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Clinical Research Article

Relationship Among Fragility Fractures and the Overall Cardiovascular Burden in Endogenous Cushing Syndrome

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Abstract

Context: Patients with endogenous Cushing syndrome (CS), in addition to significant cardiovascular morbidity, are burdened by a high prevalence of fragility fractures. Bone mineral density (BMD) alone poorly predicts the risk of fracture, and the implementation of trabecular bone score (TBS) is supported only by scant evidence. Indeed, reliable predictors of fractures in endogenous CS are still lacking.

Objective: This work aimed to analyze the prevalence and the potential predictors of fragility fractures in our patients with CS.

Methods: A monocentric, retrospective, cross-sectional study. A total of 51 patients with overt CS were enrolled. Main outcome measures included biochemical evaluation, BMD measurement, TBS evaluation, fracture presence, body composition evaluation, and arterial intima-media thickness (IMT) assessment.

Results: Fragility fractures were found in 62.7% of patients at diagnosis. Fractured patients exhibited lower spine T-score ($P = .03$), longer disease duration ($P = .025$), higher waist circumference ($P = .006$), and predominantly male sex ($P = .008$). Increased serum uric acid levels ($P = .001$), greater IMT ($P = .017$), and higher prevalence of venous thromboembolism events (31.3% vs 5.3%, $P = .037$) and atherosclerotic plaques (47% vs 5.3%, $P = .002$) were described in the fracture group.

Multivariable logistic regression identified the presence of atherosclerosis (OR 13.35; 95% CI 1.154-154.34, $P = .038$) and osteoporosis (OR 11.30; 95% CI 1.55-82.56, $P = .017$) as independent predictors. TBS values were inversely correlated with body mass index, fat and lean mass, and serum uric acid, and positively correlated with high-density lipoprotein cholesterol.

Conclusion: CS patients with higher overall burden of cardiovascular morbidity are more prone to experience fragility fractures.

Key Words: Cushing syndrome, fragility fractures, cardiovascular morbidity, TBS

Abbreviations: ACS, adrenal Cushing syndrome; ACTH, adrenocorticotropic hormone; BMD, bone mineral density; BMI, body mass index; CS, Cushing syndrome; DXA, dual-energy x-ray absorptiometry; EAS, ectopic Cushing syndrome; HDL, high-density lipoprotein; IMT, intima-media thickness; IQR, interquartile range; MACS, mild autonomous cortisol secretion; OPG, osteoprotegerin; OR, odds ratio; PTH, parathyroid hormone; UFC, urinary free cortisol; VTE, venous thromboembolism.

Endogenous Cushing syndrome (CS) arises from prolonged exposure to high circulating cortisol levels, typically resulting from either adrenocorticotropic hormone (ACTH)-dependent, primarily related to a pituitary ACTH-secreting adenoma (observed in 80% of cases), or ACTH-independent, autonomous cortisol overproduction [1]. Despite its rarity, with a reported incidence of 0.7 to 2.4 cases/million per year [2], CS is associated with several cardiovascular comorbidities, including hypertension, vascular atherosclerosis, cardiac remodeling, increased thromboembolic risk, and metabolic dysregulation such as impaired glucose and lipid metabolism, along with central obesity [3]. Consistently, patients with CS exhibited elevated intima-media thickness (IMT) and a higher prevalence of atherosclerotic plaques. These findings, coupled with impaired endothelial function, contribute to an increased risk of atherosclerosis development and subsequently elevate overall cardiovascular risk [4].

These cardiovascular complications are recognized as major contributors to the increased mortality rate observed in CS [5, 6].

Skeletal issues, encompassing structural and functional alterations of the skeletal system, are common in CS patients, potentially leading to impaired quality of life and increased mortality [7].

Cortisol excess is involved in decreasing bone mineral density (BMD) through various mechanisms including, first, the inhibition of osteoblast differentiation, function, and survival. This effect is mediated mainly through the disruption of Wnt signaling pathway and the increase in sclerostin and dickopf-1 expression. Glucocorticoids are involved in the reduced differentiation of osteoblasts through a decline in bone morphogenic protein as well. Simultaneously, glucocorticoid effect is known for hampering the osteoprotegerin (OPG) production, promoting thereby osteoclast lifespan

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and activity [8]. The final consequence is an imbalance between bone formation and resorption [9]. Moreover, osteocytes are burdened by glucocorticoids impact experiencing increased apoptosis with resultant reduction in bone strength. Despite these detrimental direct consequences, glucocorticoids are also known to exert indirect damages vs the bone, such as the secondary hypogonadism, the impediment in insulin-like growth factor 1 (IGF-1) secretion [9] and the reduced formation of 1,25-hydroxylated vitamin D as well [10]. Interestingly, the OPG has been demonstrated in some studies to have a protective effect against vascular calcifications. However, as previously mentioned, glucocorticoids' action leads to an impairment in its production. This implies a potential dual detrimental impact on both skeletal and vascular health [11].

Studies have consistently demonstrated a prevalence of osteoporosis in CS ranging from 30% to 50%, contributing to an elevated risk of fragility fractures [7, 12].

Indeed, low-energy and fragility fractures occur in 11% to 76% of CS patients, presenting occasionally as the initial sign of the disease [2]. Moreover, they occur despite normal or slightly reduced values of BMD, suggesting that cortisol excess might compromise bone quality as well [13]. Hence the risk of fractures in overt hypercortisolism is poorly predicted by BMD values alone.

Trabecular bone score (TBS) is a parameter derived from gray-level texture analysis of 2D projection images obtained during routine dual-energy x-ray absorptiometry (DXA) lumbar spine scans, and it serves as a quantitative measure of bone microarchitecture [14]. Elevated TBS values correlate with improved fracture resistance, highlighting its complementary role alongside BMD in assessing skeletal health and fracture risk [15].

TBS's efficacy has been proven in different populations, including postmenopausal women and individuals with various endocrine-related osteoporosis [16-19]; nonetheless, the exploration of bone microarchitecture using TBS in endogenous CS is supported merely by scant evidence.

Consistently, reliable predictors of fracture risk in this population are still lacking.

The objective of this retrospective observational study was to analyze the cardiovascular and bone health through clinical, biochemical, and morphological characteristics of patients with overt CS who experienced fragility fractures compared to those who did not, in order to identify potential predictors of worse skeletal outcome.

Methods

Study Design and Participants

This study followed a retrospective cross-sectional design in which we analyzed patients affected by overt CS at the moment of the diagnosis. All subjects were included among those attending the Endocrine Unit of Padua before starting any kind of cortisol-lowering medications.

The inclusion criteria were: (i) the presence of overt CS (ie, biochemical diagnosis of CS and clinically evident cortisol excess); and (ii) stage I of TNM classification in case of ectopic CS (EAS).

The exclusion criteria were: (i) CS diagnosed with mild autonomous cortisol secretion (MACS) (ie, cortisol after 1-mg dexamethasone levels >1.8 µg/dL, 50 nmol/L and absence of clinical signs and symptoms); (ii) stage > I of TNM classification in case of EAS; (iii) patients carrying adrenocortical carcinoma among adrenal CS (ACS); (iv) unavailability of DXA, vertebral morphometry examination or carotid arteries ultrasound evaluation; (v) concomitant aldosterone or catecholamine excess in case of ACS; and (vi) the presence of other diseases influencing fracture risk (primary hyperparathyroidism, hyperthyroidism, rheumatological, or hematologic disease).

Initially, 78 subjects were included in the study; however, following the application of exclusion criteria we finally enrolled 51 patients, 34 female and 17 male. Details regarding the selection process are reported in Fig. 1. For the statistical analysis, ACS and EAS were considered as one category.

The study received approval from the Ethics Committee of Padova University Hospital (Comitato Etico per la Sperimentazione Scientifica) with protocol number AOP1782 and CESC reference 4834/AO/20.

This observational study was conducted in accordance with the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines [20].

Diagnostic Evaluation

The diagnosis of CS was established according to Endocrine Society guidelines [21, 22], based on clinical suspicion and biochemical evaluation: increased 24-hour urinary free cortisol (UFC) levels (mean of 2 samples), failure of serum cortisol decrease < 50 nmol/L after 1-mg dexamethasone suppression. Plasma and urinary cortisol levels were determined in all patients by radioimmunoassay (Diagnostic Product Corp., Los Angeles, CA, USA). The diagnosis of ACTH-dependent CS was based on the presence of detectable plasma ACTH concentrations (> 10 ng/L), measured by immunoradiometric assay. The source of ACTH secretion was investigated by dynamic endocrine assessment and by pituitary magnetic resonance imaging. Endocrine assessment included overnight 8-mg dexamethasone suppression test (8 mg-DST), corticotropin-releasing hormone (CRH) stimulation test (100 µg intravenous ovine or human CRH) and desmopressin (DDAVP) test (10 µg intravenous).

Clinical and Biochemical Evaluation

Disease duration of CS was determined in all considering the time elapsed between the initial onset of signs and symptoms and the time of diagnosis.

Patients were interviewed regarding their medical history to ascertain any instances of venous thromboembolism (VTE), encompassing both pulmonary embolism and deep vein thrombosis. In our statistical analysis, VTE was regarded as a dichotomous variable (present or absent).

Body mass index (BMI) between 25 and 30 kg/m^2 was classified as overweight and BMI above 30 kg/m^2 as obesity. Waist circumference was collected as a clinical indicator for visceral obesity, and it was measured at the midpoint between the top of the iliac crest and the lower margin of the last palpable rib at the end of natural breaths. Arterial hypertension was defined as blood pressure values ≥ 140/90 mmHg or the use of antihypertensive medication. Diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance were diagnosed according to established national guidelines [23]. For our study, alterations in glucose metabolism were treated as a dichotomous variable (presence or absence) named *glucose metabolism alterations* (GMA). Hypercholesterolemia was defined as total cholesterol levels ≥ 5.2 mmol/L (200 mg/dL) with low-density lipoprotein cholesterol ≥3.37 mmol/L (130 mg/dL), while low

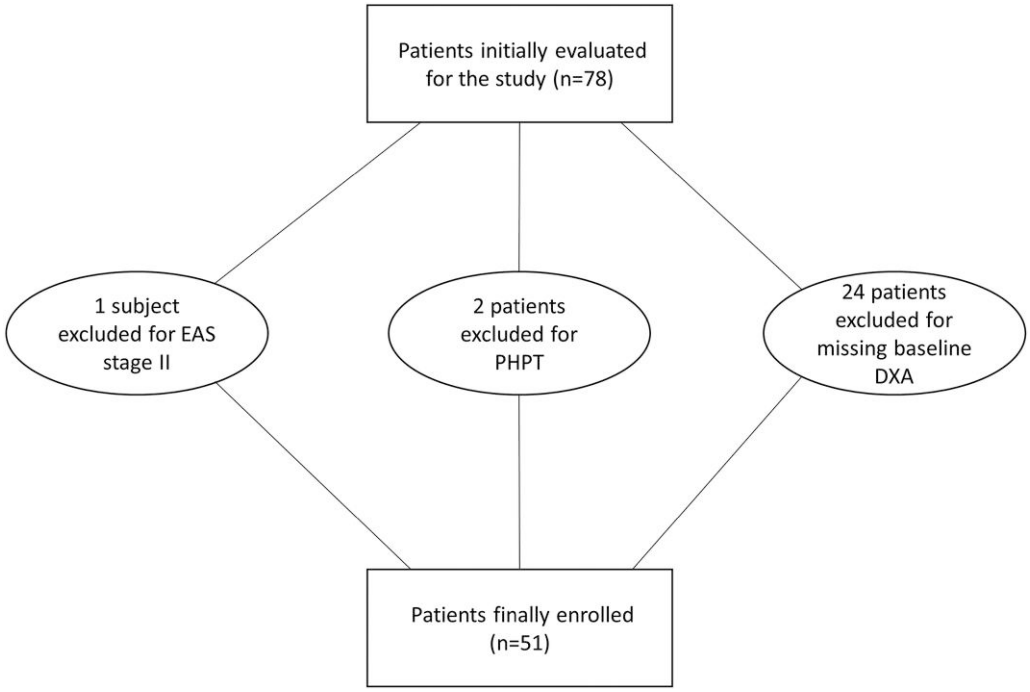

Figure 1. Flow-chart representing the inclusion process of patients in the study.

Abbreviations: DXA, dual-energy x-ray absorptiometry; EAS, ectopic Cushing syndrome; PHPT, primary hyperparathyroidism.

high-density lipoprotein cholesterol was defined as < 1 mmol/L (39 mg/dL) in men and < 1.16 mmol/L (45 mg/dL) in women. Hypertriglyceridemia was defined as triglyceride levels ≥1.7 mmol/L (150 mg/dL), according to ESC guidelines [24]. Patients were considered dyslipidemic if they exhibited alterations in any of these parameters or were receiving hypolipidemic therapy. High and very high levels of lipoprotein(a) were defined as > 500 mg/L and > 1800 mg/L, respectively [25]. Gonadal status was assessed based on clinical history, the presence of menses in female patients, and serum levels of follicle stimulating hormone, luteinizing hormone, and sex hormones.

Men were considered hypogonadal if they exhibited typical signs or symptoms and had serum total testosterone levels < 12 nmol/L and/or free calculated testosterone levels < 220 pmol/L, in agreement with national guidelines [26]. Parathyroid hormone (PTH) was measured using a whole molecule intact PTH bridge kit. Biochemical parameters related to glucose and lipid metabolism, serum and urinary ions, uric acid, serum creatinine, bone alkaline phosphatase (ALP), and 25-OH vitamin D were measured at diagnosis using standard automated laboratory methods and commercial kits.

Evaluation of Bone Parameters, Prevalence of Clinical Fractures, and Body Composition

At diagnosis, each patient underwent DXA using the Hologic QDR 4500C densitometer (Hologic Inc., Waltham, MA, USA). Bone mineral density (BMD) measurements were obtained at the lumbar spine (L1-L4), femur neck, and total femur. Individual BMD values were expressed in absolute terms (g/cm^2). For postmenopausal women and men aged > 50 years, BMD results were categorized based on T-scores: T-score ≥ −1 SD indicated normal bone density, T-score between −1 and −2.5 SD indicated osteopenia, and T-score ≤ −2.5 SD indicated osteoporosis. For patients with premenopausal status or male patients younger than 50 years, BMD was evaluated using Z-scores: Z-score values of −2.0 SD or lower were classified as "below the expected range for age," while values above −2.0 SD were categorized as "within the expected range for age" [27]. For the statistical analysis, *osteoporosis* and *below the expected range for age* were considered as a unique variable. Additionally, these variables are categorized considering only the densitometric results. Concurrently, vertebral morphometry examinations were conducted, with vertebral fractures defined as a reduction of more than 20% of anterior, middle, or posterior vertebral height according to the Genant semiquantitative method [28]. Data regarding previous fractures were obtained from medical history. Low-traumatic fractures were defined as those resulting from a fall from standing height or less [13]. Peripheral fragility fractures at the sites of ribs, wrists, and femurs were also considered. Vertebral fractures and peripheral fractures were collectively categorized as "fragility fractures."

The same examination was utilized to evaluate the body composition of the subjects, providing assessments of total body bone mineral content (BMC) and fat and lean mass. These variables were also evaluated specifically in the trunk region and expressed as absolute values in grams. Additionally, fat mass was expressed as a percentage of total body mass.

TBS values for the lumbar spine were available in 29 patients and obtained from DXA images using TBS iNsight software, version 2.1.2.0 (Med-Imaps, Pessac, France). Based on findings from previous studies [29], TBS values were categorized using the available cutoff adjusted in postmenopausal women, as follows: TBS ≥ 1.31 indicated normal microarchitecture, while values < 1.31 indicated compromised microarchitecture. Quality control for both BMD and TBS were upheld through daily calibration using specialized phantoms.

Vascular Study

We assessed the prevalence of carotid atherosclerotic plaques using echo-color Doppler ultrasonography, examining the right and left common carotid arteries, carotid bulbs, internal, and external carotid arteries. The prevalence of carotid plaques was recorded as a dichotomous variable (presence or absence). The IMT was also measured and calculated as the mean of values obtained from both sides. Ultrasonography was conducted by an experienced radiologist who was blinded to the clinical and biochemical conditions of the patients. We utilized an Advanced Technology Laboratories Apogee 800 plus instrument (Advanced Technology Laboratories, USA), equipped with an 8.5 MHz probe for echography and a 6 MHz probe for pulse-wave Doppler.

Statistical Analysis

Continuous variables were summarized using mean and SD for normally distributed data or median and interquartile range (IQR) for non-normally distributed data. Categorical variables were presented as counts, frequencies, and proportions within each category.

The normality of distribution for each variable was assessed using the Kolmogorov-Smirnov test. Group comparisons for quantitative variables were conducted using independent sample *t* tests or Mann-Whitney tests, depending on the distribution of the data. Categorical variables were compared using the chi-square test or Fisher exact test, as appropriate. Associations between quantitative variables were examined using the Pearson correlation test for normally distributed data and Spearman correlation for non-normally distributed data. Binary and multiple logistic regression analyses were performed to assess the effects of variables on fragility fractures. Variables selected for binomial regression were those exhibiting significance or near significance during group comparison. Consequently, multiple logistic regression included the variable identified as significant in binary regression. Statistical significance was defined as a P value <.05.

All statistical analyses were conducted using SPSS statistical software (version 24.0, SPSS Inc., Chicago, IL). All data analyzed during this study are securely stored in the data repositories of the University of Padova—Research Data UniPD [30].

Results

Comparison Between CS Patients With and Without Fragility Fracture

Our cohort included 51 patients, 34 female and 17 male, mean age 48.8 ± 13.34 years. Subjects were affected by overt CS; 37 (72.5%) with Cushing disease (CD), 9 (17.6%) with adrenal CS (ACS) and 5 (9.8%) with ectopic CS (EAS).

The main features of patients with and without fragility fractures are reported in Table 1. Thirty-two patients (62.7%) were found to be affected by fragility fractures at diagnosis. Twenty-five patients carried vertebral fractures, 6 patients registered peripheral fractures, and one patient experienced both vertebral and peripheral fractures.

Twenty-three (71.9%) patients with fractures were affected by CD, 5 (15.6%) by ACS, and 4 (12.5%) by EAS; while considering patients without fractures (19/51) we observed 14 (73.7%) with CD, 4 (21.1%) with ACS and only 1 patient (5.3%) with EAS. No differences in terms of fragility fractures between CD patients and other types of CS was observed (P = .311). Median levels of UFC were comparable in fractured patients (819 [IQR 517-1199] nmol/24 hours), and in nonfractured patients (718 [IQR, 262-1751.50] nmol/24 hours; P = .915). Age at diagnosis, BMI, and absolute values of systolic and diastolic blood pressure were similar among subjects with and without fragility fractures. Male patients were observed to be more affected by fragility (fractures, 88.2% vs 11.8%; P = .008). Fractured patients were found to carry a longer disease duration compared to nonfractured patients (36 [IQR, 24-60] vs 12 [IQR, 12-36] months; P = .025). Among the anthropometric parameters mean waist circumference was higher in subject with fractures, (107.61 ± 13.77 cm vs 94.50 ± 14.36 cm; P = .006).

No differences between the 2 cohorts were detected analyzing mean fasting glucose, complete lipid profile, lipoprotein(a). In concordance with these findings, the prevalence of hypertension, dyslipidemia, glucose metabolism alterations, obesity, and metabolic syndrome exhibited comparable results in both populations. The analysis of bone biochemical parameters, in particular, serum calcium, PTH, vitamin D, serum carboxy-terminal collagen crosslink (CTX) and bone alkaline phosphatase revealed no difference in patients with and without fragility fractures.

Notably, fractured patients had increased mean serum levels of uric acid (0.35 ± 0.12 mmol/L) compared to nonfractured patients (0.25 ± 0.06 mmol/L; P = .001).

As summarized in Table 1, patients with fractures showed a higher prevalence of VTE events compared with those without fractures (31.3% vs 5.3%; P = .037), and similar findings were observed considering the prevalence of atherosclerotic plaques (47% vs 5.3%; P = .002) in the same cohorts, respectively.

Osteoporosis prevalence was 43.1% (22/51) in the whole cohort, fractured patients showed a higher prevalence of osteoporosis compared to nonfractured patients (56.3% vs 21.1%, P = .014). DXA findings were concordant, indeed lumbar mean spine T-score was reduced in patients carrying fractures (−2.12 ± 1.37 vs −1.04 ± 1.72, P = .030). Conversely, nonsignificant differences were observed for BMD of lumbar spine, total hip and femoral neck BMD, and T-score.

The body composition evaluation showed similar results in terms of lean and fat mass both considering total body and trunk assessment, as well as fat percentage between fractured and nonfractured patients (Table 2).

Vascular examination revealed similar findings regarding median IMT at carotid bulbs, internal carotid, and external carotid, whereas fractured patients showed higher median IMT examined at the common carotid artery compared to nonfractured patients (0.95 mm [IQR, 0.80-1.45] vs 0.88 mm [IQR 0.70-1.18]; P = .017).

Logistic Regression

Bivariate logistic regression was employed to evaluate the individual contribution of each independent predictor concerning the presence or absence of fragility fractures. As illustrated in Table 3, the presence of atherosclerotic plaques (odds ratio [OR] 15.88; 95% CI, 1.88-133.64; P = .011), male gender (OR 0.83; 95% CI, 1.66-46.83; P = .01), waist circumference (OR 1.074; 95% CI, 1.014-1.139; P = .015), spine T-score (OR 0.602; 95% CI, 0.386-0.940; P = .025), common carotid IMT (OR 12.36; 95% CI, 1.49;102.270, P = .02), and presence of osteoporosis (OR 4.82; 95% CI, 1.307-17.79; P = .018) emerged as significant independent

Table 1. Biochemical and clinical data of patients affected by Cushing syndrome with and without fragility fractures

	CS with fragility fractures	CS without fragility fractures	*P* value
Gender (M/F)	15/17	2/17	**.008**
Mean age at diagnosis	51.4 (± 13.9)	44.5 (± 1.3)	.075
Median disease duration (month)	36 (24-60)	12 (12-36)	**.025**
Median UFC (nmol/24 hours)	819 (517-1199)	718 (262-1751.50)	.915
Median serum F h8 nmol/L	637.50 (523.75-715)	657 (552-872.10)	.806
Cushing type (CD/other)	23/9	14/5	.311
Mean BMI (kg/m^2)	29.04 (± 5.82)	27.66 (± 5.70)	.414
Obesity (yes/no)	13/19	4/15	.152
Median SBP (mmHg)	140 (130-160)	130 (120-140)	.350
Median DBP (mmHg)	90 (80-100)	85 (80-90)	.539
Hypertension (yes/no)	9/6	18/1	.639
Menopause (yes/no)	12/5	9/8	.290
Hypogonadism (yes/no)	9/6	1/1	.999
Mean waist circumference (cm)	107.61 (± 13.77)	94.50 (± 14.36)	**.006**
Median fasting glucose (mg/dL)	106 (94-117)	86 (83-118)	.899
GMA alterations (yes/no)	14/18	12/7	.180
Mean total cholesterol (mg/dL)	217.21 (± 58.20)	218.10 (± 40.00)	.954
Median triglycerides (mg/dL)	122 (64-152)	82 (59.5-132)	.520
Mean HDL (mg/dL)	51.67 (± 13.11)	55.84 (± 14.24)	.297
Mean cLDL (mg/dL)	136.13 (± 48.43)	130.97 (± 39.72)	.697
Median Lpa (mg/L)	174 (66-452)	185 (87.5-410)	.482
Dyslipidemia (yes/no)	27/5	16/3	.999
Metabolic syndrome (yes/no)	29/3	15/4	.402
Mean uric acid (mmol/L)	0.35 (± 0.12)	0.25 (± 0.06)	**.001**
Mean serum Ca (mmol/L)	2.39 (± 0.15)	2.34 (± 0.12)	.179
Mean serum phosphate (mmol/L)	1.01 (± 0.21)	1.01 (± 0.19)	.962
Median PTH (ng/L)	31 (25-46)	25 (17.5-29)	.081
Mean 25OHD (nmol/L)	57.62 (± 24.24)	44.86 (± 24.86)	.083
Mean CTX (pg/mL)	543 (± 386.27)	458.58 (± 247.69)	.497
Median BALP (ug/L)	15.9 (11-25)	10.5 (9.95-23.55)	.277
VTE (yes/no)	10/22	1/18	**.037**
Atherosclerotic plaque (yes/no)	15/17	1/18	**.002**

Mean values and median values are presented with SD and interquartile range, in brackets, respectively. Bold values indicate statistical significance.
Abbreviations: 25OHD, 25-hydroxylated vitamin D; ACTH, adrenocorticotropic hormone; BALP, bone alkaline phosphatase; BMI, body mass index; Ca, calcium; cLDL, calculated low-density lipoprotein; CS, Cushing syndrome; CTX, beta-C-terminal telopeptide; DBP, diastolic blood pressure; F, cortisol; GMA, glucose metabolism alterations; HDL, high-density lipoprotein; Lpa, lipoprotein a; PTH, parathyroid hormone; SBP, systolic blood pressure; UFC, urinary free cortisol; VTE, venous thromboembolism events.

predictors of fragility fractures, as shown in Fig. 2. Multiple logistic regression was subsequently conducted, incorporating these statistically significant predictors while separately considering spine T-score and osteoporosis, as well as the presence of atherosclerotic plaques and common carotid IMT, to mitigate multicollinearity for each model (the 4 tested models are depicted in supplementary material [30]). The model that exhibited the highest performance, as assessed by the Nagelkerke R square index, in predicting variations in the dependent variable (presence of fractures) included gender, presence of atherosclerotic plaques, waist circumference, and osteoporosis, as delineated in Table 3.

Following multiple regression, the presence of plaques and osteoporosis remained significant (OR 13.35; 95% CI, 1.154-154.34; $P = .038$ and OR 11.30; 95% CI, 1.547-82.562; $P = .017$, respectively).

TBS Assessment

TBS evaluation of spine BMD was available for 29 patients: a TBS reduction (ie < 1.31) was observed in 23 out of 29 cases (79.3%). Median TBS values were comparable in patients with and without fractures, being 1.26 (IQR, 1.045-1.351) and 1.22 (IQR, 1.084-1.276), respectively ($P = .551$). Furthermore, there were no significant differences in the prevalence of TBS reduction between the 2 cohorts (73.7% vs 90%, $P = .303$).

Bivariate correlation analysis, as presented in Table 4, demonstrated a significant inverse correlation between TBS and BMI ($rho = -0.602$, $P = .001$). Regarding body composition, TBS was inversely correlated with both fat and lean mass ($rho = -0.406$, $P = .039$; $rho = -0.428$, $P = .029$, respectively). Similar inverse correlations were observed when examining fat and lean mass specifically in the trunk region ($rho = -0.495$, $P = .010$; $rho = -0.495$, $P = .010$).

Table 2. Bone and instrumental data of patients affected by Cushing syndrome with and without fragility fractures

	CS with fragility fractures	CS without fragility fractures	*P* value
Mean lumbar spine BMD (g/cm^2)	0.836 (± 0.150)	0.931 (± 0.190)	.080
Mean lumbar spine T-score	−2.12 (± 1.37)	−1.04 (± 1.72)	**.030**
Mean lumbar spine Z-score	−1.20 (± 1.69)	−0.62 (± 1.62)	.229
Mean femoral neck BMD (g/cm^2)	0.695 (± 0.130)	0.709 (± 0.127)	.753
Mean femoral neck T-score	−1.60 (± 1.02)	−1.29 (± 1.14)	.371
Mean femoral neck Z-score	−0.46 (± 0.95)	−0.60 (± 1.08)	.655
Mean total hip BMD (g/cm^2)	0.829 (± 0.134)	0.829 (± 0.162)	.993
Mean total hip T-score	−1.22 (± 0.94)	−1.00 (± 1.29)	.525
Mean total hip Z-score	−0.51 (± 1.01)	−0.55 (± 1.02)	.895
Densitometric osteoporosis (yes/no)	18/14	4/15	**.014**
Median TBS	1.26 (1.045-1.351)	1.22 (1.084-1.276)	.551
TBS reduced (yes/no)	14/5	9/1	.303
Median IMT Cc	0.95 (0.80-1.45)	0.88 (0.70-1.18)	**.017**
Median IMT Bc	0.80 (0.65-1.40)	1.075 (0.70-1.46)	.331
Median IMT Ic	0.95 (0.75-1.10)	0.95 (0.70-1.33)	.422
Mean IMT Ec	0.89 (± 0.23)	0.82 (± 0.15)	.316
Mean fat mass (g)	30280.59 (± 10972.00)	29827.01 (± 13534.22)	.936
Mean trunk fat mass (g)	16918.45 (± 6373.79)	16044.10 (± 7846.28)	.752
Median lean mass (g)	44850.55 (39326.55-59180.88)	43340.30 (38514.40-52096.60)	.767
Mean trunk lean mass (g)	24673.86 (± 5840.20)	23785.98 (± 4198.39)	.669
Mean fat mass percentage (%)	36.93 (± 6.52)	36.80 (± 7.74)	.694
Mean trunk fat mass percentage (%)	39.37 (± 6.78)	37.89 (± 8.56)	.616

Mean values and median values are presented with SD and interquartile range, in brackets, respectively. Bold values indicate statistical significance.
Abbreviations: BMD, bone mineral density; Bc, carotid artery bulbs; Cc, common carotid artery; Ec, external carotid artery; Ic, internal carotid artery; IMT, intima-media thickness; TBS, trabecular bone score.

Table 3. Results of binomial and multiple logistic regression analysis

	OR	95% CI	*P*
VTE (yes)	8.18	0.955-70.95	.055
Atherosclerotic plaque (yes)	15.88	1.88-133.640	**.011**
Gender (male)	0.83	1.66-46.83	**.01**
Age	1.042	0.995-1.091	.08
Waist circumference	1.074	1.014-1.139	**.015**
BMI	1.045	0.942-1.160	.407
Disease duration	1.027	0.999-1.055	.056
Lumbar spine T-score	0.602	0.386-0.940	**.025**
Lumbar spine BMD	0.026	0.001-1.234	.064
IMT Cc	12.36	1.49-102.27	**.02**
Osteoporosis (yes)	4.82	1.307-17.788	**.018**
Multiple logistic regression			
Gender (male)	1.79	0.215-14.86	.591
Atherosclerotic plaque (yes)	13.35	1.154-154.34	**.038**
Waist circumference	1.069	0.993-1.151	.075
Osteoporosis (yes)	11.30	1.547-82.562	**.017**

Results of bivariate and multiple logistic regression analysis, respectively, are reported. Bold values indicate statistical significance.
Abbreviations: BMD, bone mineral density; BMI, body mass index; Cc, common carotid artery; CS, Cushing syndrome; IMT, intima-media thickness; OR, odds ratio; VTE, venous thromboembolism events;

Additionally, serum uric acid displayed an inverse relationship with TBS (rho = −0.434, $P = .024$), while a positive correlation was observed between serum high-density lipoprotein (HDL) and TBS (*rho* = 0.371, $P = .047$).

Discussion

Cortisol excess has been demonstrated to degrade both the bone mass and quality, leading to an impaired skeletal microarchitecture [31, 32]. In our study, fragility fractures were frequent, achieving almost a 63% prevalence at the diagnosis, in concordance with previous findings [13, 33, 34]. Despite DHEAS being thought to exert a protective effect on bone health, suggesting a higher propensity for bone fragility in adrenal CS [35], we did not find a significant difference in fracture prevalence when considering different etiology of CS.

In contrast to previously published papers [13, 33], serum morning cortisol and UFC levels were comparable in subjects with and without fragility fractures. Patients with fragility fractures exhibited a longer disease duration, remarking similar trends observed in other systemic comorbidities of CS, where the disease duration plays a pivotal role, beyond the absolute degree of cortisol excess [5]. Interestingly, in our cohort, fractured patients showed higher waist circumference, reflecting perhaps a deeper involvement in terms of visceral obesity, clinically portrayed by the measurement of waist circumference [2]. Furthermore, our data show that fragility fractures affect male patients significantly more than females, confirming previous reports [13, 36] despite a comparable prevalence of hypogonadism in males and menopausal status in females. Albeit a prevalence of osteoporosis ranging from

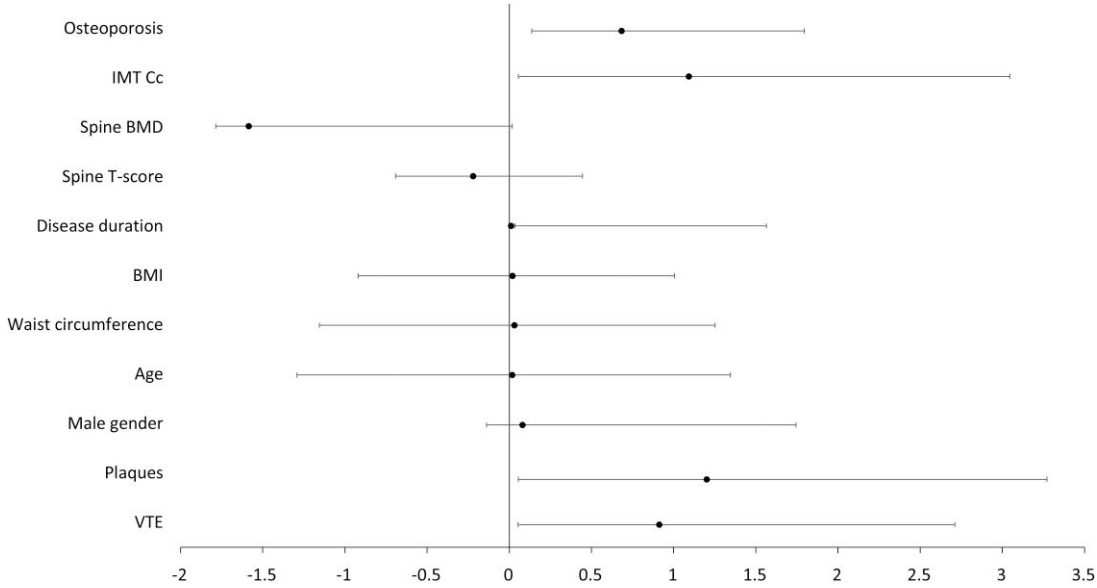

Figure 2. Forest plot showing odds ratio (OR) of binary logistic regression; OR and lower and upper limits of 95% CI are represented after transformation vs Log10.

Abbreviations: BMD, bone mineral density; BMI, body mass index; IMT Cc, intima-media thickness of the common carotid artery; VTE, venous thromboembolic events.

40% to 70% in CS patients [12], it is well known that low-trauma fractures might occur even in the presence of normal age and sex values of BMD. Eller-Vainicher et al [37] found no correlations between vertebral fractures and lumbar spine BMD in patients with MACS. Similarly, Belaya et al [13] describe a cohort of 182 patients with CS with fractures occurring in the "non-osteoporotic range." Our data highlight a reduced spine T-score in fractured patients as well as a higher prevalence of densitometric osteoporosis (56%) in the same group compared to patients without fractures (21%). Lower spine T-score among patients with CS was previously reported by Stachowska et al [38]; however, the authors did not perform a correlation analysis with fractures' incidence. Among our patients, median BMD values at the lumbar spine were lower in patients carrying fragility fractures, even if this result did not achieve statistical significance, whereas we described comparable values of BMD at total hip and femoral neck between the 2 groups, remarking the recent findings concerning the deterioration of bone quality and microarchitecture in patients with endogenous CS that might increase the risk of fractures regardless of BMD [31].

TBS values have been shown to be reduced in patients with adrenal MACS compared to healthy individuals [37] and those with nonsecreting adrenal incidentaloma [32, 39]. Similar findings were observed in patients with overt CS compared to healthy controls, although limited studies are available [31, 38]. Notably, in our study, no statistical differences were observed between patients with and without fractures, even when conducting subgroup analysis specifically considering only vertebral fractures. However, when considering the entire cohort, TBS values were reduced in 79% of patients regardless of the presence of fractures. This observation can lead us to 2 main considerations: first, the median reduction of TBS values among our whole cohort confirms the well-known detrimental effect of cortisol excess on bone quality [31, 37, 38]. On the other hand, the same homogeneous reduction of TBS in patients with and without fragility fractures might have contributed to conceal the presence of a significant difference between them, aligning with previous findings [13]. According to bivariate correlation analysis, we observed that TBS was inversely correlated to BMI, in concordance with data described by Stachowska et al [38] yet in contrast with other reports [31] [37]. Furthermore, we noted an inverse significant correlation between TBS values and both fat and lean mass, and they were also inversely related to serum uric acid levels. Conversely, TBS was positively associated with HDL cholesterol levels.

Higher BMI is traditionally associated with a protective effect against osteoporosis in postmenopausal women [40] despite that nowadays this concept is perhaps considered oversimplistic; individuals with obesity face, indeed, an increased risk of non-spine fractures, particularly those affecting the proximal humerus, upper leg, and ankle [41]. However, the effect of BMI and body composition vs TBS and bone quality is less clear. In a recent study, a negative correlation between visceral adipose tissue and TBS was found in a cohort of 56 patients, including pre- and postmenopausal women, suggesting a potential detrimental effect of increased fat mass vs bone quality [42], perhaps secondary to adipose tissue associated low-grade chronic inflammation [43]. Conversely, the link between an increased lean mass and a degraded TBS was described only by a large study among older men [44]. Moreover, the same authors suggested that this association should be carefully interpreted also considering the interference of soft tissue in TBS instrumental determination.

Intriguingly, in our cohort, beyond the connections with the trabecular microarchitecture, serum uric acid was found to be significantly increased in patients burdened by fragility fractures. Notably, while some studies suggest a protective effect of uric acid on bone health under physiological conditions, the process of uric acid degradation can actually lead to intracellular oxidative stress [45], since high levels of serum uric acid had been recently identified not only as a potential agent in determining osteoporosis but also as potentially involved in determining fragility vertebral and non-vertebral fractures [46, 47].

Table 4. Correlation analysis between TBS and many biochemical and instrumental parameters

	Spearman's rho	*P* value
BMI	−0.602	**.001**
Month of duration	−0.098	.614
Age at diagnosis	−0.175	.364
8 AM serum cortisol	−0.088	.649
UFC	0.237	.216
25OHD	0.164	.395
Total cholesterol	0.223	.244
HDL cholesterol	0.371	**.047**
cLDL cholesterol	0.348	.064
Triglycerides	−0.354	.059
Lpa	0.433	.107
Serum uric acid	−0.434	**.024**
Lumbar spine BMD	0.278	.144
Lumbar spine T-score	0.258	.176
Total hip BMD	0.102	.605
Total hip T-score	0.087	.660
Femoral neck BMD	0.206	.293
Femoral neck T-score	0.169	.391
Fat mass	−0.406	**.039**
Lean mass	−0.428	**.029**
Trunk fat mass	−0.495	**.010**
Trunk lean mass	−0.495	**.010**
IMT Cc	0.100	.613
IMT Bc	−0.031	.882
IMT Ic	−0.001	.995
IMT Ec	0.022	.910

Bold values indicate statistical significance.
Abbreviations: 25OHD, 25-hydroxylated vitamin D; Bc, carotid artery bulbs; BMD, bone mineral density; BMI, body mass index; Cc, common carotid artery; cLDL, calculated low-density lipoprotein; Ec, external carotid artery; HDL, high-density lipoprotein; Ic, internal carotid artery; IMT, intima-media thickness; Lpa, lipoprotein a; UFC, urinary free cortisol.

If we further keep in mind that TBS in our dataset showed no correlation with spine and hip BMD, we might deduce an intricate interplay between the bone quality, body composition, and BMI among patients with CS.

Specifically, since higher BMI and increased uric acid and fat mass are established cardiovascular risk factors [45], our observations might imply a close connection between those features and both a compromised bone quality and a higher rate of fragility fractures in CS patients. Contrarywise, the bone quality seems to be more preserved in patients showing protective features against cardiovascular risk, like higher HDL cholesterol.

Intriguingly, in our cohort, patients who experienced fragility fractures showed a higher rate of deep thromboembolism events. This interaction is particularly significant when considering that patients affected by fractures experienced reduced mobility; this might undoubtedly exacerbate the thromboembolic risk, a well-known and severe complication associated with CS [48]. Moreover, in the fractured cohort, we noted a higher prevalence of atherosclerotic plaques as well as a higher median IMT assessed at the common carotid artery. Thromboembolic events and vascular disease are direct features of cardiovascular morbidity that burden patients with CS [49], however, their involvement as potential predictors of fragility fracture have not been reported yet. Bivariate logistic regression analysis, as shown in Table 3, revealed that patients with atherosclerotic plaques are more than 15 times more likely to experience a fragility fracture. Male gender, increased waist circumference, common carotid IMT, decreased spine T-score, and the presence of densitometric osteoporosis were identified as predictors of fragility fractures as well. Subsequent multiple logistic regression analysis confirmed osteoporosis and particularly atherosclerotic plaques as independent predictors of low-trauma fractures, with an 11-times and 13-times higher risk, respectively.

Bone and vascular diseases commonly coexist, sharing risk factors and pathogenic mechanisms, collectively referred to as the "bone-vascular axis." Experimental evidence suggests that the excess of glucocorticoids may disrupt this axis. Specifically, exogenous glucocorticoid administration has been shown to induce vascular calcification by promoting the osteogenic differentiation of vascular cells [50]. However, the extent to which glucocorticoid excess contributes to the concurrent manifestation of bone and vascular diseases in humans remains uncertain. Geng et al [51] reported an inverse correlation, in men without CS, between coronary atherosclerotic plaques and coronary artery disease and spine BMD. However, no data regarding fracture occurrence were reported. Recently, Yano et al, explored the bone and vascular damage in adrenal CS, observing that patients with ACS experienced higher coexistence rates of vertebral fractures and arterial stiffness as well as vertebral fractures and abdominal aortic calcifications than those with non-functional adrenal tumors [52]. Nonetheless, in this study, most patients in the ACS cohort were affected by MACS and not overt CS. Our findings showed a major prevalence of cardiovascular risk factors in patients with fragility fractures, firstly uncovering a potential novel pathological link between the cardiovascular complications and fragility fractures in patients with overt endogenous CS, highlighting the need for further exploration of the relationship between skeletal and cardiovascular health. Moreover, identifying these cardiovascular factors as potential predictors of fractures could aid in stratifying fracture risk in CS, advocating for more rigorous screening and follow-up of bone health in patients with a higher burden of cardiovascular complications. Simultaneously, CS patients who have experienced fragility fractures should undergo a more intensive screening and early treatment for cardiovascular comorbidities, potentially contributing to decrease the mortality rate.

Limitations and Future Perspective

The present study, which further investigates the relationship between bone and cardiovascular health in endogenous CS, is not without limitations. First of all, the retrospective design allowed for partial collection of the biochemical, clinical, and instrumental information, and we recognize that this is one of the most important limitations of our study. Moreover, due to the retrospective design and limited available data, accurate sample size analysis was not feasible. Consequently, in some cases, for example considering the patients with available TBS examination, the accessible clinical details were scant. Undoubtably, the limitations are strictly linked to the monocentric design that hampered us in the enrollment of a larger sample size and to the missing of a prospective arm.

However, our study also possesses some strengths. In particular, the intra-cohort analysis enables a comprehensive evaluation of key features, enhancing the stratification of fracture predictors. Furthermore, to our knowledge, this is the first study to offer a holistic assessment of the interplay between fracture risk, bone quality, and cardiovascular morbidity in overt endogenous CS.

Nevertheless, this relationship necessitates further and more in-depth investigation, potentially requiring a prospective analysis. Additionally, a multicenter study would facilitate the inclusion of a larger sample size, thereby strengthening the overall results.

Conclusions

Endogenous CS represents a complex condition associated with a negative impact on long-term mortality and morbidity. Among the various complications, cardiovascular and skeletal issues stand out as particularly debilitating. However, these intriguing findings indicate the overall burden of cardiovascular morbidity as potential novel shared predictors of damage affecting the bone-vascular axis, and impacting the risk of fracture, extending beyond the mere degree of cortisol excess.

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Author Contributions

S.P. and M.C.: data collection; G.V. and F.C.: data analysis; G.V., P.M., and M.B.: writing of original draft; V.C., M.B. and C.S.: supervision, writing-review and editing. All authors approved the final version of the paper.

Research Involving Human Participants and Patient Consent

Informed consent was obtained from all participants.

Disclosures

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Data Availability

All data generated or analyzed during this study and supplementary material are included in this published article or the data repositories in the public available website of the University of Padova (doi: 10.25430/researchdata.cab.unipd.it.00001294).

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