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Bone mineral density evaluation in pediatric patients with Cystic Fibrosis

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Bone mineral density evaluation in pediatric patients with Cystic Fibrosis

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ABBREVIATIONS

25(OH)D – 25-hydroxy-vitamin D

BMD – bone mineral density

BMI – body mass index

CF – Cystic Fibrosis

CFDR – Cystic Fibrosis-related diabetes

CFTR – Cystic Fibrosis Transmembrane Conductance Regulator

CHUC – Centro Hospitalar e Universitário de Coimbra

DEXA – dual-energy X-ray absorptiometry

FEV1 – forced expiratory volume in one second

FVC – forced vital capacity

IQR – interquartile range

LS – lumbar spine

p50 – median

PI – pancreatic insufficient

PS – pancreatic sufficient

SD – standard deviation

TH – total hip

$\bar{x}$ – mean

ABSTRACT

Introduction: Cystic fibrosis (CF) is a multisystemic, potentially fatal, autosomal recessive genetic disorder. Morbidity and mortality are mostly secondary to chronic pulmonary disease. Developments in treatments, quality of life, and clinical outcomes improved median life expectancy in CF patients, increasing the prevalence of new long-term complications like CF-related bone disease. Various factors play a part in the decrease of bone mineral density (BMD), thought to originate during childhood and adolescence.

Objectives: This study aims to retrospectively evaluate BMD in CF Portuguese pediatric patients, explore its correlation to other variables in this disease, and identify patients at greatest risk of developing CF-related bone disease.

Materials and methods: Retrospective evaluation of the 20 pediatric CF patients, followed in the Pediatric Hospital of the CF Reference Center of Centro Hospitalar e Universitário de Coimbra, with at least one dual-energy X-ray absorptiometry, between 2012 and 2021, was carried out. Analyzed variables included: chronological age, age at diagnosis, genotype, weight, height, body mass index (BMI), BMD, pulmonary function tests, serum 25-hydroxy-vitamin D, physical activity, and pancreatic function.

Results: Twenty patients, with a median age of 12 years, had mean Z-scores for BMD, weight, and BMI significantly lower than the reference population. The mean BMD Z-score was -0.955±1.355 for the lumbar spine (LS), and -1.053±1.415 for the total hip (TH). Half the patients had reduced pulmonary function, 40% had vitamin D deficiency, and 85% were pancreatic insufficient. BMD was globally significantly lower in stunted, underweight patients or with reduced pulmonary function. Total hip BMD was lower in individuals with no physical activity (45% of patients). BMD was positively correlated with weight ($r=0.867$, $p<0.001$ LS; $r=0.811$, $p<0.001$ TH), height ($r=0.565$, $p=0.009$ LS; $r=0.526$, $p=0.021$ TH), BMI ($r=0.723$, $p<0.001$ LS; $r=0.677$, $p<0.001$ TH), forced expiratory volume in one second ($r=0.653$, $p=0.002$ LS; $r=0.781$, $p<0.001$ TH), and forced vital capacity ($r=0.643$, $p=0.002$ LS; $r=0.779$, $p<0.001$ TH). There was no correlation with chronological age, age of diagnosis, genotype, pancreatic function, and serum vitamin D.

Discussion and conclusion: Undernourished patients with decreased pulmonary function are at most risk of developing CF-related bone disease. Levels of physical activity also play a role in BMD. A multidisciplinary, preventive approach in CF patients, with BMD evaluation, is crucial to optimize outcomes and decrease health complications.

Keywords: Cystic Fibrosis, bone density, pulmonary function, nutritional status, pediatrics

RESUMO

Introdução: A Fibrose Quística (FQ) é uma doença genética autossómica recessiva, multissistémica e potencialmente fatal. A doença pulmonar crónica é a maior causa de morbimortalidade. Desenvolvimentos nos tratamentos, qualidade de vida e resultados clínicos melhoraram a esperança média de vida dos doentes com FQ, aumentando a prevalência de novas complicações a longo prazo, como a doença óssea. A diminuição da densidade mineral óssea (DMO) tem origem na infância e adolescência, sendo causada por vários fatores.

Objetivos: Este estudo pretende avaliar retrospetivamente a DMO em doentes portugueses com FQ, em idade pediátrica; explorar a correlação com outras variáveis e identificar os doentes em maior risco de desenvolver doença óssea.

Materiais e métodos: Foi realizada uma avaliação retrospetiva dos 20 doentes com FQ, seguidos no Hospital Pediátrico do Centro de Referência de FQ do Centro Hospitalar e Universitário de Coimbra, que fizeram pelo menos uma absorciometria radiológica de dupla energia, entre 2012 e 2021. Variáveis em estudo incluem: idade cronológica, idade de diagnóstico, genótipo, peso, altura, índice de massa corporal (IMC), DMO, testes de função pulmonar, vitamina D sérica, atividade física e função pancreática.

Resultados: Vinte doentes, com idade mediana de 12 anos, apresentavam *Z-scores* médios de DMO, peso e IMC significativamente mais baixos que a população de referência. A média dos *Z-scores* de DMO na coluna lombar (CL) foi de -0,955±1,355 e no fémur total (FT) de -1,053±1,415. Metade dos doentes tinham função pulmonar reduzida, 40% défice de vitamina D e 85% insuficiência pancreática. A DMO estava globalmente diminuída em doentes com baixa estatura, baixo peso ou função pulmonar reduzida. A DMO do fémur total era mais baixa em indivíduos sem atividade física (45% dos casos). Encontrámos correlação positiva entre a DMO e o peso (CL: $r=0,867$, $p<0,001$; FT: $r=0,811$, $p<0,001$), altura (CL: $r=0,565$, $p=0,009$; FT: $r=0,526$, $p=0,021$), IMC (CL: $r=0,723$, $p<0,001$; FT: $r=0,677$, $p<0,001$), volume expiratório forçado no primeiro segundo (CL: $r=0,653$, $p=0,002$ LS; FT: $r=0,781$, $p<0,001$) e capacidade vital forçada (CL: $r=0,643$, $p=0,002$ LS; FT: $r=0,779$, $p<0,001$). Não houve correlação com a idade cronológica, idade de diagnóstico, genótipo, função pancreática e vitamina D sérica.

Discussão e conclusão: Doentes malnutridos ou com diminuição da função pulmonar têm maior risco de ter doença óssea relacionada com a FQ. Níveis de atividade física também têm influência na DMO. Uma abordagem preventiva e multidisciplinar nos doentes com FQ, com avaliação da DMO, é crucial para otimizar os resultados e diminuir as complicações.

Palavras-chave: Fibrose Quística, densidade óssea, função pulmonar, estado nutricional, pediatria

INTRODUCTION

Cystic fibrosis (CF) is a severe, life-limiting, autosomal recessive genetic disorder. It is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene, located in chromosome 7q[1], which affects chloride, bicarbonate, and sodium transport through the epithelial cell membranes[2]. The *F508del* gene mutation is the most common among more than 2000 gene variants identified[3]. In Portugal, the estimated prevalence of CF is 0.271 per 10,000[4] and the incidence was 1:7769 new cases in newborns in 2020[5].

Diagnosis of CF can be made by a positive sweat test, when chloride concentration in sweat is > 60mmol/L, or genetic testing, by identifying two known disease-causing mutations in the *CFTR* gene[1]. In Portugal, since 2013, newborn screening was implemented by measuring the immunoreactive trypsinogen (IRT) and pancreatitis associated protein (PAP)[1,5,6]. Since 2018 CF was officially included in the national newborn screening program[5].

Cystic fibrosis is a multisystemic disease affecting various organs with multiple clinical presentations. Disease manifestations include progressive decreased respiratory function and increased infection susceptibility, pancreatic insufficiency and malabsorption, and chronic inflammatory status[2,3]. Morbidity and mortality are mostly secondary to chronic pulmonary disease[1,3]. Improvements in treatments, quality of life, and clinical outcomes increased median life expectancy in CF patients, which is now above 40 years of age[3].

The improved life expectancy of CF patients also increased the prevalence of new long-term complications, such as CF-related diabetes (CFRD), liver disease, and decreased bone mineral density (BMD)[7]. The prevalence of CF-related bone disease increases with age and includes decreased BMD and increased risk of pathologic fractures. Diminished BMD is a consequence of imbalanced bone remodeling, with increased bone resorption and decreased formation. Bone mineralization occurs mostly during infancy and adolescence and so decreased BMD in CF is thought to originate during these years[8,9].

Factors affecting bone health in CF are not entirely known. Systemic chronic inflammation, pulmonary infections, malnutrition, pancreatic insufficiency, CFRD, calcium and vitamin D and K deficiencies, hypogonadism, delayed puberty, corticoid use, and decreased levels of physical exercise are thought to play a part. Altered function of *CFTR* may also have a direct effect on bone[7,9]. Specifically, the *F508del* gene mutation may slow new bone formation[10], and individuals homozygous or heterozygous for this mutation may have lower BMD compared with other genotypes[11].

Routine bone densitometry by dual-energy X-ray absorptiometry (DEXA) is recommended for BMD evaluation in CF patients starting between the ages of 8 and 10 and should be repeated in different intervals depending on BMD value, presence of risk factors, and age[9,12,13].

Low BMD is more prevalent in children and adolescents with CF when compared to their healthy peers. CF patients can have pubertal delay resulting in suboptimal bone acquisition and lower peak bone mass accrual with premature bone loss [9]. Nevertheless, BMD could be normal in patients with good nutritional status and preserved pulmonary function[8,9]. Physical activity and nutritional programs, with adequate supplementation, should be implemented early on, as well as prompt control of pulmonary infection and systemic inflammation, and minimization of the use of bone toxic medications. Appropriate follow-up of CF patients may prevent the detrimental effect of CF in bone health during growth, diminishing CF-related bone disease in adult patients and its consequences[9].

In 2019, in Portugal, CF patients with less than 18 years were 52.47% of the CF Portuguese population[14]. To the best of our knowledge, currently, there are no studies focusing on reporting BMD, and possible correlation factors, in the CF Portuguese pediatric population. The CF Reference Center of Centro Hospitalar e Universitário de Coimbra (CHUC) follows all patients diagnosed with CF in the center region of Portugal, allowing the study of the CF pediatric population of this region. With this study we aim to retrospectively evaluate BMD in these CF pediatric patients; investigate its relationship with chronological age, age of diagnosis, genotype, organized physical activity, pancreatic function, lung function, nutritional status, and vitamin D status; and identify patients at greatest risk of developing CF-related bone disease.

MATERIALS AND METHODS

Study population

A retrospective study was conducted including all pediatric CF patients who had at least one DEXA scan in the last 10 years, between 2012 and 2021, and were followed in the Pediatric Hospital of the CF Reference Center of CHUC.

Study variables

Diagnosis of CF had been established according to the current standards: positive sweat test and identification of two known disease-causing mutations. Hospital electronic medical records (*SClínico*®) were used to collect patient data. Studied variables included gender, age at DEXA scan in years, age at diagnosis in months, genotype, weight, height, body mass index (BMI), BMD, spirometry results, serum 25-hydroxy-vitamin D (25(OH)D), physical activity, and pancreatic function. The records closest to the date of the DEXA scan were used in this study and registered in an anonymized database.

DEXA scan was used to assess lumbar spine (LS) and total hip (TH) BMD and results were expressed as Z-scores adjusted for gender and age. When Z-scores were below -2, participants were classified as having "CF-related low BMD"[9,12], Z-scores above or equal to -2 were labeled "not low".

Nutritional status was assessed by BMI (weight in kilograms/(height in meters)^2), height, and weight. Values were registered and expressed as Z-scores adjusted for gender and age calculated using the 2000 CDC Growth Charts[15]. Participants were divided into groups of Z-scores below -2 and above/equal to -2. For BMI the groups are identified as "wasted" or "not wasted", respectively. For height as "stunted" or "not stunted" and for weight as "underweight" or "not underweight"[16].

Spirometry results were used to measure lung function. Forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and the ratio FEV1/FVC (Tiffeneau index) were recorded as percentage of predicted adjusted for gender, age, weight, and height. Patients were stratified, by severity of lung disease, in 4 categories defined by FEV1 percentage of predicted. Normal when FEV1 was of 90% or greater, mild when above or equal to 70% but below 90%, moderate when above or equal to 40% but below 70%, and severe when below 40%[17].

Vitamin D status was assessed by measuring serum 25(OH)D. Patients were classified as vitamin D sufficient if 25(OH)D levels were at or above 20 ng/mL, and deficient if levels were below 20 ng/mL[13,18].

Participation, or not, in regular organized physical activity was recorded. Patients were also characterized as being pancreatic insufficient (PI) or sufficient (PS) based on measured levels of fecal pancreatic elastase-1 below/equal to 200 μg/g or above this level, respectively[12].

Statistical analysis

Statistical analysis was conducted using IBM® SPSS® Statistics v27 for MacOS. Significance level was defined as α = 0.05. Descriptive analysis of all variables was done. Data are presented as mean ($\bar{x}$) with standard deviation (SD) when quantitative variables follow a normal distribution or, in non-normally distributed variables, median (p50) with interquartile range (IQR) – difference between the 75th percentile and the 25th percentile. Maximum and minimum values are also presented. Categorical variables are presented as absolute (n) and relative (%) values. Normality of the quantitative variables was assessed by the Shapiro-Wilk test. For normally distributed variables parametric tests were used. For variables not following a normal distribution or with small sample size, inferior to 10, non-parametric tests were used.

Student's t-tests for single samples were used to evaluate differences between the BMD, BMI, height, and weight Z-scores of the children examined and those of the general population, assumed to have a mean value of 0 and SD of 1. They were also used to assess differences in mean BMI, height, weight, and FEV1 between the patients in this study and the Portuguese CF pediatric population of 2019 described in the Annual Report of the European Cystic Fibrosis Society Patient Registry of 2019[14].

Differences in BMD Z-scores between groups were calculated by Mann-Whitney U tests. A student's t-test for paired samples was employed to assess whether there was a difference in the Z-scores for BMD of the LS and TH and the correlation between these values was calculated by computing the corresponding Pearson's coefficient.

Correlations between BMD Z-scores and other variables were examined with Pearson's (r) correlation coefficient (normally distributed variables) or Spearman's (ρ) correlation coefficient (non-normal variables). Absolute values of correlation coefficients were considered as a very weak (0.000 – 0.199), weak (0.200 – 0.399), moderate (0.400 – 0.599), strong (0.600 – 0.799), very strong (0.800 – 0.999) or excellent (1.000) correlation.

Ethics approval

The study of this data was approved by the Ethics Committee for Health of CHUC in March 2017 (CHUC-080-16).

RESULTS

The study included 20 CF patients, followed in this reference center, that had had their BMD assessed at least once by DEXA scan. Demographic and clinical characteristics of the participants are shown in Table 1. Two individuals had CFRD, both with normal LS BMD. Due to the small number of patients with CFRD, its effect on BMD was not studied. Pancreatic insufficiency was present in most patients. Half of the participants had reduced pulmonary function. Other findings included reduced BMD, BMI, height, weight, and 25(OH)D levels. All patients were receiving daily supplements of cholecalciferol and vitamin K. Organized physical activities in these patients included football, judo, karate, gymnastics, running, cycling, swimming, dancing, and tennis. At the time of the DEXA scan, none of the patients were taking *CFTR* modulators.

Table 1. Characteristics of study participants (n=20).

Variables	$\bar{x}$ ± SD or p50 (IQR)	Min; Max	n (%)
Age at DEXA scan (years)	12 (6.75)	8; 17	
Gender			
Male			8 (40%)
Female			12 (60%)
Age at diagnosis (months)	11.5 (41.5)	0; 180	
Genotype			
F508del / F508del			9 (45%)
Heterozygous			
F508del / R334W			4 (20%)
F508del / 711+1G-T			3 (15%)
F508del / 2184insA			1 (5%)
F508del / 3171delC			1 (5%)
F508del / G542X			1 (5%)
N1303K / A561E			1 (5%)
Organized physical activity			
Yes			11 (55%)
No			9 (45%)
Pancreatic function			
Pancreatic sufficient (PS)			3 (15%)
Pancreatic insufficient (PI)			17 (85%)

$\bar{x}$, mean; SD, standard deviation; p50, median; IQR, interquartile range; Min, minimum; Max, maximum; DEXA, dual-energy X-ray absorptiometry.

Table 1 (continuation). Characteristics of study participants (n=20).

Variables	$\bar{x}$ ± SD or p50 (IQR)	Min; Max	n (%)
BMD of lumbar spine (Z-score)	-0.955 ± 1.355	-4.6; 0.9	
Not low			17 (85%)
CF-related low BMD			3 (15%)
BMD of total hip (Z-score) (n=19)[a]	-1.053 ± 1.415	-4.0; 0.7	
Not low			14 (70%)
CF-related low BMD			5 (25%)
Height (Z-score)	-0.508 ± 1.350	-2.61; 2.69	
Not stunted			16 (80%)
Stunted			4 (20%)
Weight (Z-score)	-0.828 ± 1.569	-5.29; 1.45	
Not underweight			16 (80%)
Underweight			4 (20%)
BMI (Z-score)	-0.648 ± 1.275	-2.96; 1.27	
Not wasted			16 (80%)
Wasted			4 (20%)
FVC (%)	81.64 ± 27.80	30.10; 128.30	
FEV1/FVC (%)	93.20 ± 11.16	71.00; 112.20	
FEV1 (%)	79.47 ± 31.31	24.90; 132.90	
Pulmonary Function			
Normal			10 (50%)
Impaired			
Mild			3 (15%)
Moderate			3 (15%)
Severe			4 (20%)
Serum 25(OH)D (ng/mL)	23.46 ± 9.28	9.80; 45.00	
Sufficient			12 (60%)
Deficient			8 (40%)

$\bar{x}$, mean; SD, standard deviation; p50, median; IQR, interquartile range; Min, minimum; Max, maximum; BMD, bone mineral density; BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in one second; 25(OH)D, 25-hydroxi-vitamin D.

[a] Only 19 of the 20 participants had BMD measurements of the total hip, one of the participants only had BMD of the lumbar spine (n=19).

Difference between age at DEXA scan for males (median = 12.5) and females (median = 11.52) was not significantly different (U = 44; p = 0.759).

There was no statistically significant difference between the mean Z-scores for BMD of the LS and TH (t (18) = -0.338, p = 0.739) and there was a strong positive correlation (r = 0.770, p < 0.001) between these variables.

In the studied patients, mean Z-scores for BMI, weight, and BMD, but not height, were significantly lower than the average value of 0 of an age- and gender-matched healthy population. No statistically significant difference was found between the mean values of BMI, height, weight, and FEV1 of the studied patients and the Portuguese CF pediatric population in 2019[14] (Table 2).

Table 2. Single sample T-tests (n=20).

Variables		$\bar{x}$ **± SD**	**Test statistic**	**p value**
Patients vs. general population (0 ± 1)				
BMD of lumbar spine (Z-score)		-0.955 ± 1.355	t (19) = -3.153	**0.005**
BMD of total hip (Z-score)		-1.053 ± 1.415	t (18) = -3.242	**0.005**
BMI (Z-score)		-0.648 ± 1.275	t (19) = -2.271	**0.035**
Height (Z-score)		-0.508 ± 1.350	t (19) = -1.681	0.109
Weight (Z-score)		-0.828 ± 1.569	t (19) = -2.359	**0.029**
Variables	$\bar{x}$ [a]	$\bar{x}$ **± SD** [b]	**Test statistic**	**p value**
Patients vs. Portuguese CF pediatric population 2019[14]				
BMI (Z-score)	-0.3	-0.648 ± 1.275	t (19) = -1.219	0.238
Height (Z-score)	-0.5	-0.508 ± 1.350	t (19) = -0.025	0.980
Weight (Z-score)	-0.6	-0.828 ± 1.569	t (19) = -0.650	0.524
FEV1 (%)	83.8	79.47 ± 31.31	t (19) = -0.618	0.544

$\bar{x}$, mean; SD, standard deviation; BMD, bone mineral density; BMI, body mass index; FEV1, forced expiratory volume in one second.

[a] Mean values for Portuguese CF pediatric (<18 years) population in 2019[14]

[b] Mean ± SD values for studied patients

As shown in Table 3, there were statistically significant differences in the median BMD Z-scores of the total hip between patients enrolled in organized physical activities and those who were not, with the last having lower BMD. The same was not verified for LS BMD.

No statistically significant difference was found in the median BMD Z-scores of both the LS and TH between different gender, genotype (homozygous and heterozygous), pancreatic function (PS and PI), BMI (not wasted and wasted), and serum 25(OH)D (sufficient and deficient) groups.

However, median BMD Z-scores of different groups based on height (not stunted and stunted), weight (not underweight and underweight), and pulmonary function (normal and impaired) were statistically significantly different, with lower BMD present in patients who were stunted, underweight, and had impaired pulmonary function (Table 3).

Table 3. Independent samples non-parametric tests.

Variables	**p50**	**Mann-Whitney U test statistic (U)**	**p value**
BMD of lumbar spine (Z-score)	(n=20)		
Gender		36	0.353
Male	-0.6		
Female	-0.9		
Genotype		47.5	0.879
F508del / F508del	-0.6		
Heterozygous	-1.0		
Organized physical activity		31.5	0.170
Yes	-0.6		
No	-1.0		
Pancreatic function		8	0.063
Pancreatic sufficient (PS)	0.6		
Pancreatic insufficient (PI)	-1.0		
Height		7	**0.018**
Not stunted	-0.6		
Stunted	-2.0		
Weight		11	**0.047**
Not underweight	-0.6		
Underweight	-1.7		

p50, median; BMD, bone mineral density.

Table 3 (continuation). Independent samples non-parametric tests.

Variables	p50	Mann-Whitney U test statistic (U)	p value
BMD of lumbar spine (Z-score)	(n=20)		
BMI		14.5	0.097
Not wasted	-0.6		
Wasted	-1.7		
Pulmonary Function (FEV1)		16.5	**0.011**
Normal	-0.1		
Impaired	-1.15		
Serum 25(OH)D		43.5	0.728
Sufficient	-0.6		
Deficient	-0.9		
BMD of total hip (Z-score)	(n=19)		
Gender		27.5	0.173
Male	-0.5		
Female	-1.2		
Genotype		22.5	0.076
F508del / F508del	-0.15		
Heterozygous	-1.5		
Organized physical activity		14.5	**0.013**
Yes	-0.15		
No	-2.4		
Pancreatic function		22.5	0.867
Pancreatic sufficient (PS)	0.0		
Pancreatic insufficient (PI)	-0.95		
Height		8	**0.028**
Not stunted	-0.3		
Stunted	-2.75		
Weight		4	**0.009**
Not underweight	-0.3		
Underweight	-2.75		

p50, median; BMD, bone mineral density; BMI, body mass index; FEV1, forced expiratory volume in one second; 25(OH)D, 25-hydroxi-vitamin D.

Table 3 (continuation). Independent samples non-parametric tests.

Variables	p50	Mann-Whitney U test statistic (U)	p value
BMD of total hip (Z-score)	(n=19)		
BMI		18.5	0.250
Not wasted	-0.6		
Wasted	-2.2		
Pulmonary Function (FEV1)		8	**0.002**
Normal	0.05		
Impaired	-2.4		
Serum 25(OH)D		19.5	0.057
Sufficient	-0.45		
Deficient	-2.0		

p50, median; BMD, bone mineral density; BMI, body mass index; FEV1, forced expiratory volume in one second; 25(OH)D, 25-hydroxi-vitamin D.

Positive very strong correlation coefficients were found between Z-scores for BMD of the LS and TH and weight Z-scores. Strong correlations were found with FVC, FEV1 (Fig. 1), and BMI (Fig. 2). Moderate correlations with height, at both BMD measuring sites, and FEV1/FVC ratio at the lumbar spine. No statistically meaningful correlations were found between TH BMD Z-scores and FEV1/FVC ratio, and with serum 25-hydroxi-vitamin D, age at diagnosis and at DEXA scan, and both BMD Z-scores of the LS and TH (Table 4).

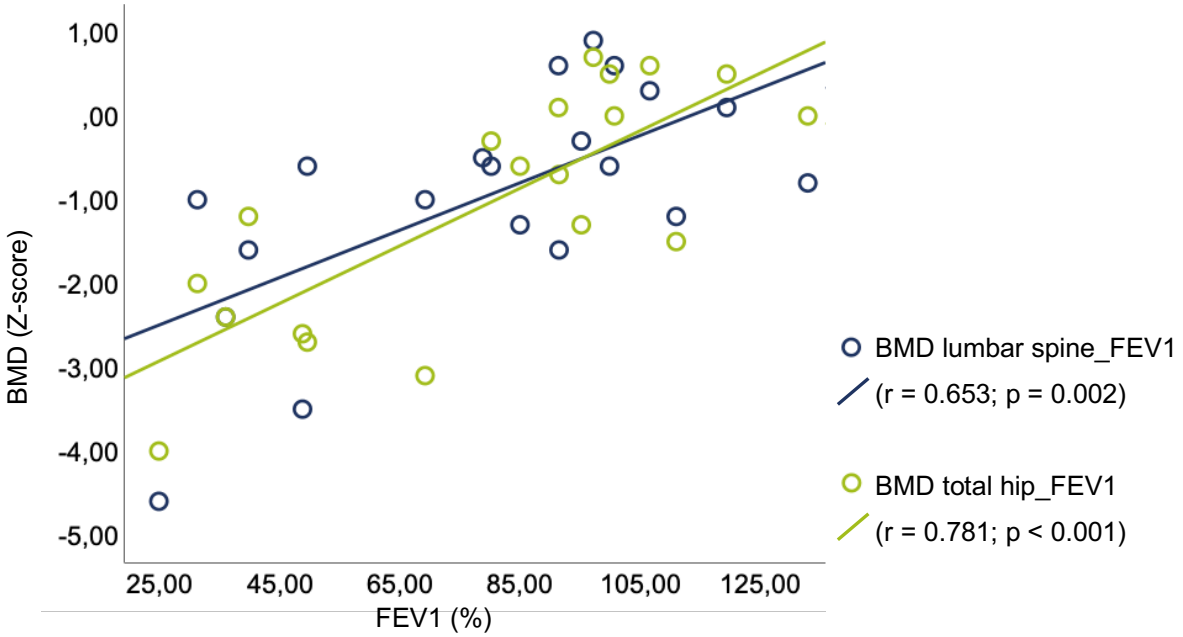

Figure 1. Correlations between Z-scores for BMD of the lumbar spine and total hip and FEV1 percent of predicted.

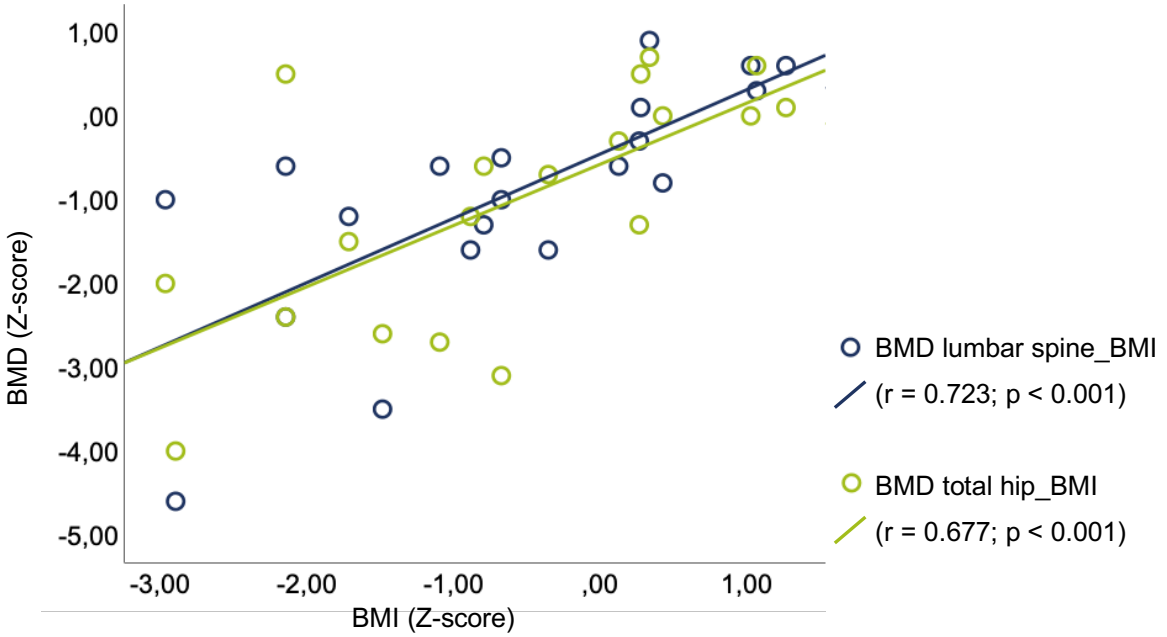

Figure 2. Correlations between Z-scores for BMD of the lumbar spine and total hip and BMI Z-scores.

Table 4. Correlations between BMD Z-scores and different variables.

Variables	**ρ**	**r**	**p value**
BMD of lumbar spine (Z-score) (n=20)			
FVC (%)		0.643	**0.002**
FEV1/FVC (%)		0.452	**0.045**
FEV1 (%)		0.653	**0.002**
BMI (Z-score)		0.723	**< 0.001**
Height (Z-score)		0.565	**0.009**
Weight (Z-score)		0.867	**< 0.001**
Serum 25(OH)D (ng/mL)		-0.016	0.947
Age at DEXA scan (years)	-0.17		0.473
Age at diagnosis (months)	0.142		0.550

r, Pearson's correlation coefficient; ρ, Spearman's correlation coefficient; BMD, bone mineral density; FVC, forced vital capacity; FEV1, forced expiratory volume in one second; BMI, body mass index; 25(OH)D, 25-hydroxi-vitamin D; DEXA, dual-energy X-ray absorptiometry.

Table 4 (continuation). Correlations between BMD Z-scores and different variables.

Variables	ρ	r	p value
BMD of total hip (Z-score) (n=19)			
FVC (%)		0.779	**< 0.001**
FEV1/FVC (%)		0.382	0.106
FEV1 (%)		0.781	**< 0.001**
BMI (Z-score)		0.677	**< 0.001**
Height (Z-score)		0.526	**0.021**
Weight (Z-score)		0.811	**< 0.001**
Serum 25(OH)D (ng/mL)		-0.289	0.230
Age at DEXA scan (years)	-0.249		0.304
Age at diagnosis (months)	0.142		0.561

r, Pearson's correlation coefficient; ρ, Spearman's correlation coefficient; BMD, bone mineral density; FVC, forced vital capacity; FEV1, forced expiratory volume in one second; BMI, body mass index; 25(OH)D, 25-hydroxi-vitamin D; DEXA, dual-energy X-ray absorptiometry.

DISCUSSION

The Pediatric department of the CF Reference Center of CHUC follows all pediatric patients diagnosed with CF in the center region of Portugal. This study illustrated the bone mineralization status of this population and characterized other related variables and their correlation with BMD.

Mean Z-scores for BMI, weight, and BMD, but not height, in our patients were significantly lower than those of the general population, similar to the results of a previous study[19]. Undernourishment is still common in CF patients even with improved follow-up[13]. In our patients mean FEV1 was of 79.47% (80.86% – 92.7% in other studies)[19-21], with 50% of them having impaired pulmonary function (similar to 49% in a previous study)[21]. Pulmonary function, as well as anthropometric measurements, were lower but not significantly different from those of the Portuguese CF pediatric population in 2019[14].

We studied available data for BMD of both the LS and TH. Currently, in individuals with less than 20 years of age, evaluation of BMD is only recommended at the LS[9,12]. Variability in the structural development of the hip meant that it was not considered an ideal DEXA site in the pediatric population. However, recent publications stated that normative data for the hip for healthy children are now available[22], and measuring of this site should be considered in CF patients[23].

There was no difference in median BMD between gender. LS BMD of the participants had a mean Z-score of -0.955, with 15% of the population having CF-related low BMD, with Z-scores inferior to -2. Results were similar in some studies. In a Polish study mean BMD was -0.95 and 17% of the population had low BMD[19]. In Australian CF patients mean BMD was -0.8 and 15.8% had low BMD[24]. In New Zealand[25] mean BMD was -0.92. Other studies had higher mean BMD (-0.4 or 0.1)[20,21] and/or lower percentage of participants with low BMD (8.1%, 8% or 0%)[20,21,26]. These disparities may be justified by differences between the selected patients' clinical status, sample size, and representability, as well as possible different reference data. Furthermore, one study used height-adjusted BMD, minimizing the effect of body size[26].

One of the limitations of our study is the lack of correction of BMD data for height. DEXA evaluation measures an "areal" bone density rather than a true volumetric BMD and is influenced by body size. BMD measured through DEXA can be underestimated in smaller bones and overestimated in larger bones and thus should be corrected for height. This influences both individual and repeated evaluations. Changes in BMD values can be mistakenly interpreted as variations only in bone content rather than also considering changes in body size[9,13,23]. While mean height Z-scores in study participants did not differ from the

expected value of 0, 20% had Z-scores below -2. The absence of correction of BMD for height means that our results may show lower values than the actual BMD of our patients.

BMD was significantly lower in stunted and underweight participants as well as those with impaired pulmonary function. We found positive correlation between BMD values and BMI, height, weight, FEV1, and FVC, reinforcing the notion that poor nutrition and pulmonary function, as markers of disease severity, are determinant factors for lower BMD. Previous published studies also report correlations between BMI and BMD[19,21,24-26], and FEV1 and BMD[19,20,24-26]. In one study conducted in Chile, no significant correlation was found between BMD and FEV1. The authors associated this lack of correlation with lower dispersion of, mostly normal, FEV1 values in their participants[21].

Although participants were taking cholecalciferol supplementation and lived in a mediterranean country with sun exposure, vitamin D deficiency was found in 40% of them. Mean serum 25(OH)D was 23.46 ng/mL which, while not considered vitamin D deficiency, is lower than optimal levels of above 30 ng/mL[18,27]. Similar means of 21.5 – 25.6 ng/mL were found in other studies[19,21,26], showing that low 25(OH)D levels are common even with supplementation and that an increase in doses might be needed. Vitamin D levels vary through the year and should be evaluated at the end of winter[27], which was not the case in all participants of our study. Despite low 25(OH)D levels being usual, we found no significant correlation between this variable and BMD, in accordance with other reports in pediatric populations[19,21,26]. There was also no significant difference in BMD Z-scores between vitamin D sufficient and deficient patients.

Pancreatic insufficiency was present in 85% of patients, which is consistent with other studied populations (67% – 100% were pancreatic insufficient)[19-21,24-26]. No significant difference in BMD was found between pancreatic sufficient and insufficient participants. Variable results were found in the literature, with reports of both correlation[28] and no correlation[21,26] between BMD and pancreatic function.

More than half of the individuals had regular organized physical activity (55%). We found no significant difference in LS BMD between patients enrolled in organized physical activities and those who were not, similar to the results of *Bravo et al*[21]. However, in our participants, TH BMD Z-scores of patients with no physical activity were significantly lower than those physically active.

We found no significant difference in BMD between homozygous and non-homozygous patients, concordant with other reports[24,26]. A study reports correlation between BMD and age at diagnosis[20], another, like us, found no correlation[21]. There was also no correlation found between BMD and age at DEXA scan, as described by *Kuczmarski et al*[19], but some studies

found negative correlation between these variables[20,24,26]. *Sharma et al* studied patients with multiple BMD evaluations and found a decrease in BMD values over time[25]. This emphasizes the impaired bone accrual in CF and suboptimal bone acquisition during adolescence.

The main limitation of our study is the small number of participants, despite including all pediatric CF patients followed in CHUC, that had at least one DEXA scan in the last 10 years. Presently there are no studies characterizing all the pediatric CF population of Portugal even though it represents 52.47% of the Portuguese CF population, with 191 patients[14]. As there is no national epidemiological study and because of the small number of patients (n=20), the sample could be unrepresentative, and the statistical inferential analysis is limited. The lack of correlation between BMD and gender, age at scan, age at diagnosis, 25(OH)D, pancreatic function, genotype, and physical activity (with LS BMD) may merely reflect the small sample size. Considering this limitation, we decided to only do univariate analysis and not to investigate partial and multiple correlations with BMD.

Other drawbacks include the poor characterization of some variables. Physical activity is inadequately reported, being only categorized as present or absent. Recommendations suggest that children and adolescents with CF engage in 20 – 30 minutes of physical activity, particularly weight-bearing exercise, three times a week[13]. In future prospective studies, more accurate, detailed, and measurable data should be obtained regarding physical activity levels and type of physical activity.

Weaknesses also include the study being retrospective and thus the absence of some information. Tanner stage and prevalence of hypogonadism were not known, preventing the evaluation of the possible effect of delayed puberty in low BMD. The use of corticoids, frequency of pulmonary exacerbations, fracture history, and inflammatory status were also not assessed. Detailed information regarding dietary intake was also lacking, as well as vitamin K, calcium, and parathyroid hormone status. All this impairs a full comprehension of the contribution of different factors to CF-related bone disease.

In the future, careful selection of variables and their measurement is needed to evaluate the impact and contribution of each one to bone health. Correction for height and delayed puberty in BMD evaluation should also be considered.

Implementation of new therapies, with *CFTR* modulators, may improve overall health in CF patients, for instance enhancing nutrition and pulmonary function, and eventually having a direct effect on bone[29]. The effect of these interventions in bone health should also be explored in the future.

Regardless of being the only study specifically characterizing BMD and correlated factors in pediatric CF patients in Portugal, the small number of patients and the retrospective design were big limitations. Future multicenter and longitudinal studies are needed to correctly characterize the Portuguese pediatric CF population, its BMD status and relation to other variables.

CONCLUSION

Despite improvement in treatment and follow-up, CF patients are still very much at risk of low BMD. In this study, we found lower TH BMD in patients who were not engaged in organized physical activity. Positive correlation was also found between BMD and pulmonary function (FVC and FEV1), and nutritional status (height, weight, and BMI). This reinforces the notion that patients at greatest risk of developing CF-related bone disease, during childhood, adolescence or earlier in adulthood, were found to be those with severe disease, having decreased pulmonary function and being undernourished.

We stress the importance of a multidisciplinary follow-up, with adequate nutrition, vitamin supplementation, treatments, and implementation of physical activity. Screening for CF-related bone disease, with BMD evaluation, is crucial, particularly in children and adolescents with deteriorating pulmonary function and poorer nutritional status. Implementation of preventive measures can decrease health complications in these vulnerable patients.

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