Osteoporosis International (2019) 30:1255–1263
https://doi.org/10.1007/s00198-019-04893-z

ORIGINAL ARTICLE

Bone involvement in young adults with cystic fibrosis awaiting lung transplantation for end-stage respiratory failure

E. Cairoli[1,2] · C. Eller-Vainicher[3] · L.C. Morlacchi[4] · P. Tarsia[4] · V. Rossetti[4] · M. Pappalettera[4] · M. Arosio[3,5] · I. Chiodini[1,5] · F. Blasi[2,4]

Received: 2 October 2018 / Accepted: 5 February 2019 / Published online: 23 February 2019

Abstract

Summary Patients with cystic fibrosis awaiting lung transplantation for end-stage respiratory failure have high prevalence of reduced bone mineral density and fragility fracture. Suboptimal 25-hydroxyvitamin D levels could significantly contribute to the development of cystic fibrosis-related bone disease.

Introduction The assessment of the prevalence of cystic fibrosis-related bone disease (CFBD) and its associated risk factors in young adults with cystic fibrosis (CF) awaiting lung transplantation for end-stage respiratory failure.

Methods Clinical characteristics, bone mineral density (BMD), the parameters of calcium metabolism, including vitamin D (25OHVitD) levels, and the presence of fragility fractures were evaluated in 42 CF patients (24 females, age 34.0 ± 8.4 years) consecutively referred as lung transplant candidates.

Results Mean 25OHVitD levels (54.9 ± 26.2 nmol/L) were below the reference range and hypovitaminosis D (25OHVitD < 75 nmol/L) was found in 34 patients (81%) and daily calcium intakes (median 550 mg/day) were lower than recommended. A BMD below the expected range for age (Z-score of − 2.0 or lower) and at least one prevalent fragility fracture were found in 22 patients (52.4%) and 18 patients (45.2%), respectively. The coexistence of low BMD and the presence of fracture was observed in 13 patients (31.0%). In these patients, the prevalence of nephrolithiasis was higher than in the remaining ones ($p = 0.046$). The presence of kidney stones was associated with a worse bone status and with severe vitamin D deficiency. In the whole sample, femoral BMD Z-scores were directly correlated with albumin-adjusted calcium ($p < 0.05$) and 25OHVitD levels ($p < 0.01$).

Conclusions Despite the improvement of CF care, CFBD is still highly prevalent in young adults awaiting lung transplantation for end-stage CF. Suboptimal 25OHVitD levels could significantly contribute to the development of CFBD. The presence of nephrolithiasis could be an additional warning about the need for a careful evaluation of bone health in CF patients.

Keywords Bone disease · Cystic fibrosis · Fracture · Lung transplantation · Nephrolithiasis · Osteoporosis

E. Cairoli and C. Eller-Vainicher contributed equally to this work.

✉ E. Cairoli
elisa.cairoli@live.it

[1] Unit for Bone Metabolism Diseases and Diabetes & Lab of Endocrine and Metabolic Research, Istituto Auxologico Italiano IRCCS, Milan, Italy

[2] Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy

[3] Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[4] Respiratory Unit & Cystic Fibrosis Adult Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[5] Department of Clinical Science and Community Health, University of Milan, Milan, Italy

Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by a mutation of the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride-conducting transmembrane channel which regulates anion transport and mucociliary clearance in the airways [1]. The CFTR dysfunction results in comorbidities involving the pancreas (malabsorption due to exocrine pancreatic insufficiency and CF-related diabetes, CFRD), liver (biliary cirrhosis), gut (distal intestinal obstruction syndrome), reproductive system (male infertility), and, finally, lung (bronchiectasis, small airways obstruction, and progressive respiratory failure) [1]. In particular, nowadays, end-stage respiratory insufficiency is the most frequent cause of death in CF patients. However, the early diagnosis through newborn screening and the

improvement of multidisciplinary CF care, included the referral to lung transplantation, have led to a progressive increase of the median age of survival of CF patients [2]. This improvement of life expectancy, which reached 45 years of age in 2016 [3], has dramatically brought out new complications of the disorder, like the CF-related bone disease (CFBD) [4]. Indeed, it is now widely acknowledged that adult CF patients are at increased risk of low bone mineral density (BMD) and of fragility fractures, particularly at the spine [5]. This bone damage is due to the combination of a low peak bone mass in adolescence and a subsequent premature bone loss during adulthood. The CFBD pathogenesis is multifactorial, including malnutrition and malabsorption, poor growth, sex steroid deficiency and delayed puberty, vitamin D and K insufficiency, lack of weight-bearing exercise, CFRD, glucocorticoid (GC) treatment, and recurrent respiratory infections [4]. With specific regard to lung involvement, the prevalence of CFBD appears to increase with the severity of pulmonary disease [6]. Thus, it is not surprising that CFBD is becoming a more and more significant matter of concern in end-stage CF patients, in whom the worsening of kyphosis and the chest pain caused by vertebral and rib fractures can accelerate the decline of respiratory function. This, in turn, may compromise in some patients the outcome of lung transplantation [7].

The available studies investigating bone mass in patients awaiting lung transplantation for end-stage respiratory failure showed a normal BMD in not more than 20% [8–14]. Conversely, a diagnosis of osteoporosis according to the WHO definition (i.e., T-score ≤ -2.5 in any site) [15] and/or of a BMD below the expected range for age (Z-score ≤ -2.0 in any site) [16] have been reported in up to 61% of patients [7, 9–14, 17–20]. Overall, these studies showed a great variability due to differences in the criteria used for both enrolment and the bone involvement evaluation. Indeed, the previous studies included patients with CF and/or with other chronic respiratory diseases and of different ages. However, when provided, a subgroup analysis limited to CF patients consistently suggested that the BMD of end-stage CF patients is lower than that of other lung transplant candidates, despite the younger age [12, 17, 18].

Even the prevalence of fragility fractures in lung transplant candidates varies highly across studies, ranging between 5 and 15% in studies which considered only the clinical fractures [17, 19, 21] and rising up to 38% if also morphometric vertebral and incidentally discovered rib fractures were evaluated [13, 18, 22]. Moreover, a dramatic fracture rate was found two decades ago in a sample of late-stage CF patients who were referred for lung transplantation [7]. Overall, data on bone involvement in CF patients awaiting lung transplantation are mostly dated or inferred from larger samples of transplant candidates for end-stage respiratory failure of any origin. Since the interest on CFBD is growing and the multidisciplinary CF care is constantly improving, the aim of our study was the assessment of BMD, morphometric fragility fractures, and vitamin D levels in a recent homogenous cohort of young adults with CF awaiting lung transplantation for end-stage respiratory failure in order to give an updated picture of bone involvement and to look for associations between the clinical or biochemical characteristics and the presence of bone complications in this population.

Methods

In this cross-sectional study, we evaluated for enrollment all consecutive CF patients aged 20 years or more with end-stage lung disease, who were referred as lung transplant candidates to the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan (Italy) from January 2009 to June 2016. Exclusion criteria included a prior transplant, a menopausal status for women, and an age ≥ 50 years old for both sexes. According to these criteria, during the study period, 49 CF patients were considered eligible for the study, of whom 7 (14%) were excluded because they did not give their informed consent or they did not complete the assessments provided by our protocol. Eventually, 42 CF patients were evaluated. The diagnosis of CF and the admission to lung transplant waiting list were made according to current international guidelines [23, 24]. Informed consent was obtained from all individual participants included in the study.

From all the enrolled patients, we collected information on daily calcium and vitamin D intake, current and past exposure to glucocorticoids, ongoing pharmacologic therapies, prevalent fragility fractures, respiratory function parameters, and presence of CF-related comorbidities (CFRD, exocrine pancreatic insufficiency, hepatic disease, hypogonadism, and nephrolithiasis). These data were confirmed by reviewing the medical records. The dietary calcium intake of our patients was assessed using a validated 7-day food frequency questionnaire [25]. A fracture was considered prevalent and due to bone fragility if occurred before our evaluation and without any evident trauma or after a low-energy trauma (e.g., a fall from a standing height), respectively. Traumatic fractures were not considered in the analysis. The presence of signs and symptoms of hypogonadism and the regularity of menses were ascertained in males and females, respectively. All patients underwent an abdominal ultrasound before the admission to lung transplant waiting list, and nephrolithiasis was considered to be present in both symptomatic (the previous occurrence of a renal colic followed by the identification of kidney stones) and asymptomatic cases (the ultrasound finding of kidney stones in the absence any previous painful event). Weight and height at the time of admission to lung transplant waiting list were recorded, and, accordingly, body mass index (BMI) was calculated.

In all patients, prior to transplantation serum calcium, albumin, phosphorus, creatinine, 25-hydroxyvitamin D (25OHVitD), and testosterone (only in males) were assessed. Serum samples were collected at the time of referral to lung transplantation, regardless of the season, and stored at − 20 °C until assayed. Serum calcium, albumin, phosphorus, and creatinine were measured by standard colorimetric techniques. Total calcium was corrected for serum albumin (Caalb adj) according to the formula Caalb adj (mg/dL) = total calcium + [(4.4 − albumin mg/dL) × 0.8] (reference interval = 8.4–10.2 mg/dL, 2.1–2.6 nmol/L). Glomerular filtration rate (GFR) was estimated according to the CKD-EPI Creatinine Equation [26]. Serum 25OHVitD and serum testosterone concentrations were measured by chemiluminescent (reference interval = 75–250 nmol/L, 30–100 ng/mL) and electrochemiluminescent immunoassay (reference interval = males 80–242 nmol/L, 2.8–8.4 ng/mL), respectively. Hypovitaminosis D was defined as a 25OHVitD below the reference range (75 nmol/L, 30 ng/mL) and in this context severe vitamin D deficiency as a 25OHVitD below 25 nmol/L (10 ng/mL) [27]. Untreated hypogonadism was defined by the presence of low testosterone levels associated with signs and symptoms of hypogonadism in males and of secondary amenorrhea of at least 3-month duration in females without hormonal replacement therapy.

Moreover, at the time of referral to lung transplantation, a dual-energy X-ray absorptiometry (DXA) scan was carried out to measure BMD (Hologic Discovery, Software version 13.3:3, Bedford MA, USA) at lumbar spine (LS; in vivo precision 1.0%), total femur (FT; in vivo precision 1.7%), and femoral neck (FN; in vivo precision 1.8%) and BMD data were expressed as Z-scores. Fractured vertebrae were excluded from BMD measurement and data from LS scans were used only if at least three vertebrae were visualized without interfering artifacts. According to the Official Positions of the International Society for Clinical Densitometry (ISCD), a BMD Z-score of − 2.0 or lower was defined as below the expected range for age [16].

In addition to DXA, at the same time, conventional radiographs of the thoracic and lumbar spine in lateral and anteroposterior projection (T4–L4) were obtained in all subjects with standardized technique. Two trained physicians, who were blinded to BMD and biochemical data, independently reviewed the radiographs, and they discussed questionable cases to agree on a diagnosis. The inter-rate reliability between the two physicians was good ($k = 0.8$). Vertebral fractures were diagnosed on visual inspection using the semiquantitative visual assessment previously described by Genant and colleagues [28] and fractures assessed on lateral thoracolumbar spine radiographs were defined in the presence of a > 20% reduction in anterior, middle, or posterior vertebral height. Accordingly, 13 vertebrae from T4 to L4 were assessed visually as intact or as having approximately mild (20 to 25% compression), moderate (25 to 40% compression), or severe (> 40% compression) deformity. According to the European CF bone mineralization guidelines, CFBD was diagnosed in the presence of a BMD Z-score of − 2.0 or lower associated with a fragility fracture history (a lower limb long bone fracture or two or more upper limb long bone fractures or a vertebral fracture) [29].

Statistical analysis

Statistical analysis was performed by SPSS version 21.0 statistical package (SPSS Inc., Chicago, IL). The normality of distribution was checked by the Kolmogorov–Smirnov test. The comparison of continuous variables was performed using one-way Student t test or Mann–Whitney U test, as appropriate. Categorical variables were compared by χ^2 test or Fisher exact test, as appropriate. The logistic regression analysis assessed the association between the presence of CFBD and potential risk factors. Bivariate associations between variables were tested by either Pearson's product moment correlation or Spearman's rank order correlation, as appropriate. p values of less than 0.05 were considered significant.

Results

The characteristics of the 42 enrolled patients are reported in Table 1. No patients were current or former smokers. About a quarter of patients were previously exposed to oral GC therapy, whereas the current use of inhaled GC treatment was much more frequent. Although the majority of patients received a vitamin D supplementation, mean 25OHVitD levels were below the reference range and hypovitaminosis D was found in 34 out of 42 patients (80%). Among these patients, 16 subjects (38.1% of the whole sample) did not even reach a more prudent threshold of 50 nmol/L and 6 (14.3% of the whole sample) were found to be severely deficient (25OHVitD < 25 nmol/L). No patients were treated with bone-active drugs before the admission to lung transplant waiting list. A BMD below the expected range for age (Z-score ≤ −2.0) and prevalent fragility fractures were found in 22 (52.4%) and 19 (45.2%) patients, respectively. Overall, 19 patients reported 34 prevalent fragility fractures: 31 vertebral (24 mild and 7 moderate) and 3 nonvertebral fractures (tibia, radius, and ribs). In 10 out of 19 fractured patients, more than one prevalent fragility fracture was observed.

CFBD was found in 13 patients (31.0%). The comparison between patients with and without CFBD is shown in Table 1. The two groups were comparable as far as age, gender, BMI, previous oral GC exposure, daily calcium intake and vitamin D supplementation, prevalence of untreated hypogonadism and of CF-related comorbidities, estimated GFR, and

Table 1 Clinical and biochemical characteristics of CF patients awaiting lung transplantation and comparison between patients with and without CF-related bone disease (CFBD)

	All patients ($n = 42$)	Patients with CFBD ($n = 13$)	Patients without CFBD ($n = 29$)	p
Age (years)	34.0 ± 8.4 (20–50)	35.3 ± 7.5 (24–47)	33.4 ± 8.8 (20–50)	0.505
Sex (females)	24 (57.1)	7 (53.8)	17 (58.6)	0.517
BMI (kg/m^2)	20.0 ± 2.0 (16.8–25.0)	20.0 ± 2.1 (16.8–24.6)	20.0 ± 2.1 (17.4–25.0)	0.998
Patients previously treated with oral GC	10 (23.8)	3 (23.1)	7 (24.1)	0.633
Prednisone cumulative exposure (g)[a]	3.5 (1.0–7.3)	3.7 (1.8–7.3)	3.4 (1.0–6.8)	0.491
Patients with current use of inhaled GC	28 (66.7)	6 (46.2)	22 (75.9)	0.059
Calcium intake (mg/day)	550 (100–1500)	600 (300–1100)	500 (100–1500)	0.510
Patients on vitamin D supplementation	34 (81.0)	10 (76.9)	24 (82.8)	0.700
Vitamin D (U/day)[a]	2000 (658–5688)	2000 (1000–5688)	2000 (658–5288)	0.607
Prevalence of untreated hypogonadism	9 (21.4)	1 (7.7)	8 (27.6)	0.232
Prevalence of CF-related diabetes	32 (76.2)	11 (84.6)	21 (72.4)	0.466
Prevalence of CF-related exocrine pancreatic insufficiency	38 (90.5)	12 (92.3)	26 (89.7)	0.637
Prevalence of CF-related hepatic disease	22 (52.4)	5 (38.5)	17 (58.6)	0.227
Prevalence of nephrolitiasis	8 (19.0)	5 (38.5)	3 (10.3)	0.046
BMD LS Z-score	− 1.93 ± 1.16 (− 4.60 to 0.20)	− 2.76 ± 1.10 (− 4.60 to − 0.70)	− 1.56 ± 0.99 (− 3.30 to 0.20)	0.001
BMD FN Z-score	− 1.68 ± 1.00 (− 3.60 to 0.90)	− 2.28 ± 0.89 (− 3.60 to − 0.80)	− 1.39 ± 0.93 (− 3.30 to 0.90)	0.006
BMD FT Z-score	− 1.48 ± 1.04 (−4.00 to 0.90)	− 2.02 ± 1.07 (− 4.00 to − 0.60)	− 1.22 ± 0.95 (− 3.00 to 0.90)	0.022
Patients with prevalent fragility fractures	19 (45.2)	13 (100)	6 (20.7)	<0.001
eGFR (mL/min/1.73 m^2)	123.5 ± 14.7 (78.6–153.6)	124.5 ± 12.5 (105.9–145.8)	123.0 ± 15.8 (78.6–153.6)	0.767
Albumin-adjusted calcium (mmol/L)	2.40 ± 0.10 (2.15–2.65)	2.38 ± 0.10 (2.23–2.53)	2.43 ± 0.13 (2.15–2.65)	0.493
Phosphorus (mmol/L)	1.20 ± 0.13 (0.90–1.52)	1.20 ± 0.15 (0.97–1.45)	1.23 ± 0.13 (0.90–1.52)	0.579
25-hydroxyvitamin D (nmol/L)	54.9 ± 26.2 (18.2–130.5)	53.9 ± 31.3 (18.2–130.5)	55.2 ± 24.0 (20.5–111.8)	0.895
Prevalence of hypovitaminosis D	34 (81.0)	11 (84.6)	23 (79.3)	0.522
Prevalence of severe vitamin D deficiency	6 (14.3)	4 (30.8)	2 (6.9)	0.063

CFBD was diagnosed in the presence of a BMD Z-score ≤ − 2 associated with a fragility fracture history. Data are expressed as mean ± standard deviation or median with range in parentheses and absolute number with percentage in parentheses, as appropriate

CF, cystic fibrosis; *BMI*, body mass index; *GC*, glucocorticoids; *BMD*, bone mineral density; *LS*, lumbar spine; *FN*, femoral neck; *FT*, femur total; *eGFR*, estimated Glomerular Filtration Rate according to the CKD-EPI Creatinine Equation

[a] Only among patients previously treated with oral GC therapy and vitamin D supplementation, respectively

parameters of calcium metabolism, including 25OHVitD levels. Even the prevalence of hypovitaminosis D was comparable between the two groups, whereas a severe vitamin D deficiency was fourfold more prevalent in patients with CFBD than in those without it, even without reaching the statistical significance, probably because of the low sample size. As expected, CFBD patients showed lower BMD Z-scores both at the lumbar spine and at the femoral site and a higher prevalence of fragility fractures (clinical and morphometric vertebral fractures taken as a whole), than patients without CFBD. It is remarkable that in the group of patients without CFBD, therefore with a relatively preserved bone health, 6 patients out of 29 (20.7%) showed at least one prevalent fragility fracture anyway. In addition, patients with CFBD tended to have a lower use of inhaled GC and had a significantly higher prevalence of nephrolithiasis.

We also compared CF patients in our sample according to further criteria defining bone involvement.

According to the suggestion that in CF adults, a significant fracture history can be considered per se an indicator of increased bone fragility, independent of BMD levels [29], we compared CF patients with and without prevalent fragility fractures. The two groups were comparable as far as age, gender, BMI, previous oral GC exposure and current use of inhaled GC, daily calcium intake and vitamin D supplementation, prevalence of untreated hypogonadism, CF-related comorbidities, hypovitaminosis D and severe vitamin D deficiency, estimated GFR, and parameters of calcium metabolism (data not shown). Despite comparable BMD Z-scores, fractured patients showed a higher prevalence of BMD below the expected range for age (68.4%) and tended to experience

a higher prevalence of kidney stones (31.6%) than CF patients without any fracture (39.1%, $p = 0.05$ and 8.7%, $p = 0.069$, respectively).

According to the ISCD criterion [16], patients with a BMD below the expected range for age (Z-score ≤ − 2.0) and those with a BMD Z-score > − 2.0 were comparable as far as age, gender, BMI, previous oral GC exposure and current use of inhaled GC, daily calcium intake and vitamin D supplementation, prevalence of untreated hypogonadism, CF-related comorbidities, hypovitaminosis D and severe vitamin D deficiency, estimated GFR, and parameters of calcium metabolism was concerned (data not shown). CF patients with a BMD below the expected range for age tended to have a higher prevalence of fragility fractures (59.1% versus 30.0%, $p = 0.059$). Nephrolithiasis was more prevalent in CF patients with low BMD than in patients with a BMD within the expected range for age (31.8% versus 5.0%, $p = 0.047$).

Finally, we compared CF patients with a BMD below the expected range for age and/or a prevalent fragility fracture ($n = 28$, 66.7%) with those without any of these criteria ($n = 14$) and we obtained very similar results (data not shown). In particular, we confirmed a significantly higher prevalence of kidney stones in CF patients with a skeletal damage independently from the criterion used to define this involvement (28.6% versus 0%, $p < 0.05$).

As nephrolithiasis was found to be a possible hallmark of bone involvement in these patients, we compared the clinical and biochemical characteristics of CF patients with and without nephrolithiasis (Table 2). The two groups were comparable as far as age, gender, BMI, GC exposure, daily calcium intake, prevalence of untreated hypogonadism, CF-related comorbidities and hypovitaminosis D, estimated GFR, albumin-adjusted calcium, and 25OHVitD levels. CF patients with nephrolithiasis had a twofold higher prevalence of fragility

Table 2 Comparison of the clinical and biochemical characteristics in CF patients awaiting lung transplantation with and without nephrolithiasis

	Patients with nephrolithiasis ($n = 8$)	Patients without nephrolithiasis ($n = 34$)	p
Age (years)	33.0 ± 10.9 (20–46)	34.2 ± 7.9 (20–50)	0.713
Sex (females)	5 (62.5)	19 (55.9)	0.527
BMI (kg/m^2)	20.0 ± 1.2 (18.0–21.6)	20.0 ± 2.2 (16.8–25.0)	0.994
Patients previously treated with oral GC	2 (25.0)	8 (23.5)	0.626
Prednisone cumulative exposure (g)[a]	4.2 (1.0–7.3)	3.5 (1.1–6.8)	1.000
Patients with current use of inhaled GC	3 (27.5)	25 (73.5)	0.092
Calcium intake (mg/day)	450 (300–1100)	600 (100–1500)	0.582
Patients on vitamin D supplementation	4 (50.0)	30 (88.2)	0.030
Vitamin D (U/day)[a]	2000 (1000–5688)	2000 (658–5288)	0.911
Prevalence of untreated hypogonadism	2 (25.0)	7 (20.6)	0.557
Prevalence of CF-related diabetes	6 (75.0)	26 (76.5)	0.626
Prevalence of CF-related exocrine pancreatic insufficiency	6 (75.0)	32 (94.1)	0.158
Prevalence of CF-related hepatic disease	4 (50.0)	18 (52.9)	0.594
BMD LS Z-score	− 2.78 ± 1.40 (−4.60 to –0.70)	− 1.74 ± 1.02 (−3.30 to 0.20)	0.021
BMD FN Z-score	− 2.44 ± 0.66 (− 3.00 to –0.90)	− 1.49 ± 1.00 (−3.60 to 0.90)	0.014
BMD FT Z-score	− 2.13 ± 0.90 (−3.00 to –0.20)	− 1.32 ± 1.0 (−4.00 to 0.90)	0.050
Patients with Z-score ≤ − 2	7 (87.5)	15 (44.1)	0.047
Patients with prevalent fragility fractures	6 (75.0)	13 (38.2)	0.069
Patients with BMD Z-score ≤ − 2 and/or prevalent fragility fractures	8 (100.0)	20 (58.8)	0.037
eGFR (mL/min/1.73 m^2)	118.2 ± 20.8 (78.6–143.8)	124.7 ± 13.0 (96.4–153.6)	0.265
Albumin-adjusted calcium (mmol/L)	2.33 ± 0.10 (2.23–2.53)	2.43 ± 0.10 (2.15–2.65)	0.067
Phosphorus (mmol/L)	1.23 ± 0.06 (1.16–1.29)	1.20 ± 0.16 (0.90–1.52)	0.632
25-hydroxy vitamin D (nmol/L)	45.9 ± 32.2 (18.2–111.3)	56.9 ± 24.7 (19.5–130.5)	0.290
Prevalence of hypovitaminosis D	7 (87.5)	27 (79.4)	0.518
Prevalence of severe vitamin D deficiency	4 (50.0)	2 (5.9)	0.008

Data are expressed as mean ± standard deviation or median with range in parentheses and absolute number with percentage in parentheses, as appropriate

CF, cystic fibrosis; *BMI*, body mass index; *GC*, glucocorticoids; *BMD*, bone mineral density; *LS*, lumbar spine; *FN*, femoral neck; *FT*, femur total; *eGFR*, estimated Glomerular Filtration Rate according to the CKD-EPI Creatinine Equation

[a] Only among patients previously treated with oral GC therapy and vitamin D supplementation, respectively

fractures, even without reaching the statistical significance, probably due to the small sample size. As expected, they also showed significantly lower BMD Z-scores both at the lumbar spine and at the femoral site and all of them exhibited at least one sign of bone involvement: one patient showed a fragility fracture associated with a BMD within the normal range for age, two patients a low BMD without any fracture and even five patients both a reduced BMD and at least one prevalent fragility fracture. Moreover, half of these patients did not take vitamin D supplementation, and, despite comparable mean 25OHVitD levels and a similar prevalence of hypovitaminosis D, the prevalence of severe vitamin D deficiency was significantly higher in the group with kidney stones than in patients without nephrolithiasis.

The independent association between nephrolithiasis and the presence of CFBD (both a Z-score ≤ -2 and at least one prevalent fragility fracture) was confirmed by the logistic regression analysis. Indeed, the presence of CFBD was significantly associated with the presence of nephrolithiasis, even after adjusting for age, gender, BMI, and previous exposure to oral GC (Table 3, panel a). However, this association was no longer present after including the presence of severe vitamin D deficiency in the multivariable logistic regression model (Table 3, panel b).

Finally, considering all CF patients as a whole, bivariate analysis showed that BMD FN Z-scores and FT Z-scores were directly associated with albumin-adjusted calcium and 25OHVitD levels and that BMD FT Z-scores were inversely associated with the prednisone cumulative exposure (Table 4). No correlations were found between BMD FN Z-scores and FT Z-scores and the other available continuous variables (data not shown). The correlation between 25OHVitD levels and BMD FT Z-scores ($r = 0.554$, $p = 0.0001$) is depicted in Fig. 1.

Table 3 Association between the presence of CF-related bone disease (both a BMD Z-score ≤ -2 and a significant fragility fracture history) and two combinations of potential risk factors using the multivariable logistic regression model

	OR	95% CI	*p*
a)			
Age (1 year increase)	1.04	0.94–1.14	0.453
Sex (female)	1.19	0.27–5.21	0.815
BMI (1 point increase)	1.03	0.72–1.47	0.873
Nephrolithiasis (yes)	6.00	1.10–32.74	0.039
Previous exposure to oral GC (yes)	1.13	0.19–6.68	0.891
b)			
Age (1 year increase)	1.05	0.95–1.17	0.303
Sex (female)	1.47	0.31–7.04	0.626
BMI (1 point increase)	1.03	0.72–1.48	0.877
Nephrolithiasis (yes)	3.19	0.46–22.10	0.240
Previous exposure to oral GC (yes)	1.43	0.24–8.70	0.698
Severe vitamin D deficiency (yes)	5.51	0.54–56.8	0.151

CF, cystic fibrosis; *BMD*, bone mineral density; *BMI*, body mass index; *GC*, glucocorticoids

Table 4 Bivariate analysis of risk factors for low BMD

	BMD Z-score FN		BMD Z-score FT	
	r	p	r	p
Prednisone cumulative exposure	−0.257	0.109	−0.383	0.015
Albumin-adjusted calcium	0.387	0.020	0.346	0.039
25-hydroxyvitamin D	0.508	0.001	0.554	0.0001

BMD, bone mineral density; *FN*, femoral neck; *FT*, total femur

Discussion

The present study confirms that CFBD is highly prevalent in young adults affected with CF and awaiting lung transplantation for end-stage respiratory failure. Consistent with previous studies, the exact prevalence of CFBD varies according to the definition used. According to the ISCD criterion of BMD below the expected range for age (i.e., a BMD Z-score ≤ -2.0), more than half of our sample showed a CF-related low BMD. [16]. These results are in line with previous studies which showed a BMD reduction (either a Z-score ≤ -2 or a T-score ≤ -2.5) in 29–61% of patients awaiting lung transplantation for end-stage respiratory failure [7, 9–14, 17–20]. It must be underlined that only one of these studies was exclusively on CF patients [7] and that sub-analysis restricted to CF patients in larger samples including end-stage respiratory failure of any origin demonstrated a BMD below the

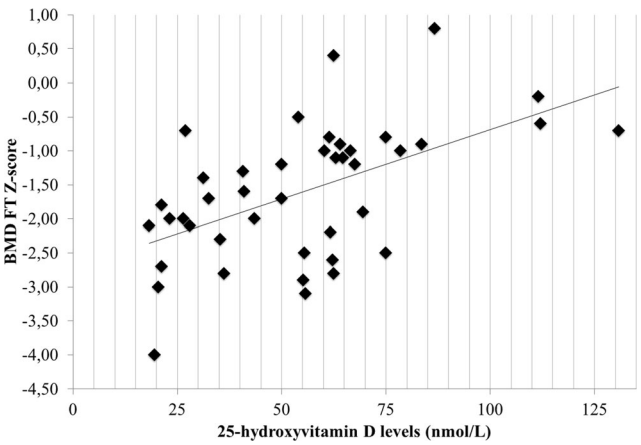

Fig. 1 The correlation between 25-hydroxyvitamin D levels and BMD FT Z-scores in our sample of young adults with CF awaiting lung transplantation for end-stage respiratory failure ($r = 0.554$, $p = 0.0001$). BMD, bone mineral density; FT, total femur; CF, cystic fibrosis

expected range for age in up to 75% of patients [17], suggesting that in previous records, the real prevalence of BMD reduction in CF patients could have been underestimated.

Importantly, almost half of our patients exhibited at least one prevalent fragility fracture. In previous records, when considering only the clinical fractures, this prevalence resulted significantly lower [17, 19, 21]. However, even in the studies considering morphometric vertebral and incidentally discovered rib fractures, this prevalence did not exceed 38% [8, 13, 18, 22]. The only exception is represented by a retrospective study published in 1998 on 70 late-stage CF patients, which documented, in addition to self-reported clinical fractures, 62 morphometric vertebral fractures, and 14 rib fractures in 33 and 10 patients, respectively [7]. This dramatic prevalence of fragility fractures, especially considered the young age of the subjects involved, is similar to that we found in the present study after two decades.

Beside the homogeneity of the sample studied (only young patients with CF), a further strength of our study was the assessment of conventional spinal radiographs instead of simple thorax radiographs for the detection of morphometric vertebral fractures. This could have allowed to a better sensitivity for detecting vertebral fractures and, thus, a more precise picture of bone involvement in CF.

Overall, about two-thirds of our patients showed a sign of bone involvement (either a BMD reduction or a prevalent fragility fracture). Consistent with the assumption that a BMD reduction can be predictive of bone fragility, in patients with a BMD Z-score ≤ -2.0, the prevalence of fragility fractures tended to be higher (approximately twofold) than in patients with a BMD Z-score > -2.0. Finally, even adopting the more conservative criterion for diagnosing osteoporosis suggested by the European CF bone mineralization guidelines (i.e., a BMD Z-score ≤ -2.0 associated with a significant fragility fracture history) [29], 13 out of 42 patients (31.0%) were diagnosed to have a CF-related bone involvement anyway.

In order to optimize bone health in CF, current guidelines recommend optimal calcium intake, which, in the age range of our sample, is about 1000 mg/day [29]). However, in our patients, the median values of daily calcium intakes were lower than suggested and these suboptimal calcium intakes could have contributed to a reduced low peak bone mass and to a subsequent bone loss. Some previous data reported calcium intakes higher than the recommended dietary requirements in CF adults [30]. It must be observed, however, that those data have been mainly collected in CF population with a good pulmonary status; the difference in the populations studied could explain these discordant results.

Moreover, despite the fact that more than 80% of patients received vitamin D supplements and that, in cholecalciferol users, median daily doses were quite in line with the recommended ones, mean 25OHVitD levels were barely greater than 50 nmol/L, and only 8 out of 42 patients reached the cut-off of 75 nmol/L. Our data, thus, suggest that multivitamin supplements specifically designed for CF patients (usually containing 1000–2000 UI of cholecalciferol, according to the dosage used) are quite insufficient to correct hypovitaminosis D and significantly larger doses should probably be used. Considering the oil vehicle of cholecalciferol supplements, in order to optimize absorption, these supplements should be taken together with pancreatic enzyme replacement therapy in those patients suffering from CF-related exocrine pancreatic insufficiency. However, there is no clear consensus about the optimal 25OHVitD levels in the general population and especially in people with CF. The cut-off of 75 nmol/L was mainly based on the evidence that over this threshold parathyroid hormone (PTH) levels begin to plateau and the intestinal calcium absorption get significantly more efficient [27], but the last European CF bone mineralization guidelines remained more prudent, recommending a minimum 25OHVitD concentration of 50 nmol/L because of the lack of data about the long-term consequences of 25OHVitD levels above 75 nmol/L in a large sample of young adults with CF [29]. In our sample, we found a significant direct correlation between 25OHVitD levels and BMD FN Z-scores and FT Z-scores (Table 4), and, as depicted in Fig. 1, no patients with 25OHVitD levels higher than 75 nmol/L showed FT Z-scores < -1.0. This finding suggests that this threshold could be safely assumed as the target to achieve in the context of cholecalciferol supplementation. The less strong direct correlation between BMD FN Z-scores and FT Z-scores and albumin-adjusted calcium levels could be interpreted as a consequence of the abovementioned correlation with 25OHVitD levels. Finally, although only a minority of patients was previously exposed to systemic oral GC treatment, we found an inverse correlation between BMD FT Z-scores and prednisone cumulative exposure, as already observed by other authors [7, 20].

A further worthy of attention result in our analysis is that the prevalence of nephrolithiasis was higher in CF patients with bone damage, regardless of the criteria used to discriminate bone involvement. There is now a wide agreement on the association between nephrolithiasis and CF. Previous studies found that 3–13% of CF patients suffer from kidney stones compared with 1–2% of healthy controls [31, 32], while the prevalence of nephrolithiasis in our sample seems to be higher (19%) than in previous reports. However, so far, an evaluation of this renal complication in our specific setting (CF patients awaiting lung transplantation for end-stage respiratory failure) was lacking. In CF, calcium oxalate is the usual stone composition and many mechanisms promoting stone formation are probably involved: low urinary volume, hyperuricosuria, hyperoxaluria, hypocitraturia, primary defects in calcium handling caused by mutation of the CFTR, and lack of colonization with an enteric oxalate-degrading bacterium [31]. However, the relative contributions of these factors are still

uncertain. It can be postulated that in our sample the reduced median calcium intake, especially in combination with a severe vitamin D deficiency, could have furtherly worsen the intestinal oxalate absorption, which is already increased in CF, therefore increasing the risk of nephrolithiasis.

The comparison between patients with and without this renal complication (Table 2) showed that the presence of kidney stones was clearly associated with a worse bone phenotype. Indeed, CF patients with nephrolithiasis displayed a significantly lower BMD both at the lumbar spine and at the femoral site, and the prevalence of fragility fractures in this subgroup was about twofold higher than in patients without nephrolithiasis, even if without reaching the statistical significance (probably because of the small number of subjects involved). Overall, we found at least one sign of bone involvement in eight out of eight patients. Interestingly, despite comparable mean 25OHVitD levels between the two groups, a higher fraction of patients with nephrolithiasis was not on vitamin D treatment at the time of evaluation and resulted severely deficient in terms of vitamin D levels. Of utmost importance, the logistic regression analysis showed that a young adult with CF awaiting lung transplantation with a history of kidney stones had a sixfold risk of CFBD than a subject who had never suffered from nephrolithiasis, regardless of potential risk factors like age, gender, BMI, and previous exposure to oral GC (Table 3, panel a). This association, however, is not independent of the presence of severe vitamin D deficiency (Table 3, panel b), which, therefore, may represent a crucial factor for the compromised bone health in CF patients. Anyway, this finding deserves a clinical interest, since the presence of nephrolithiasis could be interpreted as a warning about the need for a careful evaluation of the skeletal involvement and the prescription of appropriate treatment strategies.

The current study has several limitations. First of all, the cross-sectional design does not consent to draw conclusions about causality, but only to establish associations. Secondly, the small sample size could have reduced the statistical power of the study. However, our sample, even if small, has the value of being a homogeneous cohort of young adults with CF and awaiting lung transplantation for end-stage respiratory failure. Thirdly, the sample collection in different periods of the year and the lack of information about sunlight exposure of our patients do not allow to account for season-related vitamin D variations. However, although such an influence in our sample cannot be excluded, we could suppose that, as compared with healthy population, in CF patients the vitamin D fluctuations should be minimal, due to the poor general conditions and the limited outdoor life of end-stage CF patients approaching lung transplantation. Finally, a limitation of the study is the lack of data on other serum parameters of calcium metabolism (such as PTH levels) and on urinary samples. Indeed, these parameters could have been useful for elucidating the association between nephrolithiasis and severe vitamin D deficiency.

In conclusion, despite the improvement of CF multidisciplinary care, in our recent population of young adults with CF awaiting lung transplantation for end-stage respiratory failure a condition of CFBD is still highly prevalent and despite the young age, only one-third of patients did not show any signs of bone fragility. Furthermore, our data show that suboptimal 25OHVitD levels could significantly contribute to the development of CFBD and that the presence of nephrolithiasis is clearly associated with a worse bone health. Although severe bone disease is not an absolute contraindication to the admission to lung transplant waiting list [24], the occurrence after surgery of fragility fractures, especially rib and vertebral ones, can accelerate the decline of respiratory function and compromise the outcome of lung transplantation [7]. Therefore, the optimization of bone health should be a target to be pursued starting from the pediatric age in CF people. Further studies are needed to define the optimal prevention strategies in this population.

Compliance with ethical standards

Conflicts of interest None.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Elborn JS (2016) Cystic fibrosis. Lancet 388(10059):2519–2531
2. Hirche TO, Knoop C, Hebestreit H, Shimmin D, Solé A et al (2014) Practical guidelines: lung transplantation in patients with cystic fibrosis. Pulm Med 2014:621342
3. Cystic Fibrosis Foundation (2017) Cystic Fibrosis Foundation Patient Registry 2016 Annual Data Report Bethesda, Maryland. https://www.cff.org/Research/Researcher-Resources/Patient-Registry. Accessed 16/08/2018
4. Aris RM, Merkel PA, Bachrach LK, Borowitz DS, Boyle MP, Elkin SL, Guise TA, Hardin DS, Haworth CS, Holick MF, Joseph PM, O'Brien K, Tullis E, Watts NB, White TB (2005) Guide to bone health and disease in cystic fibrosis. J Clin Endocrinol Metab 90(3): 1888–1896
5. Paccou J, Zeboulon N, Combescure C, Gossec L, Cortet B (2010) The prevalence of osteoporosis, osteopenia, and fractures among adults with cystic fibrosis: a systematic literature review with meta-analysis. Calcif Tissue Int 86(1):1–7
6. Legroux-Gérot I, Leroy S, Prudhomme C, Perez T, Flipo RM, Wallaert B, Cortet B (2012) Bone loss in adults with cystic fibrosis: prevalence, associated factors, and usefulness of biological markers. Joint Bone Spine 79(1):73–77
7. Aris RM, Renner JB, Winders AD, Buell HE, Riggs DB, Lester GE, Ontjes DA (1998) Increased rate of fractures and severe kyphosis: sequelae of living into adulthood with cystic fibrosis. Ann Intern Med 128(3):186–193

8. Cahill BC, O'Rourke MK, Parker S, Stringham JC, Karwande SV, Knecht TP (2001) Prevention of bone loss and fracture after lung transplantation: a pilot study. Transplantation 72:1251–1255
9. Trombetti A, Gerbase MW, Spiliopoulos A, Slosman DO, Nicod LP, Rizzoli R (2000) Bone mineral density in lung-transplant recipients before and after graft: prevention of lumbar spine post-transplantation-accelerated bone loss by pamidronate. J Heart Lung Transplant 19:736–743
10. Balci MK, Ari E, Vayvada M, Salturk C, Asicioglu E, Yeginsu A, Kutlu CA (2016) Osteoporosis in lung transplantation candidates: association with 6-minute walking test and body mass index. Transplant Proc 48:2147–2151
11. Spira A, Gutierrez C, Chaparro C, Hutcheon MA, Chan CK (2000) Osteoporosis and lung transplantation: a prospective study. Chest 117:476–481
12. Lakey WC, Spratt S, Vinson EN, Gesty-Palmer D, Weber T, Palmer S (2011) Osteoporosis in lung transplant candidates compared to matched healthy controls. Clin Transpl 25:426–435
13. Shane E, Papadopoulos A, Staron RB, Addesso V, Donovan D, McGregor C, Schulman LL (1999) Bone loss and fracture after lung transplantation. Transplantation 68:220–227
14. Jastrzebski D, Lutogniewska W, Ochman M, Margas A, Kowalski K et al (2010) Osteoporosis in patients referred for lung transplantation. Eur J Med Res 15(Suppl 2):68–71
15. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy (2001) Osteoporosis prevention, diagnosis, and therapy. JAMA 285(6):785–795
16. Lewiecki EM, Gordon CM, Baim S, Binkley N, Bilezikian JP, Kendler DL, Hans DB, Silverman S, Bishop NJ, Leonard MB, Bianchi ML, Kalkwarf HJ, Langman CB, Plotkin H, Rauch F, Zemel BS (2008) Special report on the 2007 adult and pediatric position development conferences of the International Society for Clinical Densitometry. Osteoporos Int 19(10):1369–1378
17. Aris RM, Neuringer IP, Weiner MA, Egan TM, Ontjes D (1996) Severe osteoporosis before and after lung transplantation. Chest 109:1176–1183
18. Shane E, Silverberg SJ, Donovan D, Papadopoulos A, Staron RB, Addesso V, Jorgesen B, McGregor C, Schulman L (1996) Osteoporosis in lung transplantation candidates with end-stage pulmonary disease. Am J Med 101:262–269
19. Ferrari SL, Nicod LP, Hamacher J, Spiliopoulos A, Slosman DO, Rochat T, Bonjour JP, Rizzoli R (1996) Osteoporosis in patients undergoing lung transplantation. Eur Respir J 9:2378–2382
20. Tschopp O, Boehler A, Speich R, Weder W, Seifert B, Russi EW, Schmid C (2002) Osteoporosis before lung transplantation: association with low body mass index, but not with underlying disease. Am J Transplant 2:167–172
21. Hariman A, Alex C, Heroux A, Camacho P (2014) Incidence of fractures after cardiac and lung transplantation: a single center experience. J Osteoporos 2014:573041
22. Aris RM, Lester GE, Renner JB, Winders A, Denene Blackwood A et al (2000) Efficacy of pamidronate for osteoporosis in patients with cystic fibrosis following lung transplantation. Am J Respir Crit Care Med 162:941–946
23. Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F et al (2017) Diagnosis of cystic fibrosis: consensus guidelines from the Cystic Fibrosis Foundation. J Pediatr, 181S:S4, S15.e1
24. Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, Lederer DJ, Mulligan MJ, Patterson GA, Singer LG, Snell GI, Verleden GM, Zamora MR, Glanville AR (2015) A consensus document for the selection of lung transplant candidates: 2014–an update from the Pulmonary Transplantation Council of the International Society for heart and lung transplantation. J Heart Lung Transplant 34(1):1–15
25. Varenna M, Binelli L, Zucchi F, Ghiringhelli D, Sinigaglia L (2001) Unbalanced diet to lower serum cholesterol level is a risk factor for postmenopausal osteoporosis and distal forearm fracture. Osteoporos Int 12(4):296–301
26. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF III et al (2009) A new equation to estimate glomerular filtration rate. Ann Intern Med 150(9):604–612
27. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA et al (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 96(7):1911–1930 Erratum in: J Clin Endocrinol Metab 96(12):3908
28. Genant HK, Wu CY, van Knijk C, Nevitt M (1993) Vertebral fracture assessment using a semi-quantitative technique. J Bone Min Res 8:1137–1148
29. Sermet-Gaudelus I, Bianchi ML, Garabédian M, Aris RM, Morton A, Hardin DS, Elkin SL, Compston JE, Conway SP, Castanet M, Wolfe S, Haworth CS (2011) European cystic fibrosis bone mineralisation guidelines. J Cyst Fibros 10(Suppl 2):S16–S23
30. Gordon CM, Anderson EJ, Herlyn K, Hubbard JL, Pizzo A, Gelbard R, Lapey A, Merkel PA (2007) Nutrient status of adults with cystic fibrosis. J Am Diet Assoc 107(12):2114–2119
31. Gibney EM, Goldfarb DS (2003) The association of nephrolithiasis with cystic fibrosis. Am J Kidney Dis 42(1):1–11
32. Perez-Brayfield MR, Caplan D, Gatti JM, Smith EA, Kirsch AJ (2002) Metabolic risk factors for stone formation in patients with cystic fibrosis. J Urol 167(2 Pt 1):480–484