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ORIGINAL ARTICLE

Reduced bone volumetric density and weak correlation between infection and bone markers in cystic fibrosis adult patients

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Abstract
Summary In our current adult CF population, low BMD prevalence was only 20 %, lower than that historically described. We found a mild increase of serum RANK-L levels, independent from the bone resorption level. The increased fracture risk in CF may be explained by a lower tibial cortical thickness and total vBMD.
Introduction Bone disease is now well described in cystic fibrosis (CF) adult patients. CF bone disease is multifactorial but many studies suggested the crucial role of inflammation. The objectives of this study were, in a current adult CF population, to assess the prevalence of bone disease, to examine its relationship with infections and inflammation, and to characterize the bone microarchitecture using high resolution peripheral scanner (HR-pQCT).
Methods Fifty-six patients (52 % men, 26 ± 7 years) were assessed in clinically stable period, during a respiratory infection, and finally 14 days after the end of antibiotic therapy. At each time points, we performed a clinical evaluation, lung function tests, and biochemical tests. Absorptiometry and dorso-lumbar radiographs were also performed. A subgroup of 40 CF patients (63 % men, 29 ± 6 years) underwent bone microarchitecture assessment and was age- and gender-matched with 80 healthy controls.
Results Among the 56 CF patients, the prevalence of low areal BMD (T-score < −2 at any site), was 20 % (95 % CI: [10.2 %; 32.4 %]). After infections, serum RANK-L (+24 %, $p = 0.08$) and OPG (+13 %, $p = 0.04$) were increased with a stable ratio. Microarchitectural differences were mostly observed at the distal tibia, with lower total and cortical vBMD and trabecular thickness (respectively −9.9, −3.0, and −5 %, $p < 0.05$) in CF patients compared to controls, after adjustment for age, gender, weight, and height.
Conclusions In this study, bone disease among adult CF patients was less severe than that previously described with only 20 % of CF patients with low BMD. We found a mild increase of biological marker levels and an impaired volumetric density of the tibia that may explain the increased fracture risk in CF population.

Keywords Bone microarchitecture · Bone turnover · Cystic fibrosis · Fracture · Osteoporosis · RANK-L

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Introduction

Cystic fibrosis is a multisystem disease related with the Cystic Fibrosis transmembrane regulator gene (CFTR) dysfunction. With improvements in the treatment of pulmonary and gastrointestinal disorders of CF disease, the average life expectancy

of these patients has increased significantly over the past several decades, from ~16 years in the mid1970s to ~37 years currently as reported by the Cystic Foundation [1], which has led to the emergence of new complications such as bone disease, first reported in 1979 [2] and now well recognized [3–6]. This bone disease is associated with reduced bone mineral density (BMD), bone deformities including kyphosis and increased risk of fractures [6, 7]. In a recent meta-analysis [8], the authors reported a prevalence of osteoporosis and osteopenia in young adults with CF of 23.5 and 38 % respectively; moreover, they reported an increased fracture rate, particularly vertebral and rib fractures that may lead to deterioration of lung function. Bone disease can lead to severe impairment in quality of life of patients with CF and even might be an exclusion criterion for lung transplantation.

The mechanism of bone loss in CF is probably multifactorial including malnutrition and low BMI, vitamin D insufficiency, hypogonadism, glucocorticoid therapy, lack of exercise, and the possible effect of CFTR dysfunction in bone cells that expressed CFTR [9]. However, a strong association between increased bone resorption and systemic inflammation induced by chronic pulmonary infection and acute bronchial exacerbations is supported by various studies [10–13], suggesting that aggressive treatment of lung infection may prevent the progression of bone disease.

While dual-energy X-ray absorptiometry (DXA) is the gold standard method for measuring bone mineral content, this method has important limitations, as it is partly dependent on bone size, which is diminished in the CF population. High resolution peripheral quantitative computed tomography (HR-pQCT) may be a very useful tool to overcome this difficulty and also to characterizing bone microarchitecture [14] .

The aims of our study were (1) to assess the prevalence of CF related low BMD in a modern adult cystic fibrosis population, (2) to examine the relation between bone disease and systemic inflammation measured during bronchial exacerbations, and (3) to characterize the bone microarchitecture assessed with HR-pQCT in a subset of CF patients compared to controls.

Subjects and methods

Subjects

Patients were recruited in the Lyon Adult Cystic Fibrosis Centre. Diagnosis has been established by either sweat test and/or genetic testing, together with appropriate clinical signs and symptoms.

We excluded patients if they were under 18 years, posttransplanted or received oral glucocorticoids in the last 3 months before inclusion or during the studied exacerbation period. Osteoporosis medications were not exclusion criteria.

Moreover, each CF subject participating in the microarchitecture substudy was age- and gender-matched with two healthy volunteers participating in the epidemiological studies STRAMBO in men and OFELY in women [15, 16]. All CF patients and healthy controls were Caucasians.

Study protocol

The study was conducted from March 2008 to November 2011. It has been approved by the local ethical committee and informed written consent was obtained from all patients.

The CF related low BMD was assessed by DXA measurements, history of fracture, and dorso-lumbar radiographs.

To examine the relation between bone disease and systemic inflammation measured during bronchial exacerbations, patients were measured at three different clinical occasions: clinically stable period (inclusion: T0), during an exacerbation period (T1) defined by a senior clinician according to the definition of Rabin et al. [17] and requiring oral or intravenous antibiotics for 14 days, and finally 14 days after the end of antibiotic therapy (T2 , i.e. 28 days after the beginning of exacerbation). The exacerbation period (T1) happened on average 202 ± 172 days after inclusion.

At each time points, we performed a clinical evaluation including measurement of weight and body mass index (BMI), lung function tests (forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) expressed as percentage of predicted value) and biochemical tests: markers of inflammation (CRP, IL-6, TNFα), serum markers of bone turnover (serum CTX, osteocalcin), serum RANK-L, osteoprotegerin (OPG), and leptin, on blood samples mostly obtained in the morning in non-fasting condition.

All patients received routine calcium and vitamin D supplementation.

In addition, measurement of bone microarchitecture by HR-pQCT was proposed to a subset of patients and performed on average 2 years after the inclusion. A measurement of areal BMD by DXA was simultaneously performed at the radius.

BMD measurements by DXA

Lumbar spine and total hip and femoral neck BMD was measured by DXA using a GE Lunar Prodigy machine (GE-Lunar, Madison, WI, USA) within 6 months of inclusion.

Radius BMD was measured by DXA using a Hologic Discovery A machine (Hologic, Bedford, MA, USA).

According to the European guidelines on bone mineralization in cystic fibrosis [18], in young adult patients, BMD values should be expressed as Z-score or T-scores at the lumbar spine and proximal hip. CF-related low BMD was defined by a Z- or T-score below −2 and osteoporosis as a Z- or T-score below −2 and a significant fracture history.

Fracture assessment

Peripheral fractures were self-reported and vertebral fractures were ascertained on lateral X-ray films of the lumbar and thoracic spine, obtained within 6 months of inclusion.

All vertebral fractures were identified using the qualitative then the semiquantitative method of Genant [19] by two different trained rheumatologists (DG, RC).

Inflammation and bone markers

For all patients, peripheral blood samples were collected at the three previously defined periods: clinically stable (T0), during an exacerbation period (T1), and 28 days after the beginning of exacerbation (T2).

TNFα and IL-6 levels were assessed by ELISA kits from R&D systems.

CTXs were assessed by ECLIA (Elecsys automate analyzer, Roche Diagnostics). Osteocalcin was measured using TRACE technic with Kryptor automat (BRAHMS). RANK-L and OPG levels were respectively performed using Peprotech kits (900-K141) and Costar EIA plates from R&D systems. Leptin was assessed by "Human Leptin ELISA" kits from TECOmedical Group (Sissach, Switzerland).

Microarchitecture assessment

Volumetric BMD and microarchitecture were measured at the distal radius and tibia using an HR-pQCT device (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland). A stack of 110 parallel CT slices with an isotropic voxel size of 82 μm was acquired 9.5 and 22.5 mm from a reference line manually placed at the endplate of the radius and tibia respectively, as previously described [20].

A semiautomatic segmentation script was used to differentiate cortical bone from trabecular bone [21, 22]. The outcome variables used in our analyses included volumetric BMD (mg HA/cm^3) for total (Tt.BMD), trabecular (Tb.BMD), and cortical (Ct.BMD) regions; cortical tissue mineral density (Ct.TMD, mg HA/cm^3); geometry of bone: total, trabecular and cortical area (Tt.Ar, Tb.Ar, and Ct.Ar, mm^2), cortical thickness (Ct.Th, μm) and porosity (Ct.Po, %); trabecular microstructure: number (Tb.N, mm^{-1}), thickness (Tb.Th, μm), separation (Tb.Sp, μm), and intraindividual distribution of separation (Tb.Sp.SD, μm) in the refined compartments. Non-metric trabecular indices were also calculated by the manufacturer's software: the structural model index (SMI, no unit) related to the rod- or plate-like topology of the trabecular network and the connectivity density (Conn.D, no unit). Moreover, biomechanical parameters were estimated from μFEA performed on segmented HR-pQCT images using software delivered with the HR-pQCT device (IPL software v1.13, Scanco Medical AG). Bone voxels in the segmented images were further converted into equally sized brick elements and material properties were chosen to be isotropic and linear elastic, with a Young's modulus of 20 and 17 GPa for cortical and trabecular bone elements, respectively, with a Poisson's ratio of 0.3 for all elements. Applied boundary conditions represented compression tests in the axial direction [22]. μFEA-derived variables used in our study included the following: stiffness (kN/mm) and the percentage of load carried by the trabecular bone at the distal and proximal surface of the volume of interest (Tb.Dist.Load and Tb.Prox.Load, in %).

HR-pQCT measurements were performed at the nondominant limb unless there was a history of fracture, in which case, the non-fractured limb was measured.

Statistical analysis

The demographic, clinical, and biological characteristics of the patients at baseline and the biological markers in each period (stable, exacerbation, and postexacerbation) were described using absolute and relative frequencies in each modality for categorical variables and using mean and standard deviation for quantitative variables. The coefficient of Spearman adjusted on age and gender was used to quantify the correlation between these characteristics. The evolution of the serum markers (CRP, IL-6, CTX, RANK-L, OPG, TNFα, leptin, and osteocalcin) across periods (stable, exacerbation, and postexacerbation) was estimated using linear mixed models, adjusted for gender, age, BMI, ratio of the observed FEV_1 on the theoretical FEV_1 at baseline, and type of mutation. Continuous predictors were centered on their mean. CRP, IL-6, RANK-L, OPG, and osteocalcin were log-transformed to normalize their distribution. A structural equation model was used to estimate the correlations between the activity of bone formation, the activity of bone resorption, the inflammatory activity and the nutritional state. In the measurement model, the different activities and the nutritional state were considered as latent variables measured by several observed indicators at each period, i.e., osteocalcin for the activity of bone formation, RANK-L and osteoprotegerin for the activity of bone resorption, Il-6, TNFα, and CRP for the inflammatory activity, leptin, and BMI for the nutritional state. The correlations between the latent variables were estimated with their 95 % confidence interval (95 % CI).

Bone microarchitecture parameters were described by their mean and standard deviation in the groups of CF patients and controls. Differences between the two groups were expressed in percentage of the mean value of the control group. Multiple linear models with robust estimation were used to compare parameters between the two groups with adjustment on age, gender, height, and weight.

To take into account the multiplicity of comparisons, we also used the hierarchical false discovery rate (FDR) [23]. The

first level of comparison included seven parameters tested simultaneously: total vBMD at the radius and tibia; stiffness at the radius and tibia; and areal BMD, BMC, and area measured by DXA at the ultradistal radius. At the second level, cortical and trabecular vBMD, total area, cortical thickness, distal and proximal trabecular load at the radius, and tibia were tested only if the site-specific difference of total vBMD or of stiffness was statistically significant. At the third level, parameters of trabecular structure (Tb.N, Tb.Sp.SD, SMI and conn.D) were tested only if the site-specific trabecular vBMD was statistically significant; parameters of cortical structure (Ct.Po and Ct.TMD) were tested only if the site-specific cortical vBMD was statistically significant.

The statistical analyses were performed principally with the Stata statistical software, version 12. The structural equation model was performed with the LISREL 8.80 software. The comparisons using the hierarchical FDR were carried out with the SAS software, version 9.3. All tests were two-tailed and p-value < 0.05 was considered for statistical significance.

Results

Patients' characteristics

Fifty-six patients were enrolled in the study (29 men, 52 %), with a mean age of 26 ± 7 years and BMI of 20.2 ± 1.5 kg/m^2 (Table 1). Twenty-four patients (43 %) were homozygous and 25 (45 %) were heterozygous for the F508del mutation. Fifty-four patients (96 %) had pancreatic insufficiency treated with enzyme replacement and 14 (25 %) were treated for diabetes mellitus. Concerning lung function, FEV1 and FVC were respectively 64 ± 21 and 85 ± 17 %. Forty-three patients (77 %) were chronically colonized by *Pseudomonas aeruginosa*. Occasional brief courses of inhaled glucocorticoids were used by 33 (59 %) patients. Despite routine supplementation, vitamin D status was optimal (>75 nmol/l) in 13 (23 %) CF patients. Vitamin D insufficiency (between 25–75 nmol/l) was found in 31 (64 %) and vitamin D deficiency (<25 nmol/l) in 7 (13 %) patients. Two patients (3.6 %) were treated with bisphosphonates (one with alendronate, one with risedronate).

CF related low BMD

Hip and spine BMD were positively correlated with BMI, with a Spearman coefficient of correlation adjusted on age and gender of 0.33 ($p = 0.02$) and 0.28 ($p = 0.045$), respectively. We did not find significant correlation between BMD and respiratory function tests, biological markers (such as vitamin D values), or inflammatory biomarkers.

Eleven patients (20 %; 95 % CI: [10.2 %; 32.4 %]) had a CF-related low BMD, defined by a T-score <−2 at any site, and 19 patients (34 %) had a T-score between −1 and −2 SDs.

Among the women, 2 (7 %) had a T-score <−2, 10 (37 %) had a T-score between −1 and −2, and 15 (56 %) had a normal T-score. Among the men, 9 (31 %) had a T-score <−2, 9 (31 %) had a T-score between −1 and −2, and 11 (38 %) had a normal T-score. The percentage of low BMD was significantly higher in men than in women ($p = 0.04$).

CF-related low BMD was observed in 21 % (5/24) of the homozygous patients for the F508del mutation, in 20 % (5/25) of the heterozygous patients, and in 14 % (1/7) of patients without F508del mutation. The difference between the three groups of mutation was not statistically significant ($p = 0.93$).

Seven patients (13 %, three women, four men) had a history of fracture: two had a peripheral fracture, four had a vertebral fracture, and one had both peripheral and vertebral fracture. All vertebral fractures were grade I according to Genant's classification.

Among fractured patients, none had a T-score <−2 and 3 had a T-score <−1 and no clinical factor was found to discriminate fractured from non fractured CF patients.

Biological markers

- Serum markers of inflammation

  The average CRP level was higher than the normal range during the stable period (11 ± 15 mg/l, normal range <3 mg/l) It was significantly increased during the exacerbation period by 95 % (95 % CI: [42 %; 166 %], $p < 0.001$). Then, it decreased to a level comparable to baseline (14 ± 17 mg/l) (Table 2, Fig. 1).

  Il-6 level followed the same pattern of variation (Table 2, Fig. 1) with an average increase of 56 % (95 % CI: [22.4 %; 98.2 %], $p < 0.0001$) during the exacerbation period in comparison with the stable period.

  For TNFα, a significant increase was observed during the postexacerbation period in comparison with the stable period (20 %, 95 % CI: [2.3 %; 40 %], $p = 0.02$).
- Serum markers of bone turnover

  Average levels of serum CTX and osteocalcin were within the normal range at baseline (369 ± 223 pg/ml and 26.5 ± 14.5 μg/l, normal range 140–440 pg/ml and 10–52 μg/l, respectively) and remained stable during exacerbation period (Table 2, Fig. 2).
- Serum cytokine levels

  At T2, i.e., 28 days after the beginning of the exacerbation, serum RANK-L and OPG were increased by 24 % (95 % CI: [−2 %; 57 %], $p = 0.08$) and 13 % (95 % CI: [0.8 %; 27 %], $p = 0.04$) respectively, with a stable ratio (ratio before exacerbation = 0.19, ratio after exacerbation = 0.22) (Table 2, Fig. 3). This increase, statistically significant only for OPG, was delayed in comparison to the increase of inflammation markers.
- Leptin level remained stable during exacerbation period.

Table 1 Demographic, clinical, and biological characteristics of the CF patients at baseline.

Characteristics	Mean ± SD or frequencies
Age (years)	26.4 ± 6.6
Sex (*n*, (%))	
Male	29 (52 %)
Female	27 (48 %)
Weight (kg)	56.5 ± 7.3
Body Mass Index (kg m^{-2})	20.2 ± 1.5
Mutation status (*n*, (%))	
ΔF508/ΔF508	24 (43 %)
ΔF508/other	25 (45 %)
Other/other	7 (13 %)
Forced expiratory volume-1-s ratio (%)	63.5 ± 21.3
Forced vital capacity ratio (%)	85.3 ± 16.6
Pancreatic insufficiency	54 (96 %)
Diabetes mellitus	14 (25 %)
Presence of *Pseudomonas* colonies (*n*, (%))	43 (77 %)
Inhaled corticosteroids therapy (*n*, (%))	33 (59 %)
Bone status (*n*, (%))	
Normal	26 (46 %)
−1 < T-Score ≤ −2	19 (34 %)
CF-related low BMD (T-Score < −2)	11 (20 %)
25-OH vitamin D (nmol/l)	56.4 ± 28.8
>75 nmol/l (n, (%))	13 (23 %)
25– 75 nmol/l (n, (%))	31 (64 %)
<25 nmol/l (n, (%))	7 (13 %)

– Correlation between bone turnover, inflammatory activity, and nutrition state

Bone resorption was estimated from structural equation model by two markers (RANK-L and OPG), to approach the level of resorption induced by inflammation and compensated by the decoy receptor OPG. Bone resorption was negatively correlated with bone formation measured by osteocalcin, but this correlation did not reach statistical significance (−0.26, 95 % CI: [−0.69; 0.18]).

Overall, there was no significant correlation between bone turnover and inflammation or nutrition state.

Inflammatory activity estimated by Il-6, TNFα, and CRP and nutrition state (leptin, BMI) were negatively correlated (−0.51, 95 % CI: [−0.96, −0.06]).

Peripheral bone microarchitecture and density: HR-pQCT and ultradistal radius BMD by DXA

Peripheral bone microarchitecture and density were measured on a subgroup of 40 CF patients (63 % men, 29 ± 6 years) and compared with 80 age- and gender-matched healthy controls (one CF/two controls).

Ultradistal radius BMD measured by DXA was similar in CF patients and in controls (0.46 ± 0.08 and 0.46 ± 0.07 g cm^{-2}, respectively). However, BMC and area were significantly lower in CF patients than in controls (BMC 1.6 ± 0.3 vs. 1.9 ± 0.4, area 3.5 ± 0.4 vs. 4.0 ± 0.4, all $p < 0.01$) after adjustment for covariates and taking into account multiple comparisons (hierarchical FDR).

After adjustment for age, gender, weight, and height, density and microstructural differences were mostly observed at the distal tibia. We observed statistically significantly lower total and cortical tibial vBMD (respectively −10 and −3 %), trabecular thickness (−5 %) in CF compared to controls (Table 3). Moreover, cortical porosity tended to be higher in CF patients compared to controls (36 %, $p = 0.07$). At the radius, stiffness, trabecular distal load and trabecular thickness

Table 2 Biological markers evolution at three time points (stable period, exacerbation period, and postexacerbation period)

Markers	Period		
	Stable ($54 \le n \le 56$)	Exacerbation ($48 \le n \le 50$)	Postexacerbation ($44 \le n \le 46$)
RANK-L (pg/ml)	125 ± 143	151 ± 198	161 ± 201
OPG (pg/ml)	770 ± 528	762 ± 444	869 ± 733[b]
Ratio RANK-L/OPG	0.19 ± 0.23	0.21 ± 0.28	0.22 ± 0.28
Osteocalcin (μg/l)	26.5 ± 14.5	23.9 ± 12.1	26.9 ± 17.9
CTX (pg/ml)	369 ± 223	356 ± 199	361 ± 200
CRP (mg/l)	10.9 ± 14.8	25.3 ± 30.0[a]	14.3 ± 16.6
Leptin (ng/ml)	4.4 ± 4.5	3.8 ± 4.1	3.9 ± 4.7
IL-6 (pg/ml)	8.0 ± 13.5	12.6 ± 15.8[a]	7.5 ± 9.2
TNFα (pg/ml)	1.1 ± 0.7	1.4 ± 1.9	2.2 ± 4.6[b]

[a] $p < 0.05$ exacerbation compared to stable period

[b] $p < 0.05$ post-exacerbation compared to stable period

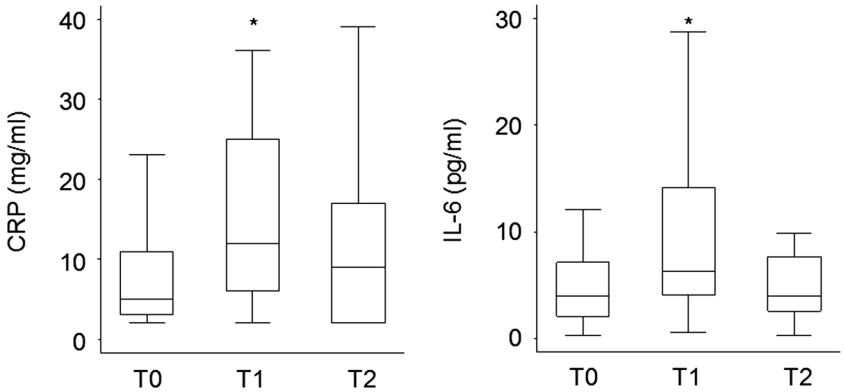

Fig. 1 Variations in markers of inflammation (CRP and IL-6) during exacerbation. T0 = clinically stable period (inclusion), T1 = exacerbation period (defined by a senior clinician according to Rabin and requiring oral or intravenous antibiotics during 14 days), T2 = 14 days after the end of antibiotic therapy (i.e., 28 days after the beginning of exacerbation). CRP and IL-6 are significantly increased (*$p < 0.001$) at T1 (exacerbation period) with a decrease by T2 to a level comparable to baseline

(respectively −13, −11, and −4 %) were lower and SMI was higher (17 %) in CF compared to controls.

After taking into account multiple comparisons (hierarchical FDR), the total tibial vBMD remained significantly lower in CF patients compared to controls ($p = 0.04$), and stiffness at the radius tended to be lower in CF patients ($p = 0.06$).

Among these 40 CF patients, 5 had a history of fracture, but their bone microarchitecture did not differ from non fractured CF patients.

Discussion

In our study, we found a CF-related low bone mineral density, defined by a T-score <−2 (lumbar spine, total hip, or femoral neck), in 20 % of our patients. Moreover, 39 % of our patients had a T-score between −1 and −2. CF patients had lower total volumetric density, assessed at the distal tibia by HR-pQCT, than healthy controls did. During the exacerbation period, serum markers of bone turnover (CTX and osteocalcin) remained stable. Serum RANK-L and OPG were increased after the exacerbation period, but this increase, statistically significant only for OPG, was delayed in comparison to the increase of inflammation markers. Moreover, the ratio of RANK-L/OPG remained stable.

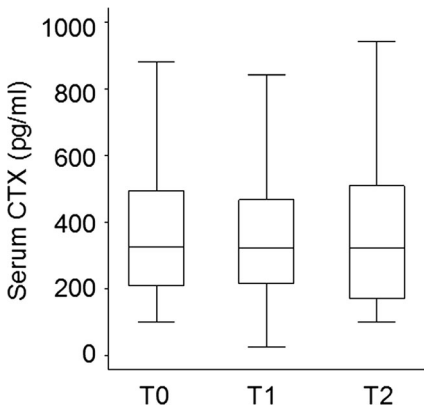

Fig. 2 Variations in bone turnover markers (CTX) during exacerbation. *T0* clinically stable period (inclusion), *T1* exacerbation period (defined by a senior clinician according to Rabin and requiring oral or intravenous antibiotics during 14 days), *T2* 14 days after the end of antibiotic therapy (i.e., 28 days after the beginning of exacerbation). Serum CTX remain stable during exacerbation

The prevalence of CF-related low BMD found in our modern adult CF population (20 %) is similar to the result of a recent Brazilian cross-sectional study in 58 adolescents and CF adults [24] where the prevalence of bone mass below the expected range (Z score <−2) was 20.7 %. According to the WHO criteria, we found a prevalence of osteopenia (T-score between −1 and −2.5) of 45 %, in-between the 38 % estimated in the meta-analysis of Paccou et al. [8], the 45 % in the recent study of Sheikh et al. [25], and the 58 % reported in another recent study by Legroux-Gérot et al. [26]. The prevalence of osteoporosis (T-score ≤ −2.5) observed in our population is as follows: 14 % was close to the prevalence reported by Sheikh et al. (14 %) but lower than that reported in the two other studies (23.5 % and 20 %). However, higher heterogeneity across patients and studies has been observed in older reports, with a prevalence of osteoporosis ranging from 9 % [7] to 59 % [10]. We suppose that many factors have improved prevention of CF bone disease such as better nutritional status, better supervision of vitamin D levels, and better control of bronchial infection. This improvement of clinical conditions in adult CF patients is supported by the collected data in French national CF registry (Registre National de la Mucoviscidose) and their evolution between 2001 and 2008 [27], the year of the beginning of the present study. Nevertheless, in a recent study by Putman et al., the prevalence of CF-related low BMD based on spine areal BMD was not different in contemporary patients with CF (9.4 %) than in patients from the late 1990s (15.6 %) [28]. However, their very low prevalence may have been underestimated as it relied only on the spine and radius; hip measurements were not available.

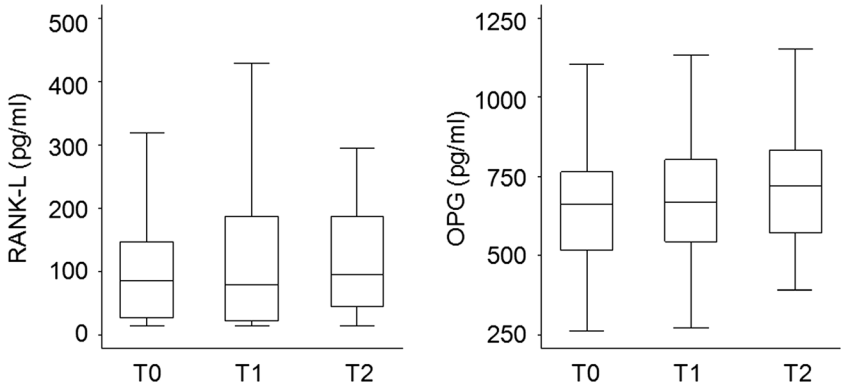

Fig. 3 Variations in serum cytokine levels (RANK-L and OPG) during exacerbation. *T0* clinically stable period (inclusion), *T1* exacerbation period (defined by a senior clinician according to Rabin and requiring oral or intravenous antibiotics during 14 days), *T2* 14 days after the end of antibiotic therapy (i.e., 28 days after the beginning of exacerbation). After infection treated with antibiotics, serum RANK-L and OPG were increased (+24 %, $p = 0.08$ and +13 %, $p = 0.04$, respectively), with a stable ratio

The interpretation of our data may also be hindered by DXA limitations and characterization of CF-related bone fragility may benefit from bone geometry, microarchitecture, and volumetric density measurements. While we found a lower ultradistal radius area by DXA (−13.9 %) in CF patients compared to controls, the difference in radius cross-sectional area assessed by HR-pQCT (−9 %) did not reach statistical significance. There was also no difference in tibia cross-sectional area. We found that volumetric density and microstructural differences were mostly observed at the load-bearing distal tibia. CF patients had reduced total vBMD as well as impaired cortical bone: reduced cortical vBMD partly explained by the 36 % higher porosity in the CF group, after adjustment for covariates. When multiple comparisons were taken into account, total vBMD remained significantly lower in CF patients compared to controls. The lower total vBMD observed in our study was consistent with results obtained by Putman et al. [14], although the impairment in their cohort was more trabecular in nature while cortical bone was mostly impaired in our CF patients. Moreover, their cohort of 30 CF patients seemed more profoundly affected as they also observed a simultaneous decline at the distal radius.

Many studies have already shown that CF bone disease is the result of decreased bone formation and increased osteoclastic bone resorption [29–31]. Recent studies in murine models and in cultured human osteoblasts suggested that CFTR dysfunction affects the balance of resorption and formation. Stalvey et al. studied a CF bone disease mouse model in which CFTR expression reduced osteoblast differentiation and enhanced osteoclastic resorption [32, 33]. Le Henaff et al. showed a reduced bone mass in F508del mice due to decreased osteoblast activity and lower bone formation rate compared with WT mice [34].

Bone turnover can be assessed by several biochemical markers and, among them, the complex of cytokines RANKL and osteoprotegerin (OPG). RANKL is expressed by osteogenic cells including osteoblasts and plays a key role in osteoclastogenesis. By binding to its receptor on osteoclast progenitor membranes, RANKL promotes osteoclast differentiation and leads to increased bone resorption [35]. Osteoprotegerin is a soluble decoy receptor that binds to RANKL and inhibits its resorptive effect. Thus, the relative concentration of RANKL and OPG in bone is a major determinant of bone resorption in several pathological states, especially in inflammatory diseases. In adult CF patients, Shead et al. reported lower serum OPG and similar RANKL levels compared to controls [13]. More recently, Ambroszkiewicz et al. showed lower serum OPG and nearly twofold higher RANKL levels in CF children aged 5–9 years compared to healthy controls [36], suggesting an increased bone resorption and a low bone formation rate.

In our study, we did not compare serum OPG and RANK-L levels in CF patients and controls but we assessed their concentration change in CF patients during three different clinical occasions (stable period, exacerbation period, and 28 days after the beginning of exacerbation). We found a mild increase of serum RANK-L and OPG levels, significant only for OPG, with a stable ratio, which was delayed compared to the pulmonary infections, i.e., observed 28 days after the beginning of exacerbation. These findings are in line with the study of Shead et al. who measured serum inflammatory cytokines and bone turnover markers at day 1 (start of exacerbation), day 14 (end of intravenous antibiotic therapy), and day 42 (follow-up) in 24 CF adult patients [13]. They observed an increase in serum levels of both cytokines at day 14, only statistically significant for OPG, and returning to baseline at day 42. From these two studies, we can speculate that serum OPG and RANK-L levels are increased from at least 2 weeks (from 14 to 28 days) after exacerbation. Yet, their design did not allow demonstrating the exact timing during which serum OPG and RANK-L levels were increased.

Table 3 Comparison of peripheral bone microarchitecture and density by HR-pQCT between CF patients and controls

	CF	Control	Difference (%)	*p* value[a]
Radius				
Density				
Tt.vBMD (mg/cm^3)	319 ± 69	330 ± 49	−3.3	0.39
Tb.vBMD (mg/cm^3)	176 ± 49	189 ± 39	−6.8	0.36
Ct.vBMD (mg/cm^3)	848 ± 67	853 ± 62	−0.6	0.49
Ct.TMD (mg/cm^3)	979 ± 48	981 ± 47	−0.2	0.68
Geometry				
Tt.Ar (mm^3)	291 ± 71	318 ± 77	−8.6	0.43
Tb.Ar (mm^3)	234 ± 70	255 ± 72	−8.3	0.64
Ct.Ar (mm^3)	60 ± 12	66 ± 13	−9.3	0.09
Ct.Th (mm)	0.897 ± 0.192	0.925 ± 0.159	−3.0	0.50
Trabecular structure				
Tb.N (mm^{-1})	1.89 ± 0.27	1.89 ± 0.24	−0.1	0.31
Tb.Th (μm)	205 ± 20	213 ± 14	−3.8	0.05
Tb.Sp (μm)	508 ± 91	499 ± 81	1.8	0.63
Tb.Sp.SD (μm)	197 ± 47	192 ± 42	2.6	0.64
SMI (−)	1.96 ± 0.52	1.68 ± 0.45	17.1	0.01
Conn.D (−)	3.48 ± 1.02	3.71 ± 0.92	−6. 4	0.83
Cortical structure				
Ct.Po (%)	1.5 ± 0.7	1.6 ± 1.1	−6.3	0.46
Biomechanical parameters				
Stiffness (kN/mm)	158.2 ± 45.5	181.9 ± 44.6	−13.0	0.03
Tb.Dist.Load (%)	45.9 ± 11.8	51.4 ± 9.8	−10.6	0.03
Tb.Prox.Load (%)	16.0 ± 8.2	19.3 ± 7.39	−16.8	0.08
Tibia				
Density				
Tt.vBMD (mg/cm^3)	298 ± 56	330 ± 50	−9.9	0.01[b]
Tb.vBMD (mg/cm^3)	178 ± 46	202 ± 40	−11.8	0.07
Ct.vBMD (mg/cm^3)	863 ± 79	890 ± 49	−3.0	0.04
Ct.TMD (mg/cm^3)	991 ± 44	1002 ± 33	−1.1	0.21
Geometry				
Tt.Ar (mm^3)	724 ± 174	737 ± 151	−1.8	0.10
Tb.Ar (mm^3)	602 ± 164	606 ± 144	−0.7	0.06
Ct.Ar (mm^3)	127 ± 33	136 ± 24	−6.6	0.31
Ct.Th (mm)	1.274 ± 0.324	1.350 ± 0.234	−5.6	0.10
Trabecular structure				
Tb.N (mm^{-1})	1.71 ± 0.29	1.82 ± 0.28	−6.0	0.52
Tb.Th (μm)	220 ± 22	231 ± 18	−4.8	0.02
Tb.Sp (μm)	576 ± 128	529 ± 96	8.9	0.43
Tb.Sp.SD (μm)	245 ± 95	215 ± 48	14.0	0.52
SMI (−)	1.53 ± 0.47	1.35 ± 0.42	13.2	0.36
Conn.D (−)	3.25 ± 1.02	3.46 ± 0.98	−6.0	0.93
Cortical structure				
Ct.Po (%)	4.9 ± 4.9	3.6 ± 2.0	36.1	0.07
Biomechanical parameters				
Stiffness (kN/mm)	423.4 ± 101.6	481.6 ± 103.3	−12.1	0.13
Tb.Dist.Load (%)	48.0 ± 9.8	50.8 ± 8.0	−5.4	0.46
Tb.Prox.Load (%)	28.8 ± 8.3	30.6 ± 7.6	−5.7	0.87

[a] Adjusted for age, gender, weight and height

[b] $p < 0.05$ after adjustment for age, gender, weight, height and taking into account multiple comparisons (hierarchical FDR methodology)

We were not able to demonstrate a significant association between inflammation cytokines and biochemical markers of bone turnover, and this increase was probably independent from the bone resorption level.

Thus, infections may have some influence on bone turnover. Perhaps, the detection of a substantial variation in proinflammatory cytokines would have required multiple measurements over time that we were not able to obtain. In addition, the baseline values of these proinflammatory cytokines may be increased with a steady pattern rather than a cyclical one, hindering the observation of an association with bone turnover. Many systemic or local chronic inflammatory conditions, such as rheumatoid arthritis, inflammatory bowel disease, and more recently, cystic fibrosis, have been associated with systemic osteoporosis. The negative bone mass balance is mostly mediated by inflammatory cytokines that activate osteoclasts, impeding simultaneously osteoblast function [37]. Augmented inflammation is believed to play a central

role in the pathogenesis of CF pulmonary disease. Among blood biomarkers reflecting disease activity in CF pulmonary exacerbations, CRP has been the most widely studied, with a significant increase as the clinical onset of pulmonary exacerbation and a decrease following treatment [38]. Unfortunately, CRP blood levels appear to be highly variable within and among individuals. Moreover, for some patients, CRP levels remain elevated both before and after exacerbations suggesting subclinical chronic inflammation. Serum TNF α has also been shown to be highly variable, sometimes with undetectable levels or no significant variations. It may be a valuable cytokine to measure in bronchial secretions but this is not a satisfactory serum biomarker. IL-6 is a promising factor to monitor CF pulmonary exacerbation but with a small number of studies [38]. As for other inflammatory diseases, it is likely that antibiotic treatment prescribed for pulmonary exacerbations lead to incomplete remission of inflammation in some patients and that smoldering inflammation continues to induce negative effects on bone remodeling.

Our study has several strengths: this is a large modern cohort of patients with CF, including inflammation biomarkers, bone biomarkers, and evaluation of areal and volumetric BMD, but it also has limitations. First, the cross-sectional design of this study for bone parameter assessment cannot capture the dynamic changes in BMD and bone microarchitecture, which are occurring over time. Moreover, there was no comparison of baseline biological values to a healthy control population, particularly for RANKL and OPG, as these may have already been altered prior to the exacerbation. Further, CF patients vary significantly from each other in terms of pulmonary and extrapulmonary disease manifestations and disease course, and this can contribute to the variability observed in outcome measures, particularly given the relatively small number of patients studied. Moreover, the sample size of our study (56 CF patients) did not allow a thorough investigation of fracture discrimination.

The interpretation of our biologic results is also limited by the impossibility to obtain bone turnover markers in fasting individuals (CF patients are usually frail, especially during exacerbation, sometimes diabetics, and may live far from the hospital). Moreover other markers of bone turnover (N-telopeptide or bone specific alkaline phosphatase) were not measured but may have potentially captured abnormalities. It would also be interesting to measure other inflammatory cytokines such as IL-17, a well-known pro-inflammatory cytokine, shown to be increased in infected CF patients and a potential risk factor for *Ps. aeruginosa* infection [39]. Serum IL-17 level was shown to induce bone loss by increasing IL-6 and RANK-L from osteoblasts in the pathogenesis of osteoporosis [40]. In our population, the presence of *Ps. aeruginosa* colonies was reported in 77 % of the CF patients and we demonstrated an increase for IL-6 and a trend for RANK-L during exacerbation. Further, as the percentage of low BMD was significantly higher in men than in women in this CF cohort, it would be interesting to measure sex hormones (17-β estradiol, testosterone) and IGF-1, as these factors are well known to modulate bone loss and formation. However, sex hormones were not evaluated in this cohort since none of these patients had clinical signs of lowered steroid hormones, and, as reported by Rousset Jablonski et al. [41], most of the women (~64 %) followed in our center from this age range take contraceptives, therefore complicating interpretation of the sex hormone levels. Moreover, low levels of testosterone have been documented in adolescents with CF without significant impact on BMD [42]. Serum hormonal profile in male adults with CF was usually considered as normal [43]. While low estradiol levels may have a significant role in CF pathogenesis [6], its role is highly complex since estradiol is also associated with inflammatory process inducing mucoid conversion of pseudomonas [44] and modifying innate and acquired immunity [45]. The homogeneity of our population made up for this limitation as only adults with an even gender distribution were included, therefore limiting the difficulties encountered with young and prepubertal patients. Similarly, low levels of IGF1 have been documented in prepubertal CF patients, correlated with low weight and height [46]. IGF-1 levels are also lower in adult CF and correlated with some inflammatory markers and nutritional condition but no direct correlation with lumbar BMD has been documented in adult CF patients [47].

Conclusion

In conclusion, our study confirms the prevalence of CF-related bone loss among adults with cystic fibrosis, but the severity of bone fragility appears much lesser than in older studies, perhaps thanks to the improvement of nutritional status and bronchial infection control in CF adult population. Impairment in lower limb bone size may still persist.

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Compliance with ethical standards The study was conducted from March 2008 to November 2011. It has been approved by the local ethical committee and informed written consent was obtained from all patients.

Conflicts of interest Déborah Gensburger, Stéphanie BOUTROY, Roland CHAPURLAT, Raphaele NOVE-JOSSERAND, Sylvain Roche, Muriel RABILLOUD, and Isabelle DURIEU declare that they have no conflict of interest.

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