Cardiovascular events in patients with subclinical hypercortisolism: analysis with artificial neural networks

Journal:	*European Journal of Endocrinology*
Manuscript ID	Draft
mstype:	Clinical Study
Date Submitted by the Author:	n/a
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Keywords:	subclinical hypercortisolism, cardiovascular risk, adrenal incidentalomas

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Cardiovascular events in patients with subclinical hypercortisolism: analysis with artificial neural networks

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Short title: Cardiovascular risk and hypercortisolism

Key words Subclinical hypercortisolism, adrenal incidentalomas, cardiovascular events

Word count: 3248

Number of figures/tables: 2/4

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Abstract

Background. The role of subclinical hypercortisolism (SH) in influencing the cardiovascular event (CVE) occurrence is debated. In this study we investigated the SH role in the CVE occurrence in patients with adrenal incidentaloma (AI) by the standard statistical analyses and the artificial neural networks (ANNs).

Methods: We analyzed the retrospective records of 518 AI patients, without symptoms of hypercortisolism. Data regarding cortisol levels after 1mg-dexamethasone suppression (1-mgDST) and the presence of obesity (OB), hypertension (AH), type-2 diabetes (T2DM), dyslipidaemia (DL), CVE familiar history, smoking habit and CVE were collected.

Results: Standard statistical analyses suggested that the 1-mgDST, at a cut-off of 1.8 μg/dl, had the best accuracy (62.9%) in detecting patients with CVE. In patients with 1mg-DST ≥1.8 μg/dl (DST+, n=223), age and prevalence of AH, T2DM, DL and CVE (66 years, 74.5%, 25.9%, 41.4%, 26.8%, respectively) were higher than in patients with 1mg-DST ≤1.8 μg/dl (61.9 years, 60.7%, 18.5%, 32.9% and 10%, respectively, $p<0.05$ for all). The CVE were associated with DST+ (OR 2.46, 95%CI 1.5-4.1, $p=0.01$), regardless of T2DM, AH, DL, smoking habit, gender, observation period and age. The presence of at least two among AH, T2DM, DL and OB had 60% accuracy in detecting patients with CVE. Adding DST+ the accuracy reached the 71.2%. By using the variables selected by ANNs (CVE familiar history, age, T2DM, AH, DL and DST+) 78.5% accuracy was reached.

Conclusions. Cortisol after 1mg-DST is independently associated with the CVE occurrence. The ANNs could assess the CVE risk in the individual AI patient.

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Introduction

Subclinical hypercortisolism (SH) is a condition of increased cortisol secretion in the absence of the classical signs of overt cortisol excess (1), and it is found in up to 20% of patients bearing an incidentally discovered adrenal mass (adrenal incidentaloma, AI) (2, 3). In adults, SH has a 0.2-2.0% prevalence and it seems to be associated with an increased risk of fragility fractures, dyslipidemia (DL), type 2 diabetes (T2DM), hypertension (AH), cardiovascular events (CVE) and mortality (4-10). However, the effect of the recovery from SH on the cardiovascular risk is debated, since the improvement of the metabolic complications after the excision of the adrenal adenoma in AI patients has been not consistently reported (11-13). Therefore, no widely accepted guidelines are available on how to define SH and to address the treatment in AI patients with subtle hypercortisolism (14-18).

However, it is likely that in SH the cardiovascular risk is influenced by different comorbidities (i.e. DL, T2DM, AH) and environmental factors interacting in nonlinear biological mechanisms. The understanding of the SH role as a CVE risk factor is difficult even because most CVE risk factors may be caused by SH itself, and, at the same time, the incidence of both SH and CVE increases with age (19). This kind of problem probably needs a specific mathematical approach, such as the artificial neural networks (ANNs), to be understood (20). Because ANNs are artificial adaptive systems, able to modify their internal structure in relation to a function objective, they are suited for solving nonlinear problems. The ability to learn in an adaptive way (i.e., extracting from the data the information needed to gather a specific task) makes the ANNs a powerful tool for data analysis (21). In the past years, ANNs have been shown to improve the predictive value of standard statistics in many areas of medicine (21-25). No studies have investigated the ability of ANNs in evaluating the factors influencing the CVE risk in patients with a low grade of cortisol excess, as is the case of SH patients.

Therefore, the aim of the present study was to investigate, in a large sample of AI patients, the factors associated with CVE events by using ANNs and the standard statistical approach.

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Patients and Method

Patients

In this observational multicenter study, we retrospectively analyzed the records regarding 1066 AI patients referred to the participating Endocrine Units between January 1996 and June 2016. Among these patients, 548 were not included due to the exclusion criteria. We excluded patients with bilateral adrenal masses, psychiatric diseases and alcoholism, or taking drugs influencing cortisol and dexamethasone metabolism or cortisol secretion, with signs or symptoms of overt cortisol excess (i.e. moon facies, striae rubrae, skin atrophy or buffalo hump), history of malignancy, infections, adrenal hemorrhage, pheochromocytoma, primary hyperaldosteronism and hyperparathyroidism, and infiltrative disease potentially affecting the adrenal glands. Eventually, 518 AI patients without signs of overt hypercortisolism were enrolled.

All AI were discovered by CT scan, ultrasonography or MRI, performed for unrelated diseases. Ultrasound findings were confirmed with CT scan. All adrenal masses were ≥1 cm of diameter, and displayed a CT pattern consistent with benign adenoma (i.e. homogeneous texture, <10 Hounsfield Units, regular margins, size <6 cm).

In all patients, we measured 24-hour urinary free cortisol (UFC), ACTH levels at 08:00 h, and serum cortisol levels at 08:00 h after 1 mg overnight dexamethasone suppression test (1mg-DST). The following parameters were recorded: age, gender, presence of obesity (OB), AH, T2DM, DL, familiar CVE history and smoking habit. We recorded the CVEs occurrence (myocardial infarction, stroke, transient ischemic attack, angina pectoris, pulmonary embolism, intracerebral hemorrhage, peripheral artery disease) during the 10 years before the AI finding AI and during a variable follow-up period (mean 161.8±45.1, range 120-426 months) after the AI finding. The observation period has been extended till 10 years before the AI finding since the diagnosis of overt hypercortisolism is done with three-eight years of delay (26), which should be probably longer in scarcely symptomatic patients (26). The study complies with the Declaration of Helsinki and it has been approved by the Ethical Committee of Fondazione IRCCS Cà Granda-Ospedale Maggiore Policlinico, Milan, Italy. An informed consent has been obtained from each patient.

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Methods

In all patients ACTH and serum and urinary cortisol levels were measured in each center using commercially available reagents. The intra- and inter-assay coefficients of variation were <10% for all the assays. Increased UFC levels (h-UFC) were defined by levels above the upper limit of the normal values of each assay.

In the diabetic, dyslipidemic and hypertensive patients the CVEs, blood pressure and metabolic control were assessed by the reports of the cardiologists and diabetologists, who annually evaluated the patients. In the remaining patients, information were obtained by their general practitioner reports. Obesity was diagnosed in the presence of body mass index (BMI) above or equal to 30 kg/m^2 and T2DM by WHO criteria (27). The diabetic patients were considered well controlled in the presence of glycated haemoglobin below 7% (28). Arterial hypertension was defined in the presence of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or antihypertensive treatment (28). Blood pressure was considered well controlled in the presence of systolic and diastolic blood pressure below 130 mmHg and 85 mmHg, respectively (29). Dyslipidemia was diagnosed in the presence of triglycerides levels ≥150 mg/dL (1.7 mmol/L), or high-density lipoprotein cholesterol levels <40 mg/dL (1.0 mmoL/L) in men and <50 mg/dL (1.3 mmol/L) in women or if any specific treatment was given (30). Dyslipidemia was considered well controlled in the presence of low density lipoprotein below 130 mg/dL, 100 mg/dL and 70 mg/dL in patients without T2DM, with T2DM and with T2DM plus cardiovascular complications, respectively. In each patient a comorbidities score was calculated by adding up the comorbidities (T2DM, AH, DL and OB) altogether. Current smokers were individuals who smoked any tobacco (including beedies, pipes, and other forms) during the observation period (31).

Statistical Analysis

Standard statistical analyses

Statistical analyses were performed using SPSS version 21.0 package (SPSS Inc, Chicago, IL). Descriptive statistics were reported as mean±standard deviation (SD), unless differently specified, or frequency and percentages. Categorical variables were compared by $\chi 2$ test. Continuous variables were compared among groups using one-way ANOVA. Multiple comparisons were addressed using or Bonferroni

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approach. The bivariate associations were assessed using Spearman or Pearson Correlation as appropriate. The receiver operating characteristic (ROC) curve analysis was used to assess the optimal cut-off of the cortisol levels after 1mg-DST or of the comorbidity score providing the best diagnostic accuracy (optimizing sensitivity, SN and specificity, SP) for detecting patients with the occurrence of CVE.

Logistic regression analyses were performed to test the independent association between the CVE occurrence and the cortisol levels after 1mg-DST above or equal to the cut-off obtained by ROC curve, adjusting for age, gender, CVE family history, duration of observation, smoking habit and the presence of T2DM, AH, and DL separately taken or as a comorbidity score equal to or above the cut-off obtained by the ROC curve. Risk were reported as odds ratios (OR) along with their 95% confidence intervals (95%CI). P-values of <0.05 were considered as statistically significant.

Unsupervised ANNs: the Auto Contractive Map

Complex mathematical networks can help us in establishing the hierarchy of variables within a specific set. We adopted the Auto Contractive Map system (Auto-CM), that is a fourth generation unsupervised ANN able to highlight the links among variables with a graph based on the minimum spanning tree (MST) theory, where distances among variables reflect the weights of the ANN (20). The Auto-CM system finds, by a specific learning algorithm, a square matrix of "similarities" (weights mathematically speaking) among the variables of the dataset (21). Once the AutoCM weights matrix is obtained, it is then filtered by MST, that shows, among the huge number of possible ways to connect the variables in a tree, the shortest possible combination. In the MST, every link able to generate a cycle into the graph is eliminated, irrespective of its strength of association, and this results in a simplified graph. The assumption is that, since all biological systems tend naturally to the minimal energetic states, this graph expresses the fundamental biological information of the system. The ultimate goal of this data mining model is to discover hidden trends and associations among variables, since this algorithm is able to create a semantic connectivity map in which non linear associations are preserved and explicit connection schemes are described. This approach gives the map of the relevant connections between and among variables and the principal hubs of the system. The learning algorithm of Auto-CM may be summarized in four orderly steps: a) signal transfer from the input into the hidden layer; b) adaptation of the connections value between the input layer and the hidden layer; c) signal transfer from the hidden layer into the output layer; d) adaptation of the connections value

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148 between the hidden layer and the output layer. The Auto-CM does not have initial weights posed at random
149 and they start always by the same value. Therefore, the resulting graph is reproducible along many possible
150 runs (20, 21).

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Results

Classical statistical analysis

We found the occurrence of myocardial infarction, stroke, transient ischemic attack, angina pectoris, pulmonary embolism, intracerebral hemorrhage and peripheral artery disease in the 38.9%, 15.6%, 13.3%, 7.8%, 3.3%, 1.1% and 20% of patients, respectively. The Table 1 reports the clinical and biochemical characteristics of patients with and without CVE (CVE+ and CVE-, respectively). The adenoma size, duration of observation period and prevalence of post-menopausal females and of subjects with positive familiar history of CVE were comparable between the two groups. As expected, CVE+ patients were elder and more frequently males, smokers, hypertensive, diabetic and dyslipidemic than CVE- patients. However, BMI, the prevalence of OB and of uncontrolled glycemia, blood pressure and LDL levels were comparable between the two groups. The cortisol levels after 1mg-DST were higher in CVE+ than in CVE- patients, while the ACTH levels and the prevalence of h-UFC levels were comparable between the two groups. The ROC curve showed that the cut-off of cortisol levels after 1mg-DST set at 1.8 μg/dl had the best compromise between SN (63%) and SP (66%) in predicting CVE (accuracy 62.9%, area under the curve, AUC, 0.673, $p<0.0001$, Figure 1).

We, therefore, compared the clinical and biochemical characteristics of patients with cortisol levels after 1mg-DST ≥1.8 and <1.8 μg/dl (DST+, n=220 and DST-, n=298, respectively, Table 2). The two groups were comparable for gender distribution, BMI, family CVE history and prevalence of OB, smokers, post-menopausal women and of not well controlled blood pressure, glycemia and LDL. As expected, DST+ patients showed higher prevalence of h-UFC and lower ACTH levels than DST- patients. Age, adenoma size and AH, T2DM, DL and CVE prevalence were higher in DST+ than in DST- patients. The CVE occurred within 2 years before or after the AI finding in 55.9% of DST+ patients and in 25.8% of DST- patients ($p=0.006$)

In the whole sample, the comorbidities score was associated with the cortisol levels after 1mg-DST ($r=0.105$, $p=0.017$). Moreover, the CVE occurrence was directly associated with the increase of the comorbidity score (0 = 2.8%, 1 = 13.6, 2 = 21.2%, 3 = 28.9%, 44.4%, p for trend <0.0001). The ROC curve analysis showed that a comorbidity score of 2 had the best compromise between SN (72.2%) and SP (57.5%) in detecting CVE patients (AUC 0.70, $p<0.0001$) and that a comorbidity score ≥2 had 60% accuracy in

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179 detecting CVE patients. Adding the DST+ presence to the comorbidity score increased the accuracy to
180 71.2% (SN 61.1, SP 73.4%). The logistic regression analysis showed that the CVE occurrence was 2.5 folds
181 increased in DST+ patients, regardless of age, gender, duration of observation, CVE family history, smoking
182 habit, T2DM, AH, and DL (Table 3). This association was confirmed also when the comorbidity score ≥2
183 was included into the regression analysis in the place of T2DM, AH and DL (Table 4).
184
185 *Artificial neural networks analysis (ANNs)*
186 The connectivity map showed that the CVE occurrence was directly associated to the presence of
187 cortisol levels after 1-DST ≥1.8 µg/dl, AH and male gender (Figure 2). The following variables were put into
188 the system: CVE familiar history, age, gender, BMI, cortisol levels after 1mgDST, smoking packs/year,
189 presence of postmenopausal status, smoking habit, obesity, hypertension, diabetes, well controlled blood
190 pressure, glycemia and LDL, low ACTH levels (i.e <10 pg/dL), h-UFC levels, 1mg-DST ≥1.8 µg/dL and
191 CVE occurrence. Among these variables, the Auto-CM system selected the CVE occurrence, CVE familiar
192 history, age, T2DM, AH, DL, low ACTH, h-UFC levels, 1mg-DST ≥1.8 µg/dL and cortisol levels after
193 1mgDST. By using this cluster of variables the Auto-CM system was able to detect patients with the
194 occurrence of CVE with 78.5% accuracy (SN 78.7%, SP 78.3%). By excluding 1mg-DST ≥1.8 µg/dL the
195 diagnostic accuracy decreased to 72.5% (SN 70.9%, SP 74.1%).

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Discussion

The present study was designed to investigate whether or not, in patients with monolateral AI, the presence of an increased, though asymptomatic, cortisol secretion was associated with the CVE occurrence independently of the common cardiovascular risk factors, such as AH, T2DM, DL, age, smoking habit and CVE familiar history. In addition, we aimed to assess if ANNs, that are suited for solving nonlinear problems, could be useful to detect AI patients with CVE.

To our knowledge this is the largest study assessing the CVE occurrence in AI patients. We found that the cut-off of cortisol levels after 1mg-DST set at 1.8 µg/dL had the best accuracy for detecting AI patients with CVE and that AI patients with 1mg-DST above or equal to 1.8 µg/dL (DST+) are more frequently hypertensive, diabetic, dyslipidemic and affected with CVE. The presence of at least two CVE risk factors (among AH, T2DM, DL and OB) was associated with the CVE occurrence, but when the presence of DST+ was added to this cluster, the association became stronger. In keeping, in the presence of DST+ the CVE occurrence is 2.5 folds increased, regardless for the presence of AH, T2DM, DL, smoking habit, age and gender. Finally, the ANNs confirmed the independent association between DST+ and the CVE occurrence and suggested that this statistical approach could be useful for assessing the CVE risk in the individual AI patient.

The present findings are in keeping with those of a previous smaller study by Di Dalmazi and co-authours (8) and with those of a previous Italian multicenter study (9) showing that AI patients with SH had an increased CVE occurrence. Although in those studies the association between SH and the CVE was present regardless for hypertension (8) and age (8, 9), the additive role of SH on the CVE occurrence was not completely clarified.

The difficulties in determining the SH influence on the cardiovascular risk have been also due to the lack of a widely accepted definition of SH itself (17, 32). Some information at this regard might be derived from the present data. Indeed, in the present study, the DST+ patients had a 2.5 folds increased CVE occurrence. Thanks to the possibility to analyze a large sample of patients, rather than arbitrarily classifying patients as affected or not with SH, we could individuate the cut-off of cortisol levels after 1mg-DST with the best accuracy in predicting the CVE occurrence and we were able to confirm that the cut-off of cortisol levels after 1mg-DST above or equal to 1.8 µg/dL is the most accurate one in detecting patients with the

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CVE occurrence. Therefore, this cut-off of cortisol levels after 1mg-DST could be proposed for diagnosing SH.

Apart from the SH definition, the main problem with the use of the biochemical tests in AI subjects is that raising the cut-offs of the cortisol secretion markers increases SP, but at the expense of a not acceptable SN decrease and *viceversa.* Interestingly, a recent metanalysis suggests a beneficial effect of adrenalectomy on cardiovascular risk factors in patients with SH compared with conservative management, even if with a low-moderate quality of evidence (33). However, to date, we cannot use a combination of clinical and biochemical markers for predicting the cardiovascular outcome in these patients, and, therefore, an algorithm for assessing the cardiovascular risk in the individual AI patient is lacking (16). These difficulties are mainly due to the fact that some comorbidities (i.e. T2DM, AH, DL and OB), that may depend even on SH itself, may worsen the SH-related cardiovascular risk and interact with SH in nonlinear biologic mechanisms.

From this point of view, the evaluation of the possible use of the ANNs to investigate the relationships between SH and the common cardiovascular risk factors is a novel approach. The ANNs are artificial adaptive systems, able to modify their internal structure in relation to a function objective, and, therefore, they are particularly suited for solving nonlinear problems (20]. The ANNs analysis demonstrates that combining several variables, including the cardiovascular risk factors, age and cortisol levels after 1mg-DST, it could be possible to further increase the accuracy (up to 80%) for detecting patients with CVE occurrence. This approach could open the field for the development of algorithms for assessing the CVE risk in the individual AI patient.

This study has some limitations. Firstly, we acknowledge that our study is retrospective and it does not report data regarding the CVE incidence during a standardized follow-up. In addition, being the SH condition asymptomatic, we cannot have information about the temporal distance between the onset of hypercortisolism and the CVE occurrence. However, at variance with DST- patients, in DST+ patients the CVE occurred mainly within 2 years before or after the AI finding. This finding suggests a temporal relationship between the CVE occurrence and the condition of subtle hypercortisolism and confirms the results of a previous smaller study showing that SH is associated with the risk of incident CVE (8).

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Furthermore, we have no information regarding the behavior of the cortisol secretion and the comorbidities of the subjects during the observation period, and, therefore, we could not assess the influence of the possible increase of the cortisol secretion and/or of the possible changes in the comorbidities control. However, since the comorbidities control assessed at the study entry was not different between patients with and without CVE and with and without DST+, this variable should not have been of particularly influence in the study. Finally, given the cross-sectional design of the study, definitive information regarding the possible causative role of a subtle increased cortisol secretion on the occurrence of CVE could not be derived. However, in consideration of the known deleterious effect of the overt hypercortisolism on the CVE risk, a similar role of SH could be affirmed.

In conclusion, the present study shows that: i) a subtle cortisol excess is associated with the CVE occurrence regardless of age, gender, familiar CVE history, AH, T2D, DL and smoke, therefore representing an additional CVE risk factor; ii) the cut-off of cortisol levels after 1mg-DST above or equal to 1.8 μg/dL has the best accuracy in detecting AI patients with CVE; iii) the application of the ANNs in evaluating the interaction between the cortisol secretion and the other CVE risk factor increases the accuracy for detecting AI patients at higher CVE risk.

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Disclosures

Declaration of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector. No author has been payed by pharmaceutical companies or other agencies.

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References

1. Chiodini I. Clinical review: Diagnosis and treatment of subclinical hypercortisolism. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 1223–1236.
2. Debono M & Newell-Price J. Subclinical hypercortisolism in adrenal incidentaloma. *Current Opinion in Endocrinology Diabetes and Obesity* 2015 **22** 185-192
3. Goddard GM, Ravikumar A & Levine AC. Adrenal mild hypercortisolism. *Endocrinology Metabolism Clinics of North America* 2015 **44** 371-379.
4. Chiodini I, Vainicher CE, Morelli V, Palmieri S, Cairoli E, Salcuni AS, Copetti M & Scillitani A. Endogenous subclinical hypercortisolism and bone: a clinical review. *European Journal of Endocrinology* 2016 **175** R265-R282
5. Giordano R, Guaraldi F, Berardelli R, Karamouzis I, D'Angelo V, Marinazzo E, Picu A, Ghigo & Arvat E. Glucose metabolism in patients with subclinical Cushing's syndrome. *Endocrine* 2012 **41** 415–423.
6. Di Dalmazi G & Pasquali R. Adrenal adenomas, subclinical hypercortisolism, and cardiovascular outcomes. *Current Opinion in Endocrinology Diabetes and Obesity* 2015 **22** 163-168
7. Di Dalmazi G, Vicennati V, Rinaldi E, Morselli-Labate AM, Giampalma E, Mosconi C, Pagotto U & Pasquali R. Progressively increased patterns of subclinical cortisol hypersecretion in adrenal incidentalomas differently predict major metabolic and cardiovascular outcomes: a large cross-sectional study. *European Journal of Endocrinology* 2012 **166** 669–677.
8. Morelli V, Reimondo G, Giordano R, Della Casa S, Policola C, Palmieri S, Salcuni AS, Dolci A, Mendola M, Arosio M, Ambrosi B, Scillitani A, Ghigo E, Beck-Peccoz P, Terzolo M & Chiodini I. Long-term follow-up in adrenal incidentalomas: an Italian multicenter study. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 827–834.
9. Di Dalmazi G, Vicennati V, Garelli S, Casadio E, Rinaldi E, Giampalma E, Mosconi C, Golfieri R, Paccapelo A, Pagotto U & Pasquali R. 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. *Lancet Diabetes Endocrinol*ogy 2014 **2** 396–405.

For Review Only

10. 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. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 4462–4467 .
11. Toniato A, Merante-Boschin I, Opocher G, Pelizzo MR, Schiavi F & Ballotta E. Surgical versus conservative management for subclinical Cushing syndrome in adrenal incidentalomas: a prospective randomized study. *Annals of Surgery* 2009 **249** 388–391.
12. Chiodini I, Morelli V, Salcuni AS, Eller-Vainicher C, Torlontano M, Coletti F, Iorio L, Cuttitta A, Ambrosio A, Vicentini L, Pellegrini F, Copetti M, Beck-Peccoz P, Arosio M, Ambrosi B, Trischitta V & Scillitani A. Beneficial metabolic effects of prompt surgical treatment in patients with an adrenal incidentaloma causing biochemical hypercortisolism. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 2736-2745.
13. Sereg M, Szappanos A, Toke J, Karlinger K, Feldman K, Kaszper E Varga I, Gláz E, Rácz K & Tóth M. Atherosclerotic risk factors and complications in patients with non-functioning adrenal adenomas treated with or without adrenalectomy: a long-term follow-up study. *European Journal of Endocrinology* 2009 **160** 647–655.
14. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, Tabarin A, Terzolo M, Tsagarakis S & Dekkers OM. 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* 2016 **175** G1-G34
15. Morelli V, Scillitani A, Arosio M & Chiodini I. Follow-up of patients with adrenal incidentaloma, in accordance with the European society of endocrinology guidelines: Could we be safe? *Journal of Endocrinological Investigation*. 2016 (epub ahead of print, doi: 10.1007/s40618-016-0558-x)
16. Chiodini I, Albani A, Ambrogio AG, Campo M, De Martino MC, Marcelli G, Morelli V, Zampetti B, Colao A, Pivonello R & ABC Group. Six controversial issues on subclinical Cushing's syndrome. *Endocrine* 2016; (epub ahead of print, doi: 10.1007/s12020-016-1017-3)
17. Shen J, Sun M, Zhou B & Yan J. Nonconformity in the clinical practice guidelines for subclinical Cushing's syndrome: which guidelines are trustworthy? *European Journal of Endocrinology* 2014 **171** 421-431

For Review Only

18. Eller-Vainicher C, Morelli V, Salcuni AS, Battista C, Torlontano M, Coletti F, Iorio L, Cairoli E, Beck-Peccoz P, Arosio M, Ambrosi B, Scillitani A & Chiodini I. Accuracy of several parameters of hypothalamic-pituitary-adrenal axis activity in predicting before surgery the metabolic effects of the removal of an adrenal incidentaloma. *European Journal of Endocrinology* 2010 **163** 925-935.
19. Di Dalmazi G, Pasquali R, Beuschlein F & Reincke M. Subclinical hypercortisolism: a state, a syndrome, or a disease? *European Journal of Endocrinology* 2015 **173** M61-M71.
20. Buscema M & Grossi E. The semantic connectivity map: an adapting self-organising knowledge discovery method in data bases. Experience in gastro-oesophageal reflux disease. *International Journal of Data Mining and Bioinformatics* 2008 **2** 362-404.
21. Buscema M, Grossi E, Snowdon D, Antuono P. Auto-Contractive Maps: an artificial adaptive system for data mining. An application to Alzheimer disease. *Current Alzheimer Research* 2008 **5** 481-498
22. Obermeyer Z, Emanuel EJ. Predicting the Future — Big Data, Machine Learning, and Clinical Medicine. *New England Journal of Medicine* 2016 **375** 1216-19
23. Eller-Vainicher C, Chiodini I, Santi I, Massarotti M, Pietrogrande L, Cairoli E, Beck-Peccoz P, Longhi M, Galmarini V, Gandolini G, Bevilacqua M & Grossi E. Recognition of morphometric vertebral fractures by artificial neural networks: analysis from GISMO Lombardia Database. *PLoS One* 2011 **6** e27277 (doi: 10.1371/journal.pone.0027277)
24. Eller-Vainicher C, Zhukouskaya VV, Tolkachev YV, Koritko SS, Cairoli E, Grossi E, Beck-Peccoz P, Chiodini I & Shepelkevich AP. Low bone mineral density and its predictors in type 1 diabetic patients evaluated by the classic statistics and artificial neural network analysis. *Diabetes Care* 2011 **34** 2186-2191
25. Baldassarre D, Grossi E, Buscema M, Intraligi M, Amato M, Tremoli E, Pustina L, Castelnuovo S, Sanvito S, Gerosa L & Sirtori CR. Recognition of patients with cardiovascular disease by artificial neural networks. *Annals of Medicine* 2004 **36** 630-40.
26. Kreitschmann-Andermahr I, Psaras T, Tsiogka M, Starz D, Kleist B, Siegel S, Milian M, Kohlmann J, Menzel C, Führer-Sakel D, Honegger J, Sure U, Müller O & Buchfelder M.From first symptoms

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to final diagnosis of Cushing's disease: experiences of 176 patients. *European Journal of Endocrinology* 2015 **172** 285-289.

27. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2003 **26** (Suppl 1) S5–S20
28. Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC); European Association for the Study of Diabetes (EASD), Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N, Deaton C, Escaned J, Hammes HP, Huikuri H, Marre M, Marx N, Mellbin L, Ostergren J, Patrono C, Seferovic P, Uva MS, Taskinen MR, Tendera M, Tuomilehto J, Valensi P, Zamorano JL. ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD - summary. *Diabetes and Vascular Disease Research* 2014 **11** 133-173.
29. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B & Zannad F; Task Force for the Management of Arterial Hypertension of the European Society of Hypertension and the European Society of Cardiology. 2013 ESH/ESC Practice Guidelines for the Management of Arterial Hypertension. *Blood Pressure* 2014 **23** 3-16
30. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *Journal of the American Medical Association* 2001 **285** 2486–2497
31. Teo KK, Ounpuu S, Hawken S, Pandey MR, Valentin V, Hunt D, Diaz R, Rashed W, Freeman R, Jiang L, Zhang X & Yusuf S; INTERHEART Study Investigators. Tobacco use and risk of myocardial infarction in 52 countries in the INTERHEART study: a case-control study. *Lancet* 2006 **19368** 647-658.
32. Morelli V & Chiodini I. Subclinical Hypercortisolism: how to deal with it. *Front Horm Res* 2016 **46** 28–38.

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33. Bancos I, Alahdab F, Crowley RK, Chortis V, Delivanis DA, Erickson D, Natt N, Terzolo M, Arlt W, Young WF & Murad MH. Improvement of cardiovascular risk factors after adrenalectomy in patients with adrenal tumors and subclinical Cushing's syndrome: a systematic review and meta-analysis. *European Journal of Endocrinology* 2016 **175** R283-R295.

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Legend to Figure 1

Title: The receiver operating characteristic (ROC) curve analysis showing the association between serum cortisol levels at 08:00 h after 1mg-dexamethasone suppression test (1mg-DST) and the occurrence of cardiovascular events

Footnotes: The ROC curve showed that the cut-off of cortisol levels after 1mg-DST set at 1.8 μg/dl had the best compromise between sensitivity (SN, 63%) and specificity (SP, 66%) in predicting CVE (area under the curve, AUC, 0.673, $p<0.0001$).

Legend to Figure 2

Title: Semantic connectivity map of studied variables.

Footnotes: The semantic connectivity map showed that the occurrence of a cardiovascular event (CVE) was directly associated to the presence of cortisol levels at 08:00 h after 1mg-dexamethasone suppression test (1mg-DST) $\geq$ 1.8 μg/dL, arterial hypertension and male gender (included within a triangle).

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Table 1. Clinical and biochemical parameters of patients with and without CVE

	CVE+ Group (n=90)	CVE- Group (n=428)	P
Age (yrs)	68.3±9.8 (20-84)	62.7±10.4 (33-86)	<0.001
Male/Female gender	47/43 (52/48)	157/271 (37/63)	0.009
Postmenopausal females	43 (47.8)	229 (53.5)	0.354
BMI (kg/m^2)	28.1±4.5 (20-40)	27.5±4.8 (18-40)	0.279
Diameter of adenoma (mm)	26±9.7 (10-55)	24.4±9.5 (10-60)	0.164
Duration of observation period (months)	164.7±49.3 (121-353)	161.2±44.2 (120-426)	0.425
Patients with familiar history of CVE	27 (30)	123 (28.7)	0.799
Smokers	47 (52)	173 (40)	0.027
ACTH (pg/mL)	14.3±9 (1.6-55)	14.8±10.4 (1-55)	0.696
1mg-DST (µg/dL)	2.5±1.4 (0.5-7.2)	1.9±1.4 (0.1-11.5)	<0.001
h-UFC patients	6 (6.7)	36 (8.4)	0.676
Hypertensive patients	83 (92)	262 (61)	<0.001
Blood pressure not at target[1]	41 (46.5)	178 (41.5)	0.557
Diabetic patients	39 (43)	73 (17)	<0.001
Glycemia not at target[2]	9 (10)	25 (5.8)	0.160
Dyslipidemic patients	51 (57)	138 (32)	<0.001
LDL not at target	25 (27.8)	91 (21.3)	0.210
Obese subjects	27 (30)	141 (33)	0.622

Data are mean ±SD with range in parenthesis or absolute number with percentage in parenthesis. BMI: body mass index; CVE: cardiovascular event; 1mg-DST: serum cortisol levels after 1–mg dexamethasone suppression test; ACTH: adrenocorticotroph hormone. h-UFC: urinary free cortisol levels above normal reference range. SI conversion factors: cortisol x 27.59; ACTH x 0.22. Smokers: individuals who smoked any tobacco (including beedies, pipes, and other forms) during the observational period. LDL: low density lipoprotein. [1]among hypertensive patients; [2]among diabetic patients.

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Table 2. Clinical and biochemical parameters of patients with and without 1mg-DST ≥1.8 μg/dL

	DST+ (n=220)	DST- (n=298)	P
Age (yrs)	66.0±10.2 (20-85)	61.9±10.3 (34-86)	<0.001
Male/Female gender	84/136 (38.2/61.8)	120/178 (40.3/59.7)	0.650
Postmenopausal females	125 (56.8)	147 (43.9)	0.109
BMI (kg/m^2)	27.4±4.9 (18-40)	27.8±4.6 (18.3-40)	0.30
Diameter of adenoma (mm)	28.8±9.5 (10-60)	21.6±8.3 (10-55)	<0.001
Duration of observation period (months)	163.7±44.9 (120-353)	160.4±45.3 (121-426)	0.420
Patients with familiar history of CVE	84 (28.2)	66 (30)	0.695
Smokers	96 (43.6)	124 (41.6)	0.654
ACTH (pg/mL)	12.3±8.5 (1-55)	16.5±10.8 (1.6-55)	<0.001
1mg-DST (μg/dL)	3.1±1.6 (1.8-11.5)	1.1±0.35 (0.1-1.7)	<0.001
h-UFC patients	27 (12.3)	15 (5.0)	0.003
Hypertensive patients	164 (74.5)	181 (60.7)	0.001
Blood pressure not well controlled[1]	96 (43.6)	123 (41.6)	0.653
Diabetic patients	57 (25.9)	55 (18.5)	0.049
Glycemia not well controlled[2]	15 (6.8)	19 (6.4)	0.859
Dyslipidemic patients	98 (41.4)	91 (32.9)	0.049
LDL not well controlled	54 (25.4)	62 (20.8)	0.338
Obese subjects	62 (28.2)	88 (29.5)	0.769
Patients with CVE	59 (26.8)	31 (10.4)	<0.001

Data are mean ±SD with range in parenthesis or absolute number with percentage in parenthesis.

DST+: patients with 1mg-DST ≥1.8 μg/dL; DST-: patients with 1mg-DST <1.8 μg/dL. BMI: body mass index; 1mg-DST: serum cortisol levels after 1–mg dexamethasone suppression test; ACTH: adrenocorticotroph hormone. h-UFC: urinary free cortisol levels above normal reference range. SI conversion factors: cortisol x 27.59; ACTH x 0.22. CVE: cardiovascular event. Smokers: individuals who smoked any tobacco (including beedies, pipes, and other forms) during the observational period. LDL: low density lipoprotein; [1]among hypertensive patients; [2]among diabetic patients.

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Table 3. Association between serum cortisol levels after 1mg-dexamethasone suppression test ≥1.8 µg/dL and the occurrence of cardiovascular event after adjustment for comorbidities and other possible contributing factors using the logistic regression analysis.

	Odds ratio	**95% CI**	**P value**
Female gender	1.6	0.9-2.7	0.13
Age (1 year increase)	1.03	1.0-1.1	0.023
Familiar history of CVE (presence vs absence)	1.1	0.6-1.9	0.724
T2DM (presence vs absence)	1.9	1.1-3.4	0.025
AH (presence vs absence)	4.3	1.8-9.8	0.001
DL (presence vs absence)	1.8	1.1-3.1	0.032
Smoke (presence vs absence)	1.8	1.1-3.2	0.03
Duration of observation period (1 month increase)	1.0	1.0-1.0	0.489
1mg-DST ≥1.8 µg/dL (presence vs absence)	2.5	1.5-4.2	<0.001

AH: arterial hypertension; DL: dyslipidemia; T2DM: type 2 diabetes mellitus; smoke: smoke of any tobacco (including beedies, pipes, and other forms) during the observational period. 1mg-DST: serum cortisol levels at 08:00 h after 1mg-dexamethasone suppression test. CVE: cardiovascular event.

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Table 4 Association between serum cortisol levels after 1mg-dexamethasone suppression test ≥1.8 µg/dL and the occurrence of cardiovascular event after adjustment for the comorbidities score and other possible contributing factors using the logistic regression analysis.

	Odds ratio	**95% CI**	**P value**
Female gender	1.7	1.0-2.9	0.04
Age (1 year increase)	1.0	1.0-1.1	0.001
Familiar history of CVE (presence vs absence)	1.1	0.6-1.9	0.740
Comorbidity score (<2 or ≥2)	2.9	1.7-4.9	<0.001
Smoke (presence vs absence)	1.6	1.0-2.9	0.053
Duration of observation period (1 month increase)	1.0	1.0-1.0	0.638
1mg-DST ≥1.8 µg/dL (presence vs absence)	2.7	1.6-4.5	<0.001

AH: arterial hypertension; DL: dyslipidemia; T2DM: type 2 diabetes mellitus; smoke: smoke of any tobacco (including beedies, pipes, and other forms) during the observational period. 1mg-DST: serum cortisol levels at 08:00 h after 1mg-dexamethasone suppression test. comorbidities score: sum of the comorbidities (type 2 diabetes, arterial hypertension, dyslipidemia

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Figure 1

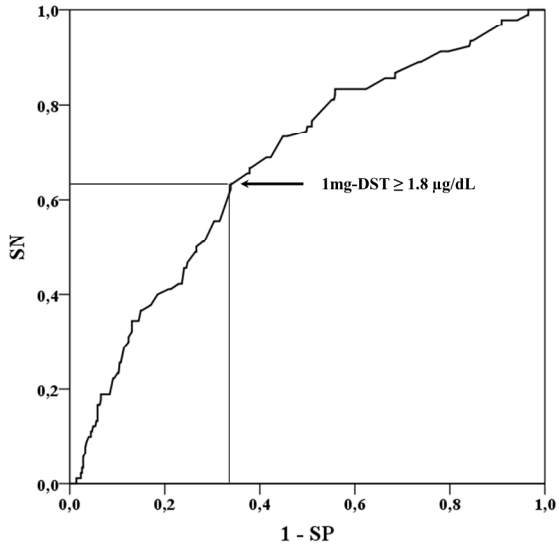

Figure 1: The receiver operating characteristic (ROC) curve analysis showing the association between serum cortisol levels at 08:00 h after 1mg-dexamethasone suppression test (1mg-DST) and the occurrence of cardiovascular events

The ROC curve showed that the cut-off of cortisol levels after 1mg-DST set at 1.8 µg/dl had the best compromise between sensitivity (SN, 63%) and specificity (SP, 66%) in predicting CVE (area under the curve, AUC, 0.673, p<0.0001).

254x190mm (300 x 300 DPI)

Figure 2

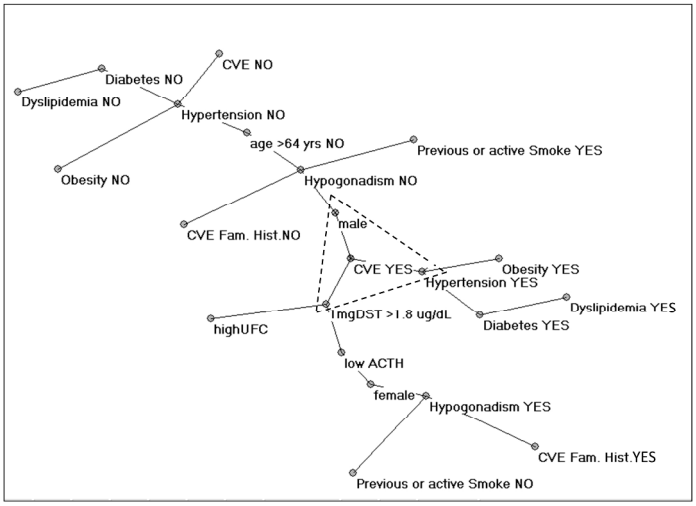

Figure 2: Semantic connectivity map of studied variables.

The semantic connectivity map showed that the occurrence of a cardiovascular event (CVE) was directly associated to the presence of cortisol levels at 08:00 h after 1mg-dexamethasone suppression test (1mg-DST) ≥ 1.8 µg/dL, arterial hypertension and male gender (included within a triangle).

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