 (65·3%)	1186 (58·6%)	944 (74·0%)	179 (75·2%)
Dyslipidaemia (n, %) [F]	1394 (40·0%)	724 (36·2%)	547 (43·8%)	123 (51·9%)
Diabetes mellitus (n, %) [G]	715 (20·5%)	365 (18·2%)	288 (23·0%)	62 (26·7%)
CV events before initial diagnosis of the adrenal incidentaloma				
Myocardial infarction and/or coronary intervention (n, %) [H]	199 (6·0%)	87 (4·6%)	96 (8·0%)	16 (7·1%)
Stroke (n, %) [I]	70 (2·1%)	31 (1·6%)	27 (2·3%)	12 (5·3%)
Deep vein thrombosis and/or pulmonary embolism (n, %) [J]	62 (1·9%)	31 (1·7%)	26 (2·2%)	5 (2·2%)
At least one CV event (n, %) [K]	319 (9·3%)	150 (7·6%)	139 (11·4%)	30 (13·2%)

If not otherwise specified, numbers are given as median (quartiles). Number of patients for whom the reported variable was available: [A] n=3565, [B] n=3219, [C] n=3354, [D] n=3656, [E] n=3537, [F] n=3486, [G] n=3484, [H] n=3305, [I] n=3299, [J] n=3293, [K] n=3415. Centre-specific data on ethnicity can be found in **Supplementary Table 1**. Abbreviations: ACS, autonomous cortisol secretion; CV, cardiovascular; DST, dexamethasone suppression test; NFA, non-functioning adenoma; PACS, possible autonomous cortisol secretion.

Table 2. Multivariable Cox regression analysis of sex- and age-specific all-cause mortality.

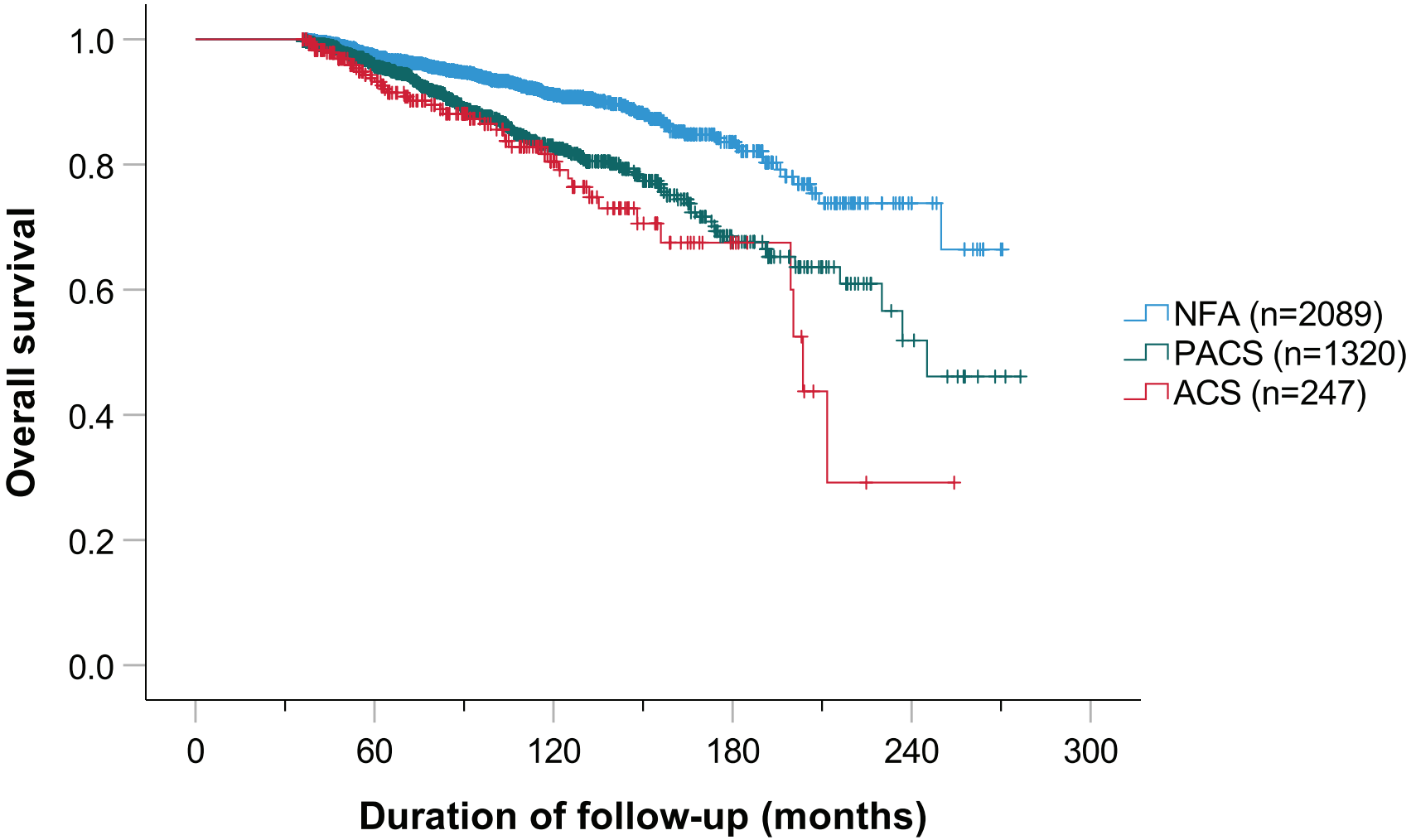
Sex	Age (years)	All Subjects (n)	All Events (n)	**PACS**				**ACS**			
				n	HR	95% CI	p	n	HR	95% CI	p
Women	< 65	1424	51	472	1·82	0·99-3·31	0·052	96	**4·39**	**1·93-9·96**	**<0·001**
	≥ 65	723	108	302	**1·99**	**1·31-3·01**	**0·001**	57	1·80	0·86-3·76	0·118
Men	< 65	734	43	222	1·35	0·70-2·59	0·370	34	1·77	0·59-5·33	0·307
	≥ 65	479	94	200	1·26	0·81-1·97	0·310	36	1·09	0·55-2·16	0·813

The analysis was adjusted for hypertension, diabetes mellitus, dyslipid**a**emia, and former CV events. Patients with missing variables were excluded from the analysis. Abbreviations: ACS, autonomous cortisol secretion; CI, confidence interval; HR, hazard ratio; PACS, possible autonomous cortisol secretion.

Table 3. Multivariate Cox regression analysis for sex- and age-specific major cardiovascular events (MACE).

Sex	Age (years)	All Subjects (n)	All Events (n)	**PACS**				**ACS**			
				n	HR	95% CI	p	n	HR	95% CI	p
Women	< 65	1377	75	466	1·20	0·74-1·95	0·463	94	1·61	0·71-3·61	0·252
	≥ 65	705	92	296	1·33	0·84-2·06	0·224	56	**2·09**	**1·08-4·05**	**0·028**
Men	< 65	694	91	218	1·05	0·67-1·63	0·831	33	0·73	0·29-1·85	0·506
	≥ 65	466	89	193	1·10	0·70-1·72	0·685	36	1·04	0·48-2·24	0·917

The analysis was adjusted for hypertension, diabetes mellitus, dyslipidaemia, and former CV events. Patients with missing variables were excluded from the analysis. Time to first MACE was defined as the time lag between the initial diagnosis of the adrenal incidentaloma and first documentation of any MACE thereafter. Abbreviations: ACS, autonomous cortisol secretion; CV, cardiovascular; HR, hazard ratio; MACE, major adverse cardiovascular event; PACS, possible autonomous cortisol secretion.



Number at risk						
NFA	2089	1504	579	123	14	0
PACS	1320	932	325	74	10	0
ACS	247	168	63	13	1	0
Number censored						
NFA	0	539	1400	1833	1935	1946
PACS	0	340	862	1085	1143	1152
ACS	0	66	154	197	205	206

Figure 1B

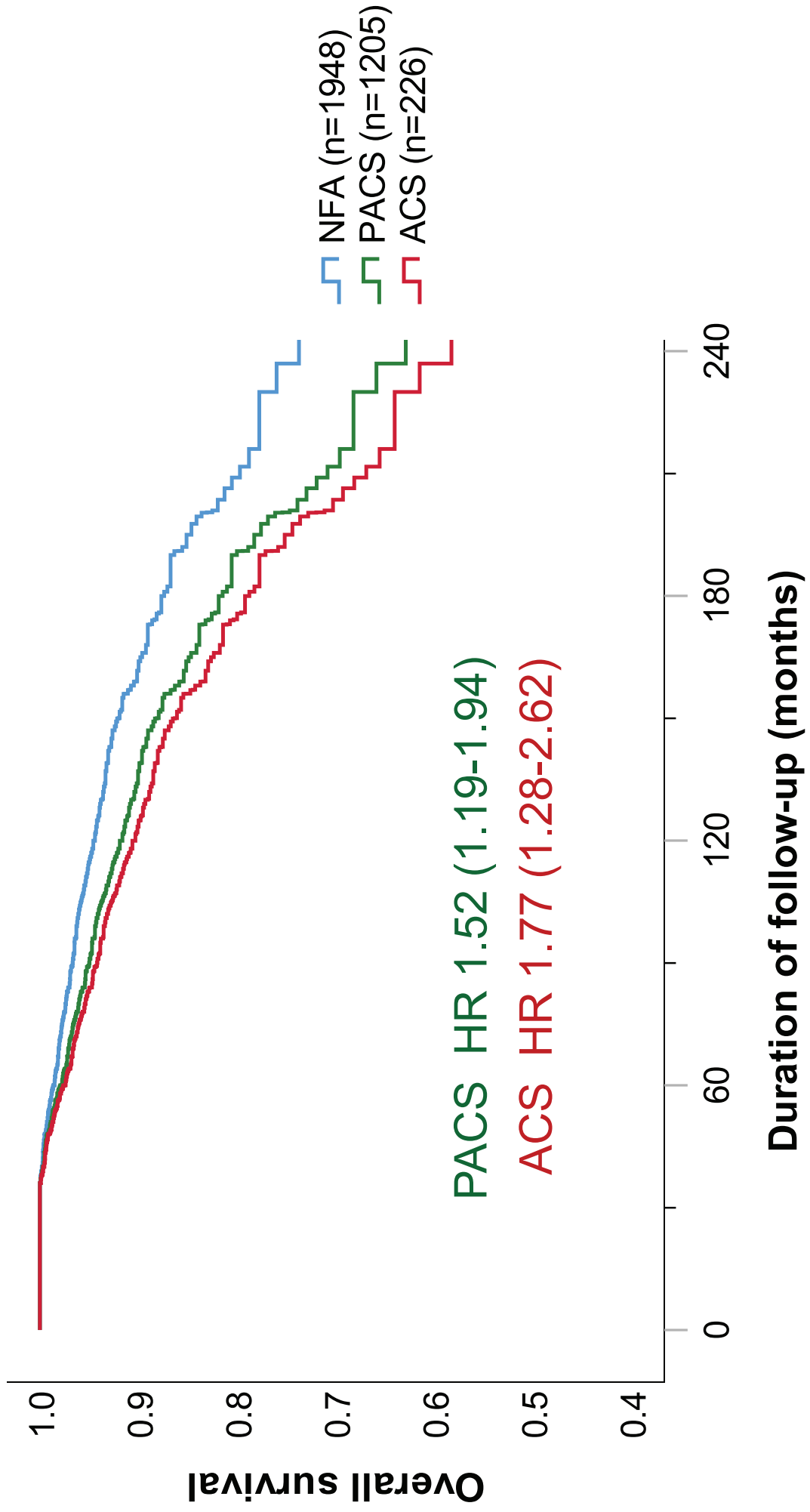

NFA (n=1948)
PACS (n=1205)
ACS (n=226)
PACS HR 1.52 (1.19-1.94)
ACS HR 1.77 (1.28-2.62)
Overall survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0
60
120
180
240
Duration of follow-up (months)

Figure 2

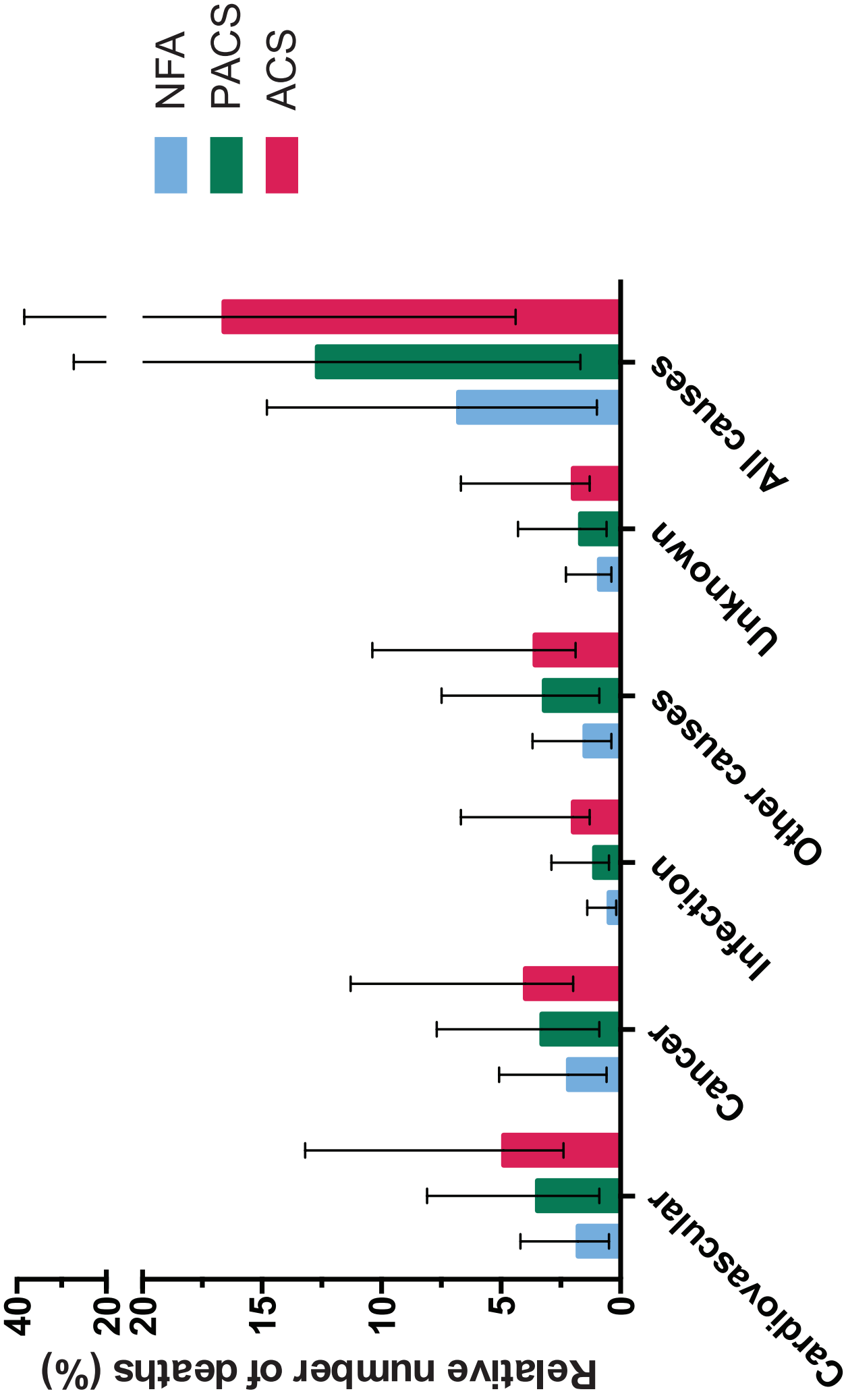
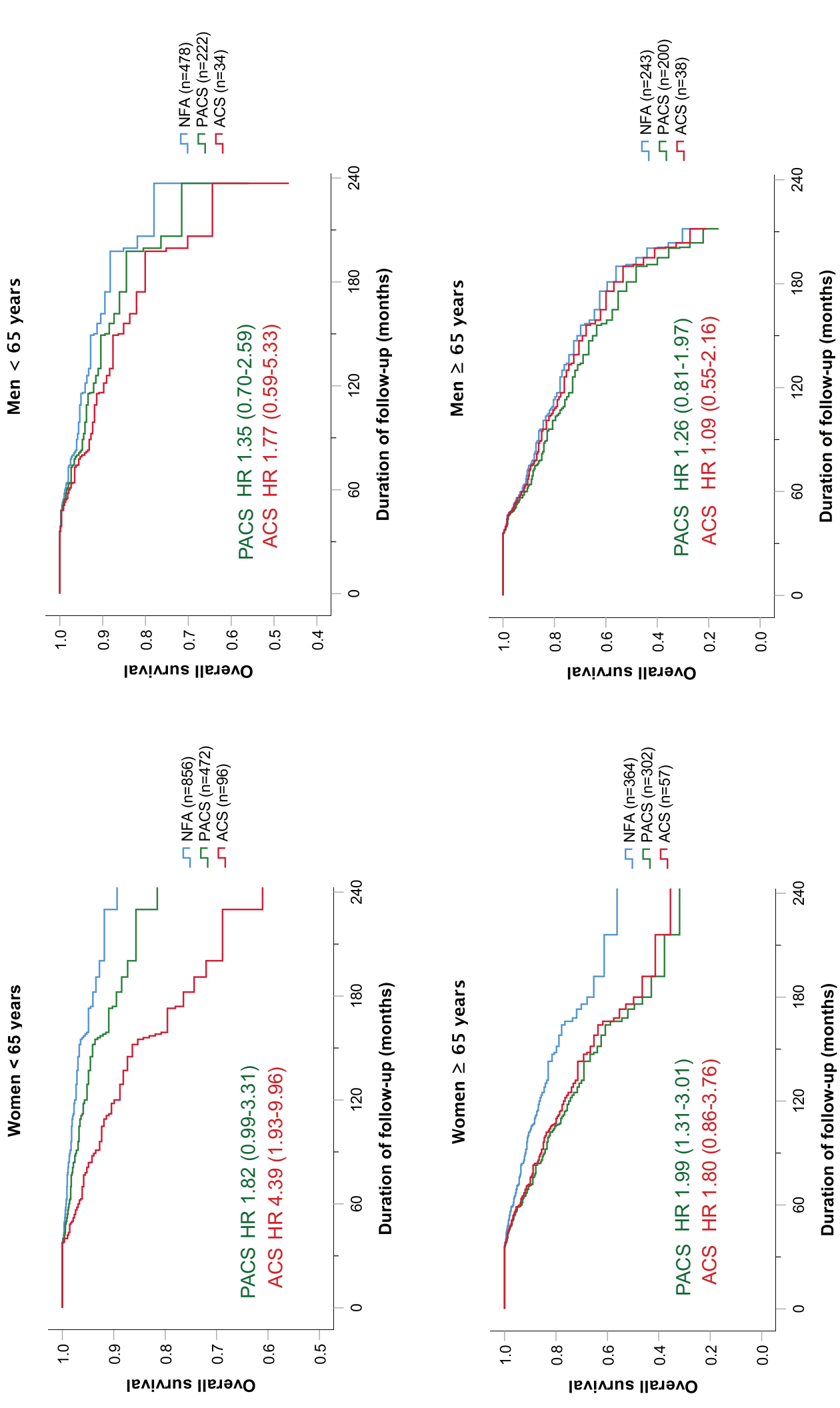

NFA
PACS
ACS
Relative number of deaths (%)
40
20
20
15
10
5
0
Cardiovascular
Cancer
Infection
Other causes
Unknown
All causes

Figure 3


Men < 65 years
NFA (n=478)
PACS (n=222)
ACS (n=34)
PACS HR 1.35 (0.70-2.59)
ACS HR 1.77 (0.59-5.33)
Overall survival
Duration of follow-up (months)
Men ≥ 65 years
NFA (n=243)
PACS (n=200)
ACS (n=38)
PACS HR 1.26 (0.81-1.97)
ACS HR 1.09 (0.55-2.16)
Overall survival
Duration of follow-up (months)
Women < 65 years
NFA (n=856)
PACS (n=472)
ACS (n=96)
PACS HR 1.82 (0.99-3.31)
ACS HR 4.39 (1.93-9.96)
Overall survival
Duration of follow-up (months)
Women ≥ 65 years
NFA (n=364)
PACS (n=302)
ACS (n=57)
PACS HR 1.99 (1.31-3.01)
ACS HR 1.80 (0.86-3.76)
Overall survival
Duration of follow-up (months)

Supplemental material

Agreement to acknowledgment