Adrenalectomy reduces the risk of vertebral fractures in patients with monolateral adrenal incidentalomas and subclinical hypercortisolism

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Abstract

Objective: Subclinical hypercortisolism (SH) is associated with increased risk of vertebral fractures (VFX). The effect on bone following recovery from SH is unknown.

Design: Of the 605 subjects consecutively referred for monolateral adrenal incidentalomas (AIs) to our outpatient clinics, 55 SH patients (recruited on the basis of the exclusion criteria) were enrolled. We suggested to all patients to undergo adrenalectomy, which was accepted by 32 patients (surgical group, age 61.3 ± 8.1 years) and refused by 23 patients, who were followed with a conservative management (non-surgical group, age 65.4 ± 7.1 years).

Methods: We diagnosed SH in patients with serum cortisol after 1 mg dexamethasone suppression test (1 mg-DST) $> 5.0 \mu\text{g/dl}$ or with greater than or equal to two criteria among 1 mg-DST $> 3.0 \mu\text{g/dl}$, urinary free cortisol $> 70 \mu\text{g/24 h}$ and ACTH $< 10 \text{ pg/ml}$. We assessed: bone mineral density (BMD) at lumbar spine (LS) and femoral neck (as Z-score) by dual-energy X-ray absorptiometry and the VFX presence by X-ray at baseline and at the end of follow up (surgical group 39.9 ± 20.9 months and non-surgical group 27.7 ± 11.1 months).

Results: The LS Z-score ($\Delta\text{Z-score/year}$) tended to increase in the surgical group (0.10 ± 0.20) compared with the non-surgical group (-0.01 ± 0.27 , $P=0.08$) and in the former, the percentage of patients with new VFX was lower (9.4%) than in the latter (52.2%, $P<0.0001$). Surgery in AI patients with SH was associated with a 30% VFX risk reduction (odds ratio 0.7, 95% CI 0.01–0.05, $P=0.008$) regardless of age, gender, follow up duration, 1 mg-DST, LS BMD, and presence of VFX at baseline.

Conclusion: In patients with monolateral AI and SH, adrenalectomy reduces the risk of VFX.

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Introduction

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). In adults, the prevalence of SH is not negligible, being estimated between 0.2 and 2.0% (2). Indeed, SH is described in up to the 30% 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 to the fact that this condition has found to be associated with an increased prevalence of hypertension and type 2 diabetes mellitus, cardiovascular events, and mortality (5, 6, 7, 8, 9, 10, 11). Moreover, some studies (12, 13), but not all (14),

showed an improvement of these metabolic complications after the surgical excision of the adrenal adenoma and the recovery from SH in AI patients.

Osteoporosis and vertebral fractures (VFX) are also well known complications of endogenous hypercortisolism (15). Several studies reported that, similarly to what happens in patients with overt cortisol excess, even in patients with SH the prevalence and incidence of VFX are increased (16, 17, 18, 19, 20) and only partially explained by the decrease of bone mineral density (BMD) (19). To date, no studies have been conducted on the effect of recovery from SH on BMD and VFX in patients with monolateral AI.

Therefore, we designed a prospective longitudinal study in AI patients with SH, aiming to assess the effect of the surgical and conservative management on the BMD modifications and the VFX risk.

Patients and methods

Subjects

The study was performed in two Italian referral centers for adrenal diseases (Endocrine Unit of 'Casa Sollievo della Sofferenza' Hospital in San Giovanni Rotondo and Unit of Endocrinology and Metabolic Diseases of Fondazione IRCCS Cà Granda Hospital in Milan). On the basis of our diagnostic protocols, we diagnosed SH by the absence of signs and/or symptoms of cortisol excess (i.e. striae rubrae, moon facies, buffalo hump, and skin atrophy) and by the presence (in at least two out of three different estimations) of cortisol levels after 1 mg overnight dexamethasone suppression (1 mg-DST) >5.0 $\mu\text{g/dl}$ (138 nmol/l) or in the presence of greater than or equal to two out of the following alterations: 1 mg-DST >3.0 $\mu\text{g/dl}$ (83 nmol/l), adrenocorticotrophic hormone (ACTH) levels <10 pg/ml (2.2 pmol/l), 24 h urinary free cortisol (UFC) levels >70 $\mu\text{g}/24$ h (193 nmol/24 h) (2).

In accordance with these criteria of the 605 subjects consecutively referred from January 2008 to June 2013 for monolateral AI to our outpatient clinics for adrenal diseases, 91 patients (15%) were diagnosed as affected with SH. Of these, 36 patients have been excluded on the basis of the following exclusion criteria: i) past or current history of hypogonadism (in men testosterone levels <300 ng/dl and in premenopausal women fewer than six menstrual cycles per year) and thyrotoxicosis, bowel diseases, chronic renal failure, chronic hepatic disease, alcoholism, eating disorders, and rheumatologic or hematological diseases; ii) administration of drugs

influencing cortisol and dexamethasone metabolism or cortisol secretion; and iii) signs or symptoms specific to cortisol excess (moon facies, striae rubrae, skin atrophy, and proximal muscle weakness). Eventually, 55 AI patients (32 post-menopausal females and 23 eugonadal males) were enrolled in to the study (Fig. 1). 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 scan. No subject had evidence of metastatic diseases. The CT scans showed that all adrenal masses were homogeneous and hypodense and with well-shaped features, consistent with the diagnosis of adrenocortical adenoma. In all patients, the diagnosis of pheochromocytoma and aldosteronoma was excluded by appropriate hormonal determinations (24 h urinary metanephrines and upright plasma renin activity and aldosterone). The contralateral adrenal gland was normal in all patients.

Figure 1

Design of the study. ^aExclusion criteria: i) past or current history of hypogonadism (in men testosterone levels <300 ng/dl and in premenopausal women fewer than six menstrual cycles per year), thyrotoxicosis, bowel diseases, chronic renal failure, chronic hepatic disease, alcoholism, eating disorders, and rheumatologic or hematological diseases; ii) administration of drugs influencing cortisol and dexamethasone metabolism or cortisol secretion; and iii) signs or symptoms specific of cortisol excess (moon facies, striae rubrae, skin atrophy, and proximal muscle weakness).

Surgery was suggested to all patients, explaining the possible advantages and disadvantages of this option. The surgical operation was accepted by 32 patients (surgical group, age 61.3 ± 8.1 years, 22 post-menopausal females per ten males) and refused by 23 patients, who were followed with a conservative management (non-surgical group, age 65.4 ± 7.1 years, ten post-menopausal females per 13 males).

From January 2008, our routine protocol for AI patients with SH includes a yearly visit to clinically evaluate the symptoms related to cortisol excess, assessment of the glycometabolic and hypertensive control and the determination of cortisol secretion and bone metabolism. BMD and the presence of asymptomatic (morphometric) VFX are routinely assessed every 2 years. For patients currently operated on, we apply the same protocol as for SH patients followed with a conservative management.

Laparoscopic or laparotomic (open) adrenalectomy was performed and no patient had complications. In all patients the histological findings were consistent with adrenal adenoma. After adrenalectomy, a precautionary steroid therapy with hydrocortisone 100 mg i.v., during surgery, and cortisone acetate *per os* (at weight related doses ranging between 25 and 37.5 mg/day in three subdivided doses during the day), immediately after surgery, was administered. The commonly used cortisone acetate dose was 25 mg/day. In all patients, cortisol secretion was re-evaluated, after 2 months, by ACTH stimulation test. The insulin tolerance test was given in the presence of inconclusive results with the ACTH stimulation test. In patients with persistent adrenal insufficiency hypothalamic-pituitary-adrenal (HPA) axis function was reassessed every 6 months. The mean duration of the steroid substitutive therapy was 12 months.

We report data at the beginning and at the end of the follow up. In all patients the BMI, the presence of arterial hypertension and of diabetes and the changes in glucose metabolism, and blood pressure were evaluated at baseline and at the end of follow up.

In the diabetic patients, the metabolic control was assessed by HbA1c. Hypertension was defined as the presence of systolic blood pressure >140 mmHg, and/or diastolic blood pressure >90 mmHg, and/or of antihypertensive treatment (21). T2DM was diagnosed using World Health Organization criteria (22). The improvement/worsening of arterial blood pressure was determined if non hypertensive patients passed from a prehypertension category to another category or hypertensive patients passed from a hypertension grade to another grade (21), or if any antihypertensive treatment was modified. Fasting

glucose levels were considered improved/worsened if they passed from one category to another (22).

In all subjects, BMD was measured by dual-energy X-ray absorptiometry (Hologic Discovery, Software version 13.3:3, Bedford, MA, USA) at lumbar spine (LS, precision 1.0%) and femoral neck (FN, precision 1.8%) and expressed as g/cm^2 and s.d. units (Z-score) in relation to reference population provided by manufacturer and as the change in Z-scores per year (Δ Z-score/year) between baseline and end of follow up. Fractured vertebrae were excluded from BMD measurement. Conventional spinal radiographs in lateral (T4–L4) and antero-posterior projection were obtained in all subjects with standardized technique. Two trained radiologists, blinded to BMD and hormonal data, independently reviewed the radiographs and discussed questionable cases. Prevalent and incident VFX were diagnosed on visual inspection using the semiquantitative (SQ) visual assessment previously described by Genant *et al.* (23, 24). According to this technique, fractures assessed on lateral thoraco-LS radiographs were defined as reductions of more than 20% in anterior, middle, or posterior vertebral height. From lateral spine radiographs, 13 vertebrae from T4 to L4 were assessed visually as intact (SQ grade 0) or as having approximately mild (20–25% compression), moderate (25–40% compression), or severe ($>40\%$ compression) deformity (SQ grades 1, 2, and 3 respectively).

All subjects provided informed consent before entering the study, which has been approved by the Ethic Committees of Fondazione IRCCS Cà Granda-Ospedale Maggiore Policlinico (Milan, Italy), and of ‘Casa Sollievo della Sofferenza’ Hospital IRCCS, San Giovanni Rotondo (Foggia, Italy).

Methods

Serum and urine samples were stored at -20°C until assayed. In all patients, serum and 24 h urinary calcium, serum albumin, phosphate, parathyroid hormone (PTH), and 25-hydroxyvitamin D (25OHVitD) levels were assessed. Calcium, albumin, and phosphate were measured by standard colorimetric techniques. Serum intact PTH was measured by electrochemiluminescence immunoassay (reference interval 15–65 pg/ml). Serum 25OHVitD concentration was measured by chemiluminescent immunoassay (reference interval 30–100 ng/ml). According to our national guidelines (25), all patients with hypovitaminosis D were supplemented with standard doses of cholecalciferol *per os* in order to achieve 25OHVitD levels above 30 ng/ml (75 nmol/l), and all

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patients with a calcium intake <1000 mg/day were supplemented with oral calcium citrate.

The need for bone active therapy was evaluated in all patients from non-surgical group on the basis of our national guidelines (26) and alendronate was offered only to the patients with a FRAX 10-year probability of a major osteoporotic fracture $\geq 10\%$ (27) ($n=11$). However, three patients refused the anti-resorptive therapy. Thus, in eight patients from non-surgical group a weekly bisphosphonate was given, but only five patients had an adherence $\geq 80\%$. No patient was treated with teriparatide.

No patient from surgical group was treated with bone active drugs. Indeed, in our protocol, the patients surgically operated on for SH are treated with bone active drugs in the absence of BMD increase or in the presence of an incident fragility fracture during the post-surgical follow up (28).

Sample size

Hypothesizing that, as suggested by previous data (20), a new VFx occurs in the 48% of not-treated SH patients during a follow up period of at least 2 years, we needed to recruit 22 patients in each group for a 90% power and 1 5% type I error.

Statistical analysis

The results are expressed as mean $\pm$ s.d. Categorical variables were compared by χ^2 test. Comparison of continuous variables among the different groups was performed using Student's *t*-test for paired or unpaired data, as appropriate, for normally distributed variables and using the Mann-Whitney *U* test or the Wilcoxon's signed-rank test, as appropriate, for not normally distributed variables.

The logistic regression analysis assessed the association between the occurrence of a new VFx after adjusting for adrenalectomy (yes/no) and for the independent variables known to be associated with increased fracture risk (i.e. age, gender, LS BMD, and VFx at baseline) as well as the independent variables that resulted to be significantly different (or tended to be) between surgically and not surgically treated patients at baseline (i.e. duration of the follow up and 1 mg-DST). These analyses were repeated also including in the model the use of bone active drugs (as independent variable).

In non-surgical group, the receiver operating characteristic curve analysis (for continuous variables) and the χ^2 test (for categorical variables) assessed the possible

association between age, gender, duration of follow up, 1 mg-DST, and LS BMD (as Z-score) at baseline and the presence of VFx at baseline with the occurrence of a new VFx during follow up.

Statistical analysis was performed by SPSS Version 21.0 Statistical Package (IBM Corporation, Armonk, NY, USA). *P* values of <0.05 were considered significant.

Results

Clinical characteristics of patients surgically and conservatively treated at the beginning and at the end of follow up are reported in Table 1. In all patients serum calcium, creatinine, phosphorous, and PTH levels and 24 h urinary calcium levels were within the normal range at both baseline and end of follow up (data not shown). At baseline, the two groups were comparable for age, BMI, adenoma size, 25OHVitD, ACTH, 1 mg-DST, and UFC levels, prevalence of hypertension and type 2 diabetes. At baseline, LS and FN BMD was lower in surgical group than in non-surgical group, while the prevalence of patients with VFx was not statistically different. All patients had 1 mg-DST levels >1.8 $\mu\text{g/dl}$ (50 nmol/l).

At the end of the follow up, non-surgical group patients showed increased prevalence of fractures as compared with baseline data. The cortisol secretion and BMD at both LS and FN were comparable between baseline and the end of follow up, but a new VFx occurred in more than 50% of patients and even in two out of the five patients with adherence to bisphosphonates treatment.

Even in surgical group patients BMD at both LS and FN did not change between baseline and the end of follow up, but, at variance with non-surgical group, in patients operated on $\Delta\text{Z-score/year}$ at LS tended to increase, even if a statistical difference of the BMD changes between the two groups was not reached ($P=0.08$). In addition in the surgical group only three patients (9.4%) experienced a new VFx. All these three subjects had a VFx at baseline. At the end of the follow up, the prevalence of fractured patients, the percentage of patients with new VFx (Fig. 2) and the absolute number of new fractures were lower in the surgical group than in the non-surgical group.

The comparison of baseline data between patients with an incident VFx during the follow up and patients without an incident VFx is reported in Table 2. Age, BMI, gender, duration of follow up, 25OHVitD, BMD at both LS and FN, prevalent VFx and prevalence of patients with hypertension and diabetes were comparable between the two groups. As expected, patients with the occurrence of

Table 1 Clinical and biochemical modifications in SH patients surgically treated and conservatively treated at baseline and at the end of follow up. Data are mean $\pm$ s.d. with range in parenthesis or absolute number with percentage in parenthesis.

Variables	Conservatively treated patients (n = 23)			Surgically treated patients (n = 32)		
	Baseline	End of FU	P	Baseline	End of FU	P
Age (years)	65.4 $\pm$ 7.05 (51 to 75)	67.7 $\pm$ 6.9 (53 to 78)	0.25	61.3 $\pm$ 8.1 (38 to 75)	64.7 $\pm$ 8.7 (40 to 79)	0.12
Gender (females)	10 (43.5)		–	22 (68.8)	–	–
BMI (kg/m ²)	26.1 $\pm$ 4.2 (19.5 to 35.5)	26.9 $\pm$ 4.2 (19.5 to 34.5)	0.53	26.5 $\pm$ 3.9 (21.3 to 35.3)	26.5 $\pm$ 4.0 (20.1 to 34.1)	0.98
Duration of FU (months)	–	27.7 $\pm$ 11.1 (24 to 72)	–	–	39.9 $\pm$ 20.9* (24 to 89)	–
Diameter of adenoma (cm)	2.8 $\pm$ 0.9 (2.0 to 5.0)	–	–	3.4 $\pm$ 1.2 (2 to 7.0)	–	–
25-Hydroxyvitamin D (ng/ml)	22.4 $\pm$ 7.3 (12.0 to 37.1)	38.7 $\pm$ 7.5 (30.1 to 56.2)	0.0001	21.9 $\pm$ 8.7 (12 to 44)	39.4 $\pm$ 8.1 (30 to 68)	0.0001
ACTH (pg/ml)	7.7 $\pm$ 1.7 (5 to 9.8)	8.4 $\pm$ 1.7 (5 to 12.5)	0.178	6.8 $\pm$ 2.4 (1.6 to 12.0)	25.9 $\pm$ 17.6 [†] (5.1 to 55.0)	0.0001
1 mg-DST (μ g/dl)	3.4 $\pm$ 0.7 (2.0 to 5.8)	3.1 $\pm$ 0.6 (1.8 to 4.3)	0.06	4.4 $\pm$ 2.3 (1.9 to 9.2)	0.8 $\pm$ 0.3 [†] (0.4 to 1.7)	0.0001
UFC (μ g/24 h)	63.7 $\pm$ 38.9 (11.5 to 175.3)	58.2 $\pm$ 29.8 (15.3 to 116)	0.59	75.1 $\pm$ 33.0 (15.5 to 171.4)	33.0 $\pm$ 14.7 [†] (19.0 to 62.1)	0.0001
LS BMD (Z-score)	0.23 $\pm$ 1.4 (–1.8 to 2.7)	0.25 $\pm$ 1.5 (–2.1 to 2.8)	0.96	–0.86 $\pm$ 1.27 [‡] (–2.9 to 1.9)	–0.49 $\pm$ 1.17* (–2.7 to 2.1)	0.23
LS Δ Z-score/year	–	–0.01 $\pm$ 0.3 (0.03 to 0.17)	–	–	0.10 $\pm$ 0.2 (0.0 to 1.0)	–
FN BMD (Z-score)	0.14 $\pm$ 1.2 (–2.4 to 2.7)	0.12 $\pm$ 1.2 (–2.4 to 2.6)	0.97	–0.54 $\pm$ 0.9 [†] (–1.9 to 1.7)	–0.41 $\pm$ 0.9 [†] (–1.7 to 1.8)	0.57
FN Δ Z-score/year	–	–0.58 $\pm$ 2.3 (–0.83 to 0.64)	–	–	–0.09 $\pm$ 2.0 [†] (–4.26 to 4.76)	–
Patients with VFx (%)	15 (65.2)	21 (91.3)	0.03	15 (46.9)	15 [†] (46.9)	1.00
Absolute number of VFx	21	33	–	22	25*	–
Patients with new VFx (%)	–	12 (52.2)	–	–	3 ^a (9.4)	–
Patients with hypertension (%)	14 (60.9)	15 (65.2)	0.76	21 (65.6)	18 (56.3)	0.44
Blood pressure control (stable/better/worse)	–	11/1/11 (47.8/4.3/47.8)	–	–	23/9 [†] /0 [†] (71.9/28.1/0.0)	–
Patients with type 2 diabetes (%)	9 (39.1)	9 (39.1)	1	5 (15.6)	5 (15.6)	1.00
Glycemic control (stable/better/worse, %)	–	15/0/8 (65.2/0.0/34.8)	–	–	25/6 [†] /1 [†] (78.1/18.8/3.1)	–

FU, follow up; Δ Z-score/year, change of Z-score per year between baseline and end of FU. * $P < 0.05$, $^{\dagger}P < 0.0001$, and $^{\ddagger}P < 0.06$ vs conservatively treated patients at the end of FU. $^{\S}P < 0.005$ vs conservatively treated patients at baseline; $^{\parallel}P = 0.08$, $^{\text{a}}P = 0.41$, and $^{\text{b}}P < 0.0001$ vs conservatively treated patients. SH was diagnosed by the presence of 1 mg-DST $> 5 \mu\text{g/dl}$ (138 nmol/l) or by the presence of at least two of the following alterations: 1 mg-DST $> 3 \mu\text{g/dl}$ (83 nmol/l), UFC $> 70 \mu\text{g/24 h}$ (193 nmol/24 h), and ACTH $< 10 \text{ pg/ml}$ (2.2 pmol/l). SI conversion factors: 1 mg-DST 27.59, ACTH 0.22, and UFC 2.759. LS and FN BMD measured by dual X-ray absorptiometry at spine (L1–L4) and FN respectively.

Figure 2

Prevalence of new vertebral fractures (VFX) in patients with adrenal incidentalomas (AIs) and subclinical hypercortisolism (SH) surgically treated (surgical group) or followed with a conservative management (non-surgical group). In patients with AI and SH the prevalence of new VFX was significantly higher in subjects conservatively followed than in those surgically treated.

an incident VFX during the follow up have been less frequently surgically treated than patients without the occurrence of an incident VFX.

In the whole sample of patients, the logistic regression analysis showed that the surgical treatment of SH was significantly associated with a reduced risk of a new VFX regardless of age, gender, duration of follow up, degree of hypercortisolism (as expressed by 1 mg-DST), LS BMD (as expressed as Z-score), and prevalence of VFX at baseline (Table 3).

In the non-surgical group age, BMI, gender, duration of follow up, 25OHVitD, BMD at both LS and FN, and prevalent VFX at baseline were not different between patients with an incident VFX ($n=12$) and patients without an incident VFX ($n=11$) during the follow up (age 65.8 ± 7.4 years vs 64.9 ± 7.0 years, BMI 25.3 ± 3.4 kg/m² vs 26.9 ± 4.9 kg/m², females 41.7% vs 45.5%, duration of follow up 31 ± 14.9 months vs 24 ± 0.9 months, 25OHVitD 23.0 ± 6.7 ng/ml vs 21.9 ± 8.2 ng/ml, LS Z-score 0.40 ± 1.6 vs 0.05 ± 1.4 , FN Z-score 1.6 ± 0.5 vs 0.08 ± 0.5 , and prevalent VFX 50% vs 81.8%; $P > 0.1$ for all comparisons). In this group, the logistic regression analysis showed that age, gender, duration of follow up, 1 mg-DST, LS BMD, and the VFX prevalence at baseline were not predictive of a new VFX during follow up (data not shown).

The results were substantially identical even after the inclusion of the presence of bone active drugs among the independent variables in the logistic regression models (data not shown).

Discussion

This study was aimed to assess the effect of surgical or conservative management on BMD and the risk of VFX in AI patients with SH. We found that the recovery from SH led to an important reduction in the occurrence of new VFX (Fig. 2) and, in turn, that the SH persistence was associated with a 15-fold increased risk of a new VFX regardless of age, gender, duration of follow up, degree of hypercortisolism, LS BMD, and prevalence of VFX at baseline (Table 3).

Table 2 Clinical and biochemical characteristics at baseline in AI patients with or without the occurrence of an incident fracture during the follow up. Data are mean $\pm$ s.d. with range in parenthesis or absolute number with percentage in parenthesis.

Variables	Patients without incident VFX ($n=40$)	Patients with incident VFX ($n=15$)	P
Age (years)	62.1 ± 8.1 (38 to 75)	65.5 ± 6.7 (51 to 75)	0.16
Gender (females)	25 (62.5)	8 (53.3)	0.54
BMI (kg/m ²)	26.6 ± 4.3 (19.3 to 35.6)	25.6 ± 3.2 (21.5 to 32.2)	0.39
Duration of FU (months)	35.5 ± 18.9 (24 to 89)	32.7 ± 17.3 (23 to 72)	0.62
25-Hydroxyvitamin D (ng/ml)	22.0 ± 8.3 (12.0 to 44.0)	22.6 ± 7.6 (12.0 to 37.1)	0.80
LS BMD (Z-score)	-0.63 ± 1.4 (-2.9 to 2.1)	0.22 ± 1.5 (-1.8 to 2.7)	0.06
FN BMD (Z-score)	-0.40 ± 0.9 (-1.9 to 1.7)	0.13 ± 1.5 (-2.4 to 2.7)	0.11
Patients with prevalent VFX at baseline (%)	22 (55)	8 (53.3)	0.91
Not surgically treated/surgically treated patients (%)	12/28 (30/70)	12/3 (80/20)	0.001
Patients with hypertension (%)	24 (60)	11 (73.3)	0.36
Patients with type 2 diabetes (%)	10 (25)	4 (26.7)	0.89

Δ Z-score/year, change of Z-score per year between baseline and end of FU. SH was diagnosed by presence of 1 mg-DST > 5 μ g/dl (138 nmol/l) or by the presence of at least two of the following alterations: 1 mg-DST > 3 μ g/dl (83 nmol/l), UFC > 70 mg/24 h (193 nmol/24 h), and ACTH < 10 pg/ml (2.2 pmol/l). SI conversion factors: 1 mg-DST 27.59, ACTH 0.22, and UFC 2.759, LS and FN BMD measured by dual X-ray absorptiometry at spine (L1–L4) and FN respectively.

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Table 3 Odds ratio (OR) for new VFX for potential risk factors using logistic regression analysis.

Model 1	OR	P value	95% CI
Age (1 year increase)	1.031	0.67	0.89–1.19
Gender (female vs male)	1.16	0.86	0.24–5.68
Duration of follow up (1 month increase)	1.02	0.44	0.97–1.07
LS BMD (1 Z-score unit decrease)	1.07	0.85	0.56–2.01
1 mg-DST (0.1 µg/dl decrease)	1.97	0.11	0.85–4.54
VFX at baseline (presence vs absence)	1.27	0.37	0.33–9.43
Surgical treatment of SH (absence/presence)	14.9	0.008	2.05–111.10

The prevalence of VFX at the end of the follow up and the magnitude of the VFX risk in not surgically treated SH patients may be considered surprising. However, these data are comparable with those of a previous study, in which a 82% VFX prevalence and a 12-fold increased risk of VFX in a group of SH conservatively followed up for 2 years were found (20). No prospective longitudinal study has been specifically designed for assessing the VFX incidence in overt hypercortisolism. The only information on fracture risk in overt hypercortisolism come from a study by Vestergaard *et al.* (29), in which the self-reported risk of clinical fracture was increased sixfold 2 years before the diagnosis of Cushing's syndrome. Therefore, considering that in the present study we assessed the incidence of asymptomatic VFX and not of clinical VFX, the 15-fold increased risk of VFX that we found in not surgically treated SH patients is not surprising.

In a previous study by Toniato *et al.* (12), the surgical treatment for SH did not result in an improvement in osteoporosis. At variance, in the present study we found that in surgically treated patients, but not in conservatively followed ones, the BMD tended to increase. These apparent discordant findings may be explained by the fact that in the previous study by Toniato *et al.* only the prevalence of osteoporosis was reported, while the BMD changes were not assessed. However, several studies have documented the usefulness of BMD in predicting the fracture risk in patients with SH (18, 19, 20, 30, 31). In keeping with a previous investigation, we demonstrated that risk of VFX in SH patients is independent of the BMD changes (20). Thus, the fact that in the present study SH patients who will experience an incident VFX have a similar, if not higher, spinal BMD than SH patients who will not have a new VFX during the follow up (Table 2), confirms once more the reduced usefulness of BMD in predicting the risk of VFX in SH patients.

Besides confirming the deleterious effect of SH persistence on the risk of new VFX, this study most importantly suggests that in AI patients with SH the surgical treatment is able to significantly reduce the risk of new VFX. This finding has never been reported so far and it is in accordance with the fact that the recovery from SH is also associated with the improvement of blood pressure and glycometabolic control, as previously suggested by larger studies (2, 12, 13).

However, it must be considered that the diagnosis of SH is still a matter of debate. Indeed, while almost all authors agree on the fact that SH is certain in the presence of 1 mg-DST > 5 µg/dl, the diagnosis of SH is uncertain in the presence of 1 mg-DST levels between 1.8 and 5 µg/dl and further biochemical parameters are often requested at least in patients with some possible consequences of cortisol excess, such as osteoporosis (32). For this reason, in patients with uncertain SH we diagnose SH in the presence of greater than or equal to two criteria out of 1 mg-DST > 3.0 µg/dl (83 nmol/l), ACTH levels < 10 pg/ml (2.2 pmol/l), and 24-h UFC levels > 70 µg/24 h (193 nmol/24 h). Indeed, this 'combination' criterion and very similar ones have been already validated on clinical ground by our and other groups (16, 20, 33, 34, 35).

Besides the intrinsic limit related to the diagnosis of SH, this study has some other limitations.

First, the lack of randomization might have introduced some selection biases. Indeed, the surgical group and non-surgical group are not fully comparable for baseline BMD and duration of follow up. Moreover, the patients followed-up with a conservative management showed a higher (though not statistically significant) prevalence of VFX at baseline as compared with surgically treated patients (65.2% vs 46.9%, respectively, $P=0.18$), and, therefore, they could have been at higher risk of new VFX. However, the fact that at baseline, BMD was lower in surgically treated than in conservatively followed patients, and, most importantly, that the risk of new VFX was increased in conservatively followed subjects regardless of the prevalence of VFX at baseline and of BMD levels, as shown by the logistic regression analysis, is against this hypothesis. On the other hand, the duration of follow up, that was longer in surgically treated patients than in conservatively followed ones, and the age, gender and level of cortisol secretion may be considered potential biases linked to the lack of randomization. However, the fact that among surgically treated patients the fracture risk was persistently reduced even during a long follow up reinforces the idea that surgery is beneficial in SH patients. Overall, the logistic regression analysis, that corrects, at

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least in part, for these potential biases, showed that surgery was associated with a reduced risk of fracture also regardless of age, gender, and duration of follow up.

Secondly, during the follow up we did not measure 25OHVitD levels, and therefore we could not ascertain the vitamin D status. However, at the end of the follow up the 25OHVitD levels were normal and not different between patients surgically treated and those conservatively followed up, and, therefore, the presence of a different vitamin D status between the two groups during the study seems unlikely.

Finally, we could not measure the trabecular bone score and the markers of bone turnover that could have been more informative in predicting the risk of new Vfx in SH (30).

Notwithstanding these limits, in our opinion these findings have some important clinical implications. First, the demonstration that the recovery from SH is beneficial even for bone besides glucose metabolism and blood pressure reinforces the idea that the surgical treatment in AI patients has to be considered a possible therapeutic option, at least in patients with hypertension and/or diabetes and/or osteoporosis (36). Secondly, since the presence of Vfx increases the risk of clinical vertebral and non-Vfxs (37), the finding that SH patients are at risk of Vfx, if not surgically treated, suggests that all conservatively followed patients with SH have to be screened for Vfx.

In conclusion, the present study shows for the first time that the surgical treatment importantly reduces the Vfx risk in SH patients with monolateral AI and confirms the deleterious effect on bone from SH persistence.

Further randomized prospective studies are needed in order to definitively demonstrate the beneficial effect of the surgical approach in adrenal SH and to personalize the treatment of choice in AI patients with SH.

Declaration of interest

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

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Author contribution statement

A S Salcuni and I Chiodini contributed to data collection, data analysis and interpretation, preparation of the manuscript. V Morelli, S Palmieri, and

E Cairoli contributed to data collection, data analysis, and interpretation. C Eller Vainicher, A Scillitani, and A Spada contributed to data analysis and interpretation of the data, review of the manuscript.

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