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ORIGINAL ARTICLE

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High bone marrow fat in patients with Cushing's syndrome and vertebral fractures

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Abstract

Purpose The evaluation of skeletal fragility in Cushing's syndrome (CS) is a clinical challenge, since dual-energy X-ray absorptiometry (DXA) does not capture abnormalities in bone microstructure induced by glucocorticoid excess. Hypercortisolism was shown to increase bone marrow adiposity, but it is still unknown whether high bone marrow fat (BMF) as measured by vertebral magnetic resonance spectroscopy may predict fracture risk in this clinical setting. In this cross-sectional study, we evaluated the association between BMF and vertebral fractures (VFs) in patients with CS.

Methods Twenty patients (5 M, age 44 ± 13 years) with active CS were evaluated for morphometric VFs, lumbar spine BMF, and bone mineral density (BMD). Fifteen healthy volunteers (4 M, age 43 ± 12 years) acted as control group for BMF evaluation.

Results BMF was significantly higher in CS patients vs. controls (52.0% vs. 27.0%, $p < 0.01$), and was directly correlated with patients' age ($p = 0.03$), 24-hours urine-free cortisol ($p = 0.03$), midnight serum cortisol ($p = 0.02$), and serum CTX ($p = 0.01$). Patients with VFs (13 cases) showed significantly higher BMF vs. patients without VFs (65.0% vs. 24.0%, $p = 0.03$). Fractured patients with either normal BMD or osteopenia showed comparable BMF to fractured patients with either osteoporosis or low BMD for age ($p = 0.71$). When the analysis was restricted to patients with normal BMD or osteopenia, VFs were still significantly associated with higher BMF ($p = 0.05$).

Conclusions This study provides a first evidence that vertebral adiposity may be a marker of hypercortisolism-induced skeletal fragility and measurement of spine BMF could have a role in the diagnostic work-up for the assessment of fracture risk in CS.

Keywords Cushing's syndrome · Vertebral fractures · Bone marrow fat · Osteoporosis · Cortisol

Introduction

Chronic hypercortisolism has multiple severe deleterious systemic effects [1–3]. Cushing's syndrome (CS) frequently causes skeletal fragility as consequence of multiple direct and indirect effects of glucocorticoid excess on bone remodeling and calcium metabolism [4]. Patients with CS characteristically have a low-bone turnover osteoporosis with severe suppression of bone formation and an increased risk of fragility fractures, mainly involving vertebrae and ribs [5, 6]. Noteworthy, vertebral fractures (VFs) may be diagnosed in up to 75% of patients with CS, half of them being clinically symptomatic associated with pain, functional limitation, and height shortening by 3–10 cm of final stature [7].

In the general population, measurement of bone mineral density (BMD) by DXA is used as the primary surrogate of

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bone strength for clinical diagnosis of osteoporosis [8]. However, in patients with secondary osteoporosis the prediction of fracture risk may be a clinical challenge, since bidimensional DXA measurement of BMD is not able to capture the deterioration of bone quality primarily affected in this clinical setting [9]. As a matter of fact, a remarkable number of patients with CS may fracture in presence of low-normal or even normal BMD values [7, 10–12].

Despite the effects of glucocorticoid excess on bone quality being well known and characterized [13–15], data on bone microstructure in endogenous hypercortisolism are scant and controversial [12, 16–18]. Bone marrow adiposity has been proposed as a reliable biomarker of bone quality and quantity reflecting, the process of mesenchymal stem cell allocation in bone microenvironment and osteoblast differentiation in the basic multicellular units [19, 20]. Using either proton magnetic resonance spectroscopy (MRS) or bone biopsy, some studies reported an association between high bone marrow fat (BMF), low BMD, and high prevalence of VFs in patients with osteoporosis or aging [21–24]. Noteworthy, high BMF was reported in patients exposed to glucocorticoid excess [25, 26] but is it still unknown whether high bone marrow adiposity may predict fracture risk in this clinical setting [27].

The aim of this cross-section study was to evaluate the association between BMF, as assessed by MRS, and morphometric VFs in patients with active endogenous CS.

Materials and methods

Twenty patients (5 M and 15 F, age 44 ± 13 yrs) with active endogenous CS referring to the Endocrine Unit of the University Hospital "AOU Policlinico G. Martino" of Messina were consecutively enrolled from January 2015 to December 2018. Sixteen patients had ACTH-dependent CS whereas the remaining four patients had ACTH-independent. Diagnosis of CS was performed according to the current guidelines [28]. Thirteen patients had newly diagnosed CS, whereas seven were uncontrolled despite multiple treatments.

Fifteen healthy volunteers (4 M and11 F, age 43 ± 12 yrs), matched for sex and age with the patients, acted as control group for the BMF evaluation.

The exclusion criteria for both patients and controls were: (1) age below 18 years; (2) pregnancy or breast-feeding; (3) BMI above 30 Kg/m^2 or below 21 Kg/m^2; (4) chronic therapies with drugs potentially causing osteoporosis [29]; (5) treatment with bone active-drugs in the 12 months prior study, except for calcium and vitamin D (2 females and 1 man received supplementation); (6) chronic diseases potentially causing osteoporosis except for CS.

As primary end-point of the study, we evaluated the association between BMF and VFs in patients with active CS. We also explored the following secondary end-points: (1) the association between BMF and VFs in relationship to different BMD categories in patients with CS; (2) determinants of BMF in patients with CS; (3) the difference in BMF between CS and control subjects.

All the patients and volunteers gave their written informed consent, and the study protocol was approved by the Ethic Committee of Messina (Italy).

Evaluation of BMD and VFs

BMD of the lumbar spine was measured by DXA (Hologic Discovery WI). Fractured vertebrae were excluded from the BMD analysis. DXA results were expressed in BMD (g/cm^2). In patients aged ≥50 years, BMD was also expressed as *T*-score, comparing the results with those obtained in a sex-matched Caucasian population at peak of bone mass [8]. A *T*-score less than or equal to −2.5 SD at the hip or spine was defined as osteoporosis, whereas osteopenia was defined as a *T*-score between −1 and −2.5 SD. In subjects aged <50 years, the results were expressed as *Z*-score, comparing the results with those obtained in an age and sex-matched Caucasian population [8]. A *Z*-score ≤−2.0 SD was used to define a BMD "below the expected range for age".

VFs were assessed by a quantitative morphometric approach [30]. Using a translucent digitiser and a cursor, six points were marked on each vertebral body to describe vertebral shape. Anterior (Ha), middle (Hm), and posterior (Hp) vertebral heights were measured and height ratios (Ha/Hp, Hm/Hp, Hp/Hp of the above vertebrae, Hp/Hp of the below vertebrae) were calculated for each vertebra from T4 to L4; the fractures were defined mild, moderate and severe based on a height ratio decrease of 20–25%, 26–40%, and more than 40%, respectively [30]. The morphometric analysis was performed by a single operator (G.M.). The intra-observer coefficient of variation, evaluated on a series of 10 measurements, was between 4 and 8%.

Measurement of BMF

All participants underwent MRI acquisition with a 1,5-Tesla unit (Ingenia, Philips Healthcare, Best, Netherlands), in a head-first supine position. Before the MRS analysis, sagittal and coronal T2-weighted images of the lumbar spine were acquired to identify the spectroscopic volume of interest.

Single-voxel MR spectroscopy (SV-H-MRS) was acquired from vertebral body of L3 using point-resolved single-voxel proton spectroscopy (PRESS) acquisition to establish the content of lipids and water. PRESS box (voxel size 4.5 mm^3) was positioned in the middle of the vertebral body.

SV-H-MRS was performed using the following parameters: echo time = 37 ms, repetition time = 3000 ms.

Spectral data, after baseline, phase and frequency shift rectifications, were elaborated using SIVIC, an extensible, open-source, freely available, and cross-platform software suite designed to support all aspects of MRS data analysis and visualization [31]. Four peaks were well resolved using the present technique: olefinic, double bond -CH=CH- protons at 5.31 ppm, water protons at 4.65 ppm, the CH_2methylene protons α- to a double bond (-CH=CHCH_2-), at 2.03 ppm, and the bulk CH_2methylene protons at 1.3 ppm. The BMF was expressed in percentage and calculated as previously described [32]: Fat content = Imethylene/(Imethylene + Iwater) × 100%, where Imethylene and Iwater were the signal amplitudes of the bulk methylene peak (1.3 ppm) and the water peak (4.65 ppm), respectively.

Biochemical assays

Peripheral blood samples were collected after overnight fasting. All samples were processed in the laboratory of the University Hospital of Messina. Calcium and phosphorus were measured by standard analytical methods. ACTH (Medical Systems SPA, Genova, Italy), cortisol (Beckman Coulter, USA), 24-hours urinary-free cortisol (Beckman Coulter, USA), DHEAS (Beckman Coulter, USA) were measured by commercial chemiluminescence immunoassays, while PTH and osteocalcin (Cisbio Bioassays, France) by radioimmunoassays. CTX and 25(OH)D3 were measured by HPLC (Bio-Rad Laboratories S.r.l., Milano, Italy).

Statistical analysis

All data were expressed as the median and range. Un-paired data were compared using Mann–Whitney test. Multiple comparisons were performed by Kruskal–Wallis test. Frequencies were compared using χ^2 test with Fisher correction, when appropriate. Spearman correlation test was applied in order to assess the existence of significant association between L3 BMF and several variables evaluated in the study. The receiver operating characteristic (ROC) curve analysis was performed to assess the best cut-off of BMF associated with VFs. Statistical significance was assumed when p-values were ≤0.05.

Results

In CS patients aged ≥50 years (11 cases), osteoporosis was found in three patients (27.3%), whereas osteopenia and normal BMD were found in five (45.5%) and three (27.7%) patients, respectively. In CS patients aged <50 years (9 cases), the prevalence of lumbar spine BMD "below the expected range for age" was 33.3%.

VFs were found in 13 CS patients (65.0%); five patients had a single fracture, whereas in eight patients two or more vertebral fractures were found. The fractures were mild in six patients, while the remaining seven patients had moderate or severe fractures. Patients with VFs were significantly older as compared to patients who did not fracture, without significant differences in other demographical and clinical features (Table 1). Moreover, fractured patients had significantly lower lumbar spine BMD as compared to those without fractures (Table 1). Among patients with VFs, 7 (53.8%) had either osteoporosis or "low BMD form age" and 6 (46.2%) had either osteopenia or normal BMD ($p = 0.05$ vs. non-fractured patients).

Patients with CS had significantly higher BMF as compared to control subjects (52.0%, range: 11–84 vs. 27.0%, range: 10–58; $p < 0.01$). In CS, BMF was significantly associated with patients's age (rho 0.48; $p = 0.03$), 24-hours urine free cortisol values (rho: 0.48; $p = 0.03$), midnight serum cortisol values (rho: 0.50; $p = 0.02$) and serum CTX (rho: 0.54; $p = 0.01$). No significant association was found between BMF and lumbar spine BMD ($p = 0.13$ for both T-score and Z-score). BMF resulted to be significantly ($p = 0.03$) higher in patients with VFs as compared to those who did not fracture (Fig. 1). These latter patients showed no significant difference in BMF vs. controls subjects (Fig. 1).

The ROC analysis showed that BMF have enough accuracy in identifying patients with VFs (area under the ROC curve: 0.797). The cut-off with the best compromise between sensitivity (61.5%) and specificity (71.4%) was set at 52%.

Looking at the individual data and stratifying the patients for BMD categories (Table 2), fractured patients with either normal BMD or osteopenia showed comparable BMF to fractured patients with either osteoporosis or "low BMD for age" (66.0%, range: 38–84 vs 59.0%, range: 27-84; $p = 0.71$). When the analysis was restricted to patients with either normal BMD or osteopenia (Table 2), VFs were still significantly associated with higher BMF (66.0%, range: 38–84 vs. 24.0%, range: 11–66; fractured vs. non-fractured patients, $p = 0.05$).

Discussion

This study showed that patients with active CS and VFs had higher BMF as compared to patients who did not fracture.

In vitro studies have demonstrated that glucocorticoids are essential for the differentiation of mesenchymal cells (usually derived from bone marrow) into mature osteoblasts [33]. However, when glucocorticoids are in excess, osteoblastogenesis is impaired and the differentiation of stromal cells is redirected toward adipogenesis [4]. Mechanisms involved are induction of peroxisome proliferator activated

Table 1 Demographical and clinical features of Cushing's syndrome (CS) patients with vertebral fractures (VFs) as compared to those who did not fracture

Features	CS patients without VFs	CS patients with VFs	*p*-values
N	7	13	
Age (yrs)	44 (29–50)	52 (35–71)	0.04
Sex (F/M)	6/1	9/4	0.61
ACTH-dependent/ACTH-independent (cases)	5/2	9/4	0.48
ACTH (pg/ml)	50.7 (4–120)	96.4 (5–237)	0.24
Midnight serum cortisol (μg/dL)	7.2 (4.1–17.6)	14.4 (5.2–33.3)	0.09
Urinary-free cortisol (μg/24 h)	450.0 (319.0–970.0)	628.0 (132.0–5680.0)	0.11
1-mg DEX serum cortisol[a] (μg/dl)	11.8 (3.7–19.7)	10.8 (1.8-41.0)	0.84
Serum DHEA-S (mcg/dl)	160.8 (32.0–520.0)	83.2 (4.8–663.8)	0.33
Serum calcium (mg/dL)	9.5 (8.8–9.8)	9.3 (8.3–10.1)	0.48
Serum phosphorus (mg/dL)	3.2 (2.9–3.9)	3.2 (2.5–4.3)	0.82
Serum PTH (pg/mL)	54.8 (20.1–70.1)	43.6 (31.0–150.0)	0.96
Serum 25OHvitamin D (ng/mL)	18.0 (15.2–49.4)	24.4 (11.6–43.30)	0.21
Serum osteocalcin (ng/ml)	9.9 (4.7–12.1)	26 (1.2–69.9)	0.24
Serum CTX (μg/L)	0.30 (0.14–0.67)	0.60 (0.21–1.62)	0.16
Lumbar spine BMD *T*-score (SD)	−1.0 (from−2.5 to +0.6)	–2.5 (from −3.5 to −0.6)	0.006
Lumbar spine BMD *Z*-score (SD)	−0.5 (from −1.5 to +0.8)	−1.6 (from −2.8 to −0.30)	0.008

Continuous data were presented as median and ranges and the comparisons were performed using non-parametric tests

ACTH adenocorticotropic hormone, *BMD* bone mineral density, *CS* Cushing syndrome, *CTX* C-telopeptides of type-1 collagen, *DHEA-S* dehydroepiandrosterone sulfate, *F* female, *M* male, *PTH* parathyroid hormone, *SD* standard deviation, *VFs* vertebral fractures

[a]morning serum cortisol following 1 mg of dexamethasone overnight

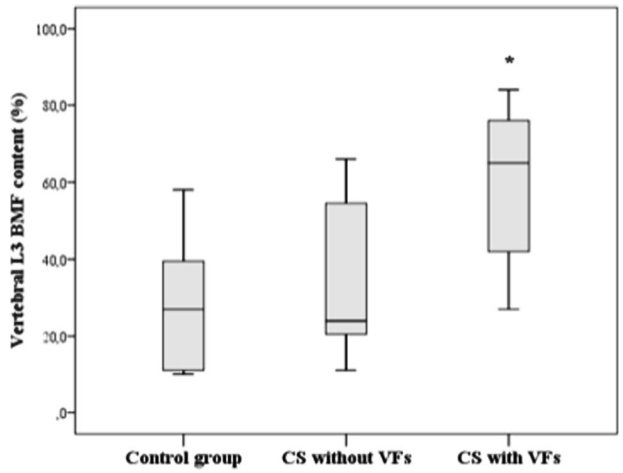

Fig. 1 Bone marrow fat (BMF) content in vertebra L3 of Cushing's syndrome (CS) patients with or without vertebral fractures (VFs) as compared to control subjects. Data were presented as median, 25th and 75th percentile and range. Data were compared by non-parametric tests. *$p < 0.05$ CS with VFs vs. CS without VFs and control subjects. The difference between CS without VFs and controls was not statistically significant

receptor γ2, the regulation of nuclear factors of the CAAT enhancer-binding protein family [34, 35], inhibition of Wnt/beta-catenin signaling and repression of BMP-2 [36, 37]. A recent study reported high bone marrow adiposity in patients with active hypercortisolism [26]. This finding was confirmed by our study which also reported a significant association between BMF and the severity of hypercortisolism, consistently with the concept that glucocorticoid excess was directly responsible for the increased number of adipocytes in the bone microenvironment. Noteworthy, the increase in BMF in our patients was demonstrated using a less sensitive but more widespread MR machine than those used in the previous study [26], suggesting that the spectroscopy analysis of bone marrow adiposity may be a feasible diagnostic tool in the real-life clinical practice.

Applying a morphometric approach, more than 60% of patients were found to have VFs, seven of them being either severe or multiple. This finding is remarkable if considering the potential negative effects of VFs on quality of life of patients with CS [7], such as already demonstrated in the general population [38]. Although the small size of study group did not allow to reach the statistical significance, we found a clinically significant difference in midnight serum cortisol values between fractured and non-fractured patients, consistent with the concept that skeletal fragility and VFs were a direct consequence of hypercortisolism. For the first time, we reported an association between VFs and higher BMF at lumbar spine, providing a first evidence that bone marrow adiposity may be a marker of skeletal fragility in patients with CS. This hypothesis was also supported by

Table 2 Individual data of patients with Cushing's syndrome stratified for BMD categories, according to WHO criteria (8)

Sex	Age (yrs.)	BMD *T*-score (SD)	BMD *Z*-score (SD)	BMF (%)	VFs
Patients with either normal BMD or osteopenia					
F	71	−1.2	−1.1	79	Yes
M	65	−1.3	−0.3	38	Yes
F	59	−1.6	−0.6	66	Yes
F	54	−1.8	−0.8	49	Yes
F	54	−1.6	−0.6	68	Yes
M	50	−2.4	−1.9	84	Yes
F	50	+0.6	+0.8	51	No
F	50	−1.0	−0.4	11	No
M	45	−0.8	−0.6	66	No
F	44	−1.0	−0.4	24	No
F	43	−2.5	−1.0	58	No
M	35	−2.5	−1.4	42	Yes
F	29	−0.5	−0.5	19	No
F	29	−1.5	−1.5	22	No
Patients with either osteoporosis or "low BMD for age"					
F	69	−2.8	−2.3	27	Yes
M	52	−3.5	−2.6	84	Yes
F	50	−2.7	−1.6	76	Yes
F	47	−3.1	−2.8	65	Yes
F	40	−2.6	−2.1	53	Yes
F	38	−3.0	−2.8	41	Yes

BMD bone mineral density, *BMF* bone marrow fat, *F* female, *M* male, *SD* standard deviation, *VFs* vertebral fractures, *yrs* years

the finding of association between BMF and CTX, a reliable marker of bone resorption [39, 40], suggesting that bone marrow adiposity may influence not only osteoblastogenesis but also osteoclastogenesis and bone resorption [41].

Lumbar spine BMD was shown to be one of the predictors of fractures in untreated CS [7]. Consistently with this finding, overall our fractured patients had lower lumbar spine BMD as compared to patients who did not fracture. However, looking at the individual data about one-half of our patients with VFs had low-normal or even normal BMD values. Similarly to other forms of secondary osteoporosis [42–44], the prediction of VFs in CS is a clinical challenge [45]. As a matter of fact, DXA analysis of BMD does not allow to capture the abnormalities of bone quality induced by glucocorticoid excess. In fact, trabecular [12, 18, 46] and cortical [16] bone microstructure was shown to be deteriorated more than BMD in patients with endogenous hypercortisolism. Noteworthy, abnormalities in trabecular bone structure as measured by trabecular bone score (TBS), that is a gray-level textural metric extracted from the two-dimensional lumbar spine DXA images [47], were shown to be associated with higher risk of fractures in patients with endogenous hypercortisolism regardless of BMD values [18, 48]. Consistently with this finding, in our patients with active disease BMF was not associated with BMD and fractured patients showed higher BMF even in presence of normal and low-normal BMD.

The present study has some limitations. Firstly, the cross-sectional design did not allow to investigate the timing of VFs development and the temporal relationship between the increase in BMF and the occurrence of fractures. Since information on body composition were not available, we cannot completely rule out that BMF values in our patients may be influenced by the increase in visceral fat induced by glucocorticoid excess [49, 50]. However, the exclusion of obese patients from the study may have weakened the potential effect of visceral fat on bone marrow adiposity. Moreover, the contribution of menopause to BMF in CS women with fractures cannot be quantified although it's worth of noting that 4 out of 5 post-menopausal women had osteopenia. An uncoupled low-bone formation and slightly increase in bone resorption was reported in patients with CS [46, 51], but the impact of this biochemical phenotype on fracture risk is unknown. In our study, no statistically significant differences in osteocalcin and CTX between fractured and non-fractured patients were found. However, unexpectedly, median osteocalcin was shown to be more than twice higher in patients with VFs as compared to those who did not fracture. This finding may reflect the heterogeneity of study group in terms of age, gonadal status and etiology of CS which did not allow to properly analyze the diagnostic value of biochemical markers of bone turnover in identifying patients with skeletal fragility. Indeed, the use of bone markers in clinical practice is still complicated mainly due to analytical variability of these substances [52]. Furthermore, the small number of patients did not allow to perform a multivariate analysis to reliably assess the effect of BMF on risk of fractures independent of BMD, age, and severity of hypercortisolism which were shown to be associated with VFs in the univariate analysis. We partially resolved this methodological shortcoming by analyzing the patients stratified for BMD values. Such an approach allowed us to hypothesize that MRS measurement of BMF may be more informative than DXA measurement of BMD in identifying patients with skeletal fragility induced by glucocorticoid excess. Specifically, based on the results of this study, MRS measurement of BMF may be useful in CS patients with either osteopenia or normal BMD in order to identify subjects with otherwise undiagnosed by DXA skeletal fragility who are at high risk of fractures. Moreover, in these cases, MRI images may be also used for morphometric diagnosis of VFs [53].

In conclusion, this preliminary study provides first evidence that vertebral adiposity is a marker of hypercortisolism-induced skeletal fragility. Future

prospective studies on larger CS populations are needed to assess the accuracy of MRS in the diagnostic work-up of skeletal fragility induced by glucocorticoid excess.

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Compliance with ethical standards

Conflict of interest G.M. received consultant fees by Ipsen and Novartis; A.L. received grants from Ipsen, Pfizer, Novartis and speaker honoraria from Ipsen and Pfizer; S.C. served in medical advisory boards of HRA. The remaining authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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