Prediction of Vertebral Fractures in Patients With Monolateral Adrenal Incidentalomas

Valentina Morelli,* Cristina Eller-Vainicher,* Serena Palmieri, Elisa Cairoli, Antonio Stefano Salcuni, Alfredo Scillitani, Vincenzo Carnevale, Sabrina Corbetta, Maura Arosio, Silvia Della Casa, Giovanna Muscogiuri, Anna Spada, and Iacopo Chiodini

Department of Medical Sciences and Community Health (V.M., S.P., E.C., M.A., A.S.), University of Milan, Milan, Italy; Unit of Endocrinology and Metabolic Diseases (V.M., C.E.-V., S.P., E.C., A.S., I.C.), IRCCS Cà Granda-Ospedale Maggiore Policlinico Milan, Italy; Unit of Endocrinology (A.S.S., A.S.) and Unit of Internal Medicine (V.C.), "Casa Sollievo della Sofferenza," Hospital, IRCCS, San Giovanni Rotondo, Foggia, Italy; Endocrine Unit (A.S.S.), Department of Medical Sciences, University of Cagliari, Italy; Unit of Endocrinology (S.C.), Department of Biomedical Sciences for Health, University of Milan, Policlinico San Donato IRCCS, San Donato Milanese, Milan, Italy; Unit of Endocrine Diseases and Diabetology (M.A.), Ospedale San Giuseppe, Gruppo Multimedica, Milan, Italy; Unit of Endocrinology and Metabolic Diseases (S.D.C., G.M.), Università Cattolica del Sacro Cuore, Rome, Italy

Context: Subtle hypercortisolism is associated with an increased risk of vertebral fracture (VFx).

Objective: The objective was to determine the best parameters of cortisol secretion for detecting the VFx risk in patients with adrenal incidentalomas (AI).

Design: This was a retrospective (cross-sectional arm) and prospective (longitudinal arm) design. In the cross-sectional arm, we assessed the accuracy of the cortisol secretion indexes in identifying the patients with VFx (prevalent VFx). In the longitudinal arm, we tested the cortisol secretion parameters, which were able to identify the prevalent VFx, for the prediction of the occurrence of a new VFx (incident VFx) in AI patients followed-up for at least 2 years.

Setting: Four referral Italian endocrinology units participated in this study.

Patients: A total of 444 and 126 AI patients without symptoms of hypercortisolism enrolled in the cross-sectional arm and longitudinal arm, respectively.

Main Outcome Measures: Serum cortisol after a 1-mg dexamethasone suppression test (1 mg DST), urinary free cortisol, ACTH, bone mineral density at lumbar spine and femoral neck (by dual-energy x-ray absorptiometry), and the VFx presence (by x-ray).

Results: The cortisol levels after 1 mg DST that were greater than 2.0 μg/dl (55 nmol/liter) were the best criteria for detecting patients with both prevalent (73.6% sensitivity, 70.5% specificity) and incident VFx (80% sensitivity, 68.8% specificity) and were associated with a 10-fold increased risk of a new VFx (odds ratio, 10.27; 95% confidence interval, 3.39–31.12; $P < .0001$), regardless of age, gender, bone mineral density at lumbar spine, and prevalent VFx.

Conclusions: In AI patients without symptoms of overt hypercortisolism, the cortisol levels after 1 mg DST greater than 2.0 μg/dl (55 nmol/liter) represent the best criterion for detecting prevalent and incident VFx. **(*J Clin Endocrinol Metab* 101: 2768–2775, 2016)**

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* V.M. and C.E.-V. contributed equally to this study.

Abbreviations: AI, adrenal incidentaloma; AUC, area under the curve; BMD, bone mineral density; BMI, body mass index; CI, confidence interval; CRH, corticotroph-hormone releasing: CT, computed tomography; DST, dexamethasone suppression test; FN, femoral neck; HPA, hypothalamic-pituitary-adrenal; LS, lumbar spine; OR, odds ratio; ROC, receiver operating characteristic; SH, subclinical hypercortisolism; SN, sensitivity; SP, specificity; UFC, urinary free cortisol; VFx, vertebral fracture.

Subclinical hypercortisolism (SH) is defined as a condition of increased cortisol secretion in the absence of the classical signs or symptoms of overt hypercortisolism (1, 2). This condition is estimated to affect up to the 30% of patients bearing an incidentally discovered adrenal adenoma (adrenal incidentaloma [AI]), which, in turn, is thought to be present in up to 4–7% of adults (3,4). The clinical importance of SH is related not only to its high prevalence, but also to its chronic complications, because SH has been suggested to be associated with an increased prevalence of hypertension, diabetes mellitus, cardiovascular events, and mortality (5–11), and some studies showed that the recovery from SH ameliorates diabetes and hypertension in AI patients (12–14). Notwithstanding the high clinical importance of SH, the diagnosis of this condition is still a matter of debate. Indeed, although the assessment of serum cortisol after 1 mg overnight dexamethasone administration (1 mg dexamethasone suppression test [DST]) is widely considered the most reliable and easy-to-perform parameter (1) for diagnosing SH, the best cutoff to be used is still unknown, some authors suggesting a high threshold (ie, 1 mg DST 5 μg/dl, 138 nmol/liter) to increase specificity (15, 16), whereas others a much lower cutoff (ie, 1 mg DST 1.8 μg/dl, 50 nmol/liter) to have high sensitivity (17–19). Interestingly, recent data show that in AI patients, a serum cortisol after 1 mg DST above 1.8 μg/dl (50 nmol/liter) is associated with "hard" cardiovascular end-points, such as the increased risk of cardiovascular events and mortality (9–11).

Several studies suggest that SH is associated also with an accelerated bone loss and with an increased prevalence and incidence of vertebral fragility fractures (VFx) (20–26). Recently, the recovery from SH has been suggested to reduce the VFx risk (27). However, it is still unknown which cutoff for cortisol after 1 mg DST has to be used for deciding which patient has to be considered at risk for VFx. It is important to consider that the threshold of cortisol secretion used for identifying the patients at risk for cardiovascular events might not be reliable for identifying patients at risk for VFx. Indeed, bone tissue is extremely sensitive to cortisol excess and the fragility VFx may be the presenting symptom of an otherwise asymptomatic hypercortisolism (28). However, if we could find a parameter of cortisol secretion reliable in predicting the risk of both cardiovascular events and VFx, we could consider this index as a useful clinical tool for diagnosing a condition of subtle cortisol excess.

Therefore, the present study was aimed to define which are the best parameters of cortisol secretion for predicting the presence of VFx and the occurrence of a new VFx in AI patients.

Study design; patients and methods

Design of the study

In the present study, we first analyzed the data-sets of three previous studies reporting data on both the VFx prevalence and the markers of hypothalamic-pituitary-adrenal (HPA) axis activity in patients with AI (24, 26, 27). Therefore, in this cross-sectional arm, we assessed in 444 AI patients (271 females, 173 males) the diagnostic accuracy of the most frequently used markers of HPA axis activity in identifying the patients with a VFx (prevalent VFx). Among the 444 patients included, 287 subjects had already been included in the first study (24), 102 subjects in the second study (26), and 55 subjects in the third study (27).

Subsequently, in the longitudinal arm of the study, we investigated if the parameters of HPA axis activity, that in the cross-sectional arm of the study were more reliable for identifying the prevalent VFx, were also useful for predicting the occurrence of a new VFx (incident VFx) in a group of 126 AI patients that have been followed up for at least 24 months. Among the patients included in the longitudinal arm of the study, 103 had already been included in a previous studies assessing in AI patients with and without SH the incidence of VFx (25) and 23 subjects had already been included in a recently published study evaluating the effect of surgery in reducing the VFx risk (27).

Subjects

The enrollment criteria have been already described elsewhere (24–27). Briefly, the AI patients included in the cross-sectional arm of the study were enrolled from January 1997 to June 2013 in four referral Italian Endocrinology Units: Scientific Institute "Casa Sollievo della Sofferenza" in San Giovanni Rotondo, "San Giuseppe" Hospital in Milan, Scientific Institute "Fondazione Cà Granda-Ospedale Maggiore Policlinico" in Milan, and Scientific Institute "Policlinico San Donato" in San Donato Milanese. The patients included in the longitudinal arm were enrolled in the same centers from January 2005 to June 2013.

All AI were discovered by computed tomography (CT) scan, ultrasonography, or magnetic resonance imaging performed for unrelated diseases. Ultrasound findings were confirmed with unenhanced CT. No subject had evidence of metastatic diseases. At CT scan, all adrenal masses were more than 1 cm in diameter, homogeneous and hypodense (Hounsfield units ≤10), and with well-shaped features consistent with the diagnosis of adrenocortical adenoma. In all patients, the diagnosis of aldosteronoma was ruled out.

The exclusion criteria were the following: 1) the presence of signs and/or symptoms of cortisol excess (ie, striae rubrae, moon facies, buffalo hump, proximal muscle weakness, and skin atrophy); 2) history of hypogonadism (in men testosterone levels <300 ng/dl and in premenopausal women fewer than six menstrual cycles/year) and thyrotoxicosis, bowel diseases, chronic kidney and hepatic disease, alcoholism, eating disorders, rheumatologic or hematological diseases; 3) administration of drugs influencing cortisol and dexamethasone metabolism or cortisol secretion; or 4) the presence of bilateral adrenal masses (29).

In all patients, we measured serum cortisol levels after 1 mg DST, plasma ACTH levels (normal values 10–55 pg/ml, 2.2–12 pmol/liter), and 24-hour urinary cortisol (UFC) levels (normal values, 10–70 μg/24 hours, 28–193 nmol/24 hours). In all patients with cortisol levels after 1 mg DST higher than 1.8 μg/dl

(50 nmol/liter) and basal ACTH levels at morning above 10 pg/ml (2.2 pmol/liter), a corticotroph-hormone releasing (CRH) test was performed to rule out an ACTH-dependent SH (2). In addition, in all patients the body mass index (BMI) and the presence of diabetes were evaluated because VFx are more frequent in diabetic patients (30). Diabetes mellitus was diagnosed using World Health Organization criteria (31).

Finally, in all subjects, bone mineral density (BMD) was measured by dual-energy x-ray absorptiometry (Hologic Discovery, software version 13.3:3) at lumbar spine (LS, precision 1.0%) and femoral neck (FN, precision 1.8%) and expressed as SD units (Z-score). Conventional spinal radiographs in lateral (T4-L4) and anteroposterior projection were obtained in all subjects with standardized technique. A trained radiologist, who was blinded to BMD and hormonal data, reviewed the radiographs. Prevalent and incident VFx were diagnosed on visual inspection using the semiquantitative visual assessment previously described by Genant and colleagues (32, 33). According to this technique, fractures assessed on lateral thoracolumbar spine radiographs were defined as reductions of more than 20% in anterior, middle, or posterior vertebral height.

All subjects signed the informed consent before entering the study, which has been approved by the Ethic Committees of the participating centers.

Materials and Methods

Serum and urinary samples were stored at –20 C until assayed. According to our national guidelines (34), all patients included in the longitudinal arm of the study with hypovitaminosis D were supplemented with cholecalciferol per os to achieve normal (>30 ng/dl, 75 nmol/liter) vitamin D levels, and all patients with a calcium intake less than 1000 mg/d were supplemented with oral calcium carbonate.

In each patient, plasma ACTH levels (mean of three determinations at 20-minute intervals) were measured by immunoradiometric assay (BRAHMS Diagnostica GmbH); serum cortisol and UFC levels (after dichloromethane extraction) were determined immunofluorimetrically by TDxFLx kits (Abbott GmbH Diagnostika) at the study entry. The intra- and interassay coefficients of variation were less than 15% for ACTH and less than 10% for all other assays.

Statistical analysis

Statistical analysis was performed by SPSS, version 21.0, statistical package (SPSS Inc.). The results were expressed as mean ± SD. The normality of distribution was tested by Kolmogorov-Smirnov test. The comparison of continuous variables between patients with and without prevalent VFx and between those with and without incident VFx was performed using the Student's *t* test or the Mann-Whitney *U* test as appropriate. Categorical variables were compared by χ^2 test.

The receiver operating characteristic (ROC) curve analysis was performed to assess the cutoff values for the parameters of HPA axis activity found to be statistically different between patients with and without prevalent VFx (in the cross-sectional arm of the study) or with and without incident VFx (in the longitudinal arm of the study) for predicting the presence of prevalent and incident VFx, respectively. On the basis of the obtained cutoffs, the sensitivity (SN), specificity (SP), positive and negative predictive values for detecting patients with prevalent VFx (in the cross-sectional arm of the study), and incident VFx (in the longitudinal arm of the study) were assessed.

The logistic regression analysis assessed in the cross-sectional arm of the study the association between the presence of prevalent VFx and the serum cortisol levels after 1 mg DST or ACTH at the cutoffs with the best compromise between SN and SP (as indicated by the ROC curve analysis), after adjusting for the independent variables known to be associated with increased fracture risk, such as age, gender, LS-BMD, and VFx. The same analysis assessed in the longitudinal arm of the study the association between the occurrence of a new VFx and the serum cortisol levels after 1 mg DST at the cutoff with the best compromise between SN and SP (as indicated by the ROC curve analysis) after adjusting for age, gender, LS-BMD, and VFx at baseline. The *P* values <.05 were considered significant.

Results

Cross-sectional arm

The clinical characteristics of AI patients with and without a prevalent VFx at baseline are reported in Table 1. Gender, BMI, UFC levels, and the prevalence of diabetes mellitus were comparable between the two groups. The patients with prevalent VFx were older and showed higher adenoma size and cortisol levels after 1 mg DST and lower ACTH levels and BMD at both LS and FN than patients without a prevalent VFx at baseline.

The ROC analysis showed that the cortisol levels after 1 mg DST (area under the curve [AUC], 0.730; $P < .0001$; Figure 1) and ACTH levels (AUC, 0.633; $P < .0001$; Figure 2) were significantly associated with the prevalent VFx, whereas UFC levels were not (AUC, 0.495; $P = .864$). The cutoff for cortisol after 1 mg DST and ACTH with the best compromise between SN and SP for detecting AI patients with VFx were set at 2.0 μg/dl (55 nmol/liter, Figure 1) and 10 pg/ml (2.2 pmol/liter, Figure 2), respectively.

The diagnostic accuracy of cortisol levels after 1 mg DST (with different cutoffs) and of ACTH levels below 10 pg/ml (2.2 pmol/liter) for identifying AI patients with prevalent VFx is reported in Table 2. The cortisol levels after 1 mg DST of at least 2.0 μg/dl (55 nmol/liter) showed a 73.6% SN and a 70.5% SP for identifying fractured AI patients. Specificity and SN greater than 90% were reached by cortisol levels after 1 mg DST more than 3.3 μg/dl (91 nmol/liter) and less than 1.0 μg/dl (27.6 nmol/liter), respectively. The SN and SP of ACTH levels less than 10 pg/ml (2.2 pmol/liter) for detecting fractured AI patients were sharply below 70% and the combination of cortisol levels after 1 mg DST of at least 2.0 μg/dl (55 nmol/liter) together with ACTH levels less than 10 pg/ml (2.2 pmol/liter) did not improve the diagnostic accuracy.

Table 1. Clinical and Biochemical Characteristics of Patients With Adrenal Incidentalomas (n = 444) With or Without a Prevalent Vertebral Fragility VFx Included in the Cross-Sectional Arm of the Study

	Patients Without Prevalent VFx (n = 251)	Patients With Prevalent VFx (n = 193)	*P*
Age (y)	59.5 ± 12.3 (21–89)	64.7 ± 9.5 (32–83)	.0001
Gender (females)	154 (61.4)	117 (60.6)	.875
BMI (kg/m^2)	29.3 ± 5.1 (19.5–40.9)	29.0 ± 4.5 (20.3–40.9)	.753
Diameter of the adenoma (cm)	2.4 ± 1.1 (0.8–8.0)	2.8 ± 1.1 (0.8–7.0)	.0001
1 mg DST (μg/dl)	1.8 ± 1.4 (0.5–9.2)	3.1 ± 2.2 (0.5–12)	.0001
UFC (μg/24 h)	53.9 ± 29.8 (10.0–169.1)	55.6 ± 34.8 (10.0–175.3)	.572
ACTH (pg/ml)	14.3 ± 8.7 (2.3–48.3)	11.3 ± 7.7 (1.6–48.3)	.0001
LS BMD (Z-score)	0.25 ± 1.38 (−3.60 to 3.61)	−0.33 ± 1.39 (−4.50 to 3.61)	.0001
FN BMD (Z-score)	0.14 ± 1.08 (−2.80 to 5.33)	−0.23 ± 1.02 (−2.80 to 2.70)	.0001
Patients with type 2 diabetes (%)	42 (16.7)	31 (16.1)	.850

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

SI conversion factors: cortisol after 1 mg DST 27.59, ACTH 0.22, UFC 2.759.

Because adrenalectomy is generally recommended for tumors larger than 4 cm (15–18), to verify if these results could be really useful in the clinical practice, we repeated the analyses after the exclusion of patients (n = 40) with tumors larger than 4 cm and the results were confirmed. Indeed, the cutoff for cortisol after 1 mg DST and ACTH with the best compromise between SN (70.9% and 59%, respectively) and SP (71.4% and 65%, respectively) for detecting fractured patients were set by the ROC analysis (AUC, 0.721, $P < .0001$; 0.620, $P < .0001$, respectively) at 2.0 μg/dl (55 nmol/liter) and 10 pg/ml (2.2 pmol/liter), whereas UFC levels were not associated with VFx (AUC, 0.500; $P = .995$).

Finally, the logistic regression analysis showed that the presence of a prevalent VFx was associated with cortisol levels after 1 mg DST of at least 2.0 μg/dl (55 nmol/liter; Table 3, model 1) or ACTH levels less than 10 pg/ml (2.2 pmol/liter, Table 3, model 2) regardless of age, gender, and LS-BMD.

In the four patients showing cortisol levels after 1 mg DST that were higher than 1.8 μg/dl (50 nmol/liter) together with ACTH levels above 30 pg/liter (6.6 pmol/liter) a CRH test showed a blunted ACTH response and the magnetic resonance failed to individuate pituitary adenomas. However, given that in these patients an ACTH-dependent form of SH could not be definitely ruled out, we

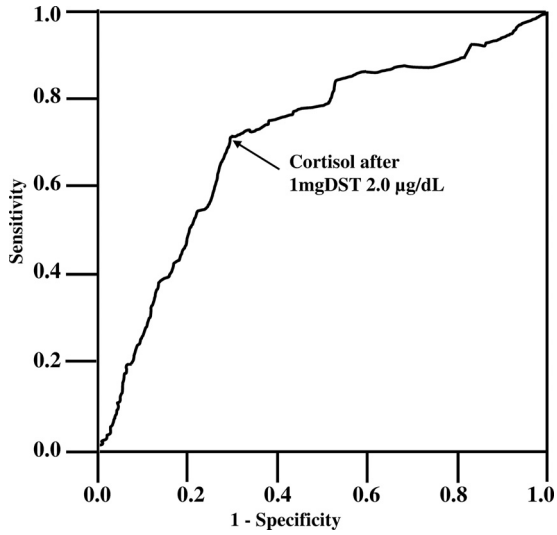

Figure 1. The ROC curve analysis assessing the association between the cortisol levels after 1 mg DST and the presence of a prevalent vertebral fracture in patients with adrenal incidentaloma. The ROC analysis showed that cortisol levels after 1 mg DST were associated with the presence of VFx (AUC, 0.73; $P < .0001$). The cutoff of cortisol levels after 1 mg DST with the best compromise between sensitivity (73.6%) and specificity (70.5%) is set at 2.0 μg/dl (55 nmol/liter), as indicated by the arrow.

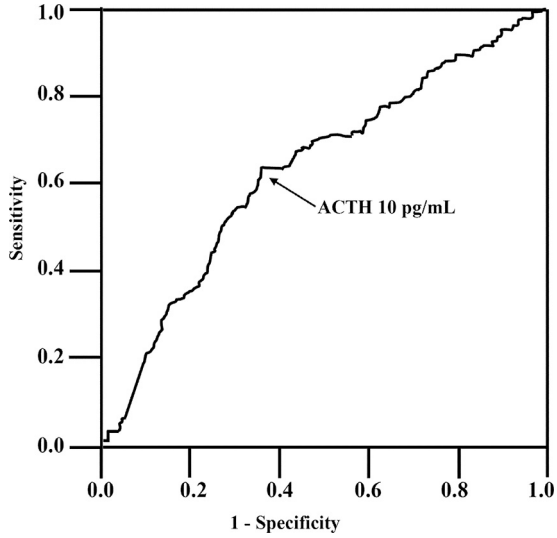

Figure 2. The ROC curve analysis assessing the association between the basal ACTH levels at morning and the presence of a prevalent vertebral fracture in patients with adrenal incidentaloma. The ROC analysis showed that cortisol levels after 1 mg DST were associated with the presence of VFx (AUC, 0.63; $P < .0001$). The cutoff of cortisol levels after 1 mg DST with the best compromise between sensitivity (62.7%) and specificity (64.5%) is set at 10 pg/ml (2.2 pmol/liter) as indicated by the arrow.

Table 2. Accuracy of the Parameters of Hypothalamic-Pituitary-Adrenal Axis Activity in Identifying a Prevalent Vertebral Fragility Fracture in the 444 Patients With Adrenal Incidentalomas Included in the Cross-Sectional Arm of the Study

Criteria	SN (%)	SP (%)	NPV (%)	PPV (%)	Accuracy (%)
1 mg DST <1.0 μg/dl	90.7	19.9	73.5	46.5	50.7
1 mg DST ≥2.0 μg/dl	73.6	70.5	77.6	65.7	71.8
1 mg DST >3.3 μg/dl	29.5	90.4	62.5	70.4	63.9
ACTH <10 pg/ml	62.7	64.5	69.2	57.6	63.7
1 mg DST ≥2.0 μg/dl and ACTH <10 pg/ml	51.8	82.1	68.9	69.0	68.9

SI conversion factors: cortisol after 1 mg DST 27.59, ACTH 0.22.

repeated the analysis after having excluded these four subjects and the results did not change (data not shown).

Longitudinal arm: validation study

The age, gender, prevalence of type 2 diabetes, duration of follow-up, BMI, size of the adrenal mass, UFC and ACTH levels, and BMD at both LS and FN were comparable between AI patients without and with incident VFx during the follow-up. These latter patients had higher cortisol levels after 1 mg DST and VFx prevalence at baseline (Table 4).

The ROC analysis showed that the cortisol levels after 1 mg DST were associated with incident VFx (AUC, 0.730; $P < .0001$), whereas ACTH (AUC, 0.597; $P = .108$) and UFC levels (AUC, 0.622; $P = .06$) were not. The cutoff for cortisol after 1 mg DST with the best compromise between SN and SP for predicting the incident VFx was 2.0 μg/dl (55 nmol/liter) and the cortisol levels after 1 mg DST cutoffs at more than 3.3 μg/dl (91 nmol/liter) and less than 1.0 μg/dl (27.6 nmol/liter) had an SP greater than 90% and an SN greater than 90%, respectively (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). These results were confirmed even after the exclusion of six patients with tumors larger than 4 cm (data not shown).

Finally, the logistic regression analysis showed that the occurrence of an incident VFx was independently predicted by the presence of cortisol levels after 1 mg DST of at least 2.0 μg/dl (55 nmol/liter; odds ratio [OR], 10.3; 95% confidence interval [CI], 3.4–31.1; $P < .0001$) after adjusting for age (OR, 1.0; 95% CI, 0.8–1.1; $P = .97$), female gender (OR, 1.3; 95% CI, 0.4–3.8; $P = .783$), prevalent VFx (OR, 1.0; 95% CI, 0.4–2.8; $P = .973$), and LS-BMD (OR, 1.3; 95% CI, 0.9–2.0; $P = .132$).

In the two patients showing cortisol levels after 1 mg DST greater than 1.8 μg/dl (50 nmol/liter) together with ACTH levels above 30 pg/liter (6.6 pmol/liter), a CRH test showed a blunted ACTH response and the magnetic resonance failed to individuate pituitary adenomas. However, we repeated the analysis after the exclusion of these two subjects and the results did not change (data not shown).

Discussion

This study aimed to assess in AI patients which indexes of cortisol secretion were associated with prevalent and incident VFx and to individuate the best cutoff to be used for detecting patients with VFx and for predicting the occurrence of an incident VFx during follow-up. We found that cortisol levels after 1 mg DST are independently associated with both the presence of VFx and the occurrence of a new VFx during follow-up regardless of confounders. Moreover, the cutoff of cortisol levels after 1 mg DST with the best compromise between sensitivity and specificity was

Table 3. Association Between the Presence of a Vertebral Fragility Fracture and Cortisol After 1-mg Dexamethasone Suppression Test or ACTH, Age, Gender, and Lumbar Spine Bone Mineral Density

Model 1	OR	*P* Value	95% CI
Age (1-y increase)	1.05	.0001	1.03–1.07
Gender (female vs male)	1.14	.574	0.73–1.78
Serum cortisol after 1 mg DST ≥2 μg/dl (presence vs absence)	6.07	.0001	3.92–9.38
LS-BMD (1 Z-score decrease)	1.36	.0001	1.16–1.61
Model 2	**OR**	***P* Value**	**95% CI**
Age (1 y increase)	1.05	.0001	1.03–1.08
Gender (male vs female)	1.02	.900	0.68–1.56
ACTH <10 pg/ml (presence vs absence)	1.10	.01	1.08–1.20
LS-BMD (1 Z-score decrease)	1.38	.0001	1.18–1.60

SI conversion factors: cortisol after 1 mg DST 27.59.

Table 4. Clinical and Biochemical Characteristics at Baseline of the AI patients (n = 126) With or Without an Incident VFx During the Follow-up Included in the Longitudinal Arm of the Study

	Patients Without Incident VFx (n = 96)	Patients With Incident VFx (n = 30)	*P*
Age (y)	62.9 ± 9.5 (27–80)	65.5 ± 9.2 (41–83)	.182
Gender (females)	64 (66.7)	16 (53.3)	.185
Duration of the follow-up (mo)	24.8 ± 5.5 (24–72)	25.2 ± 4.8 (24–48)	.686
BMI (kg/m^2)	27.1 ± 4.2 (19.0–40.0)	26.6 ± 4.6 (19.0–37.0)	.636
Diameter of the adenoma (cm)	2.3 ± 0.9 (0.8–5.0)	2.5 ± 0.9 (1–4.2)	.154
1 mg DST (μg/dl)	2.0 ± 1.2 (0.5–7.5)	2.7 ± 1.1 (0.5–6.2)	.004
UFC (μg/24 h)	46.4 ± 29.1 (10.0–150.7)	59.6 ± 35.6 (10.0–175.3)	.06
ACTH (pg/ml)	13.8 ± 8.6 (3.0–35.0)	11.1 ± 6.2 (5.0–32)	.111
LS BMD (Z-score)	−0.02 ± 1.29 (−2.80 to 4.1)	0.37 ± 1.29 (−2.0 to 2.70)	.146
FN BMD (Z-score)	0.06 ± 0.75 (−1.60 to 2.1)	−0.13 ± 1.16 (−2.40 to 2.70)	.715
Patients with prevalent VFx (%)	32 (33)	16 (53.3)	.049
Patients with type 2 diabetes (%)	24 (25)	8 (26.7)	.566

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

SI conversion factors: cortisol after 1 mg DST 27.59, ACTH 0.22, UFC 2.759.

found to be 2.0 μg/dl (55 nmol/liter) for both individuating fractured patients and predicting the occurrence of a new VFx. Finally, the determination of UFC or ACTH levels does not add advantages in the diagnostic accuracy of the VFx risk.

The finding of the association between the degree of cortisol secretion in AI patients and the prevalence and incidence of VFx was expected, being in accordance with the idea that even the subtle cortisol excess is deleterious for bone tissue, as already shown by several studies (20–27). Moreover, that this association is independent of bone density reinforces the theory that even in the subclinical form of hypercortisolism (as in overt cortisol excess) the reduction of bone quality is among the main mechanisms underlying the skeletal damage (26).

The novel finding of this study is that the cortisol levels after 1 mg DST greater than 2.0 μg/dl (55 nmol/liter) represent the best criterion for predicting both the prevalent and the incident VFx. This result is in keeping with recent data showing that the cardiovascular risk and mortality begins to increase for cortisol after 1 mg DST levels above 1.8–2.0 μg/dl (50–55 nmol/liter) (9–11). Overall, these data suggest that we should consider a cutoff of cortisol after 1 mg DST as low as at least 2.0 μg/dl (55 nmol/liter) for diagnosing the presence of subclinical hypercortisolism in AI patients (17, 18). However, this is in contrast with the theory that, in AI patients, a cortisol level after 1 mg DST greater than 5 μg/dl (138 nmol/liter) is the most reliable criterion for diagnosing SH because it increases specificity and therefore avoiding an excessive number of false-positive results (16, 17, 35). Nevertheless, our data show that using the VFx risk as the end-point for diagnosing SH in AI patients, a specificity above 90% is obtained using a cutoff of cortisol levels after 1 mg DST as high as more than 3.3 μg/dl (91 nmol/liter).

Interestingly, the determination of UFC and ACTH levels for detecting patients at risk for VFx does not seem useful. Although most authors agree that UFC levels are not enough sensitive in patients with subtle cortisol excess (19, 36), the possible usefulness of ACTH in diagnosing a condition of subclinical hypercortisolism is debated (18, 36, 37) because low ACTH levels may indicate the suppression of the HPA axis activity and a functional adrenal autonomy. However, in the present study, the ACTH determination appears to be of scarce value in predicting the effect of SH on bone. This is in contrast with the results of a previous study showing that the ACTH levels below 10 pg/ml (2.2 pmol/liter) may be an additional tool in patients with cortisol levels after 1 mg DST between 2 and 5 μg/dl (50–183 nmol/liter) for detecting AI patients with possible SH, who could benefit from surgery (38). However, the parameters of the metabolic syndrome but not the bone complications were considered study outcomes. Thus, the discordance between the good reliability of ACTH levels in reflecting the HPA axis activity suppression or in individuating AI patients who could ameliorate their metabolic syndrome after the recovery from SH and their scarce usefulness in predicting the SH related risk of fragility fractures may be explained by the fact that the sensitivity to glucocorticoids is different among the different tissues (39, 40) and that a slight cortisol excess may affect bone tissue before the suppression of HPA axis activity is present (20, 28). Finally, it must be observed that that plasma ACTH levels are probably not fully reliable, particularly in patients with ACTH levels below the lower limits of the normal range (41).

This study has some limitations. First, it is based also on retrospective data and, therefore, an unknown bias may have been occurred. Second, in AI patients, the cortisol secretion often fluctuates; therefore, the parameters of dis-

ease activity measured at the beginning of a follow-up may be not fully representative of the real disease activity over time. Finally, we did not measure serum or salivary cortisol at midnight, which could have been potentially informative. However, the determination of midnight serum cortisol, which has been suggested to be useful in diagnosing SH (42), requires the hospital admission and, it is therefore, not easy to perform. At variance the determination of salivary cortisol levels at midnight can be obtained on outpatient basis, but it does not seem useful for the SH detection (43).

Notwithstanding these limitations, the present study for the first time tries to establish a cutoff for cortisol after 1 mg DST for detecting SH by using an "hard" end-point such as the VFx. The most important clinical implication of the present data is that, because the presence of VFx increases the risk of clinical vertebral and nonvertebral fractures (44), the finding that AI patients with cortisol after 1 mg DST of at least 2.0 μg/dl (55 nmol/liter) are at risk of VFx, suggests that all conservatively followed AI patients with cortisol after 1 mg DST of at least 2.0 μg/dl (55 nmol/liter) should be screened for prevalent VFx at baseline and incident VFx during the follow-up.

In conclusion, the present study shows that in AI patients without signs and/or symptoms of hypercortisolism: 1) the cortisol levels after 1 mg DST are associated with the presence of prevalent VFx and with the occurrence of new VFx during the follow-up; 2) the cortisol level after 1 mg DST of at least 2.0 μg/dl (55 nmol/liter) represents the best cutoff for detecting fractured patients and for predicting the risk of new VFx during the follow-up.

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Address all correspondence and requests for reprints to: Iacopo Chiodini, MD, Unit of Endocrinology and Metabolic Diseases, Padiglione Granelli, Fondazione IRCCS Cà-Granda, Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milan, Italy. E-mail: iacopo.chiodini@policlinico.mi.it.

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