CLINICAL STUDY

Bone mineral density and quantitative ultrasound in adults with cystic fibrosis

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Abstract

Objective: With increasing life span osteoporosis becomes a more recognized problem in patients with cystic fibrosis (CF). The aim of this cross-sectional study in 75 adult patients with CF (mean age 25.3 years) was to assess the prevalence of low bone mineral density (BMD) by dual-energy x-ray absorptiometry (DEXA) and, for the first time, by quantitative ultrasound (QUS), and to identify predicting factors.

Design and Methods: Bone status was assessed at the lumbar spine (L2-L4) and the femoral neck by DEXA, and at the calcaneus by QUS (stiffness index). These data were correlated with a variety of clinical and anthropomorphic variables. Biochemical markers of bone turnover such as osteocalcin, bone-specific alkaline phosphatase, crosslinks in urine, 25-hydroxy vitamin D (25-OH vitamin D), parathyroid hormone, calcium and free testosterone were determined by standard assays.

Results: The mean BMD T score ($\pm$ S.E.M.) was -1.4 ± 0.17 at the lumbar spine, and -0.54 ± 0.16 at the femoral neck. The mean T score of the calcaneal stiffness index was -0.83 ± 0.19 . Based on a lumbar spine T score < -2.5 by DEXA, 27% of the patients had osteoporosis. Multiple regression analysis showed that the forced expiratory volume in one second (FEV₁) and the use of oral glucocorticoids were independent predictors of low lumbar spine BMD, whereas body mass index (BMI) and the use of oral glucocorticoids were independent predictors of low femoral neck BMD. The stiffness index correlated moderately with BMD ($0.49-0.62$, $P < 0.0001$). QUS had a sensitivity and specificity of only 57% and 89% respectively for diagnosing 'osteoporosis' (based on a femoral neck T score < -2.5 by DEXA). Positive and negative predictive values were 36% and 95% respectively.

Conclusions: Low BMD is frequent in adults with CF and is most strongly correlated with disease severity (BMI, FEV₁) and the use of glucocorticoids. Calcaneal QUS might help to screen out patients with a normal BMD, but sensitivity and specificity were not sufficiently high to replace DEXA in these patients.

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Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disease with fatal outcome in Caucasians (1). The incidence is about 1:2000 newborns in Europe. Due to improved therapy, life expectancy has increased to more than 30 years nowadays (2). With increasing age, patients with CF develop a number of new and unusual sequelae, including the CF-associated osteoporosis. Numerous studies have shown a high prevalence of low bone mineral density (BMD) in these patients (3–6). They have an increased fracture rate compared with healthy age-matched controls (7, 8) and rib fractures in particular may lead to deterioration in their lung function.

The pathogenesis of low BMD in these patients is incompletely understood. A variety of potential risk

factors may contribute to the development of osteoporosis, such as calcium and vitamin D malabsorption, malnutrition, low body mass index (BMI), delayed puberty, hypogonadism, reduced physical activity, glucocorticoid therapy and increased levels of osteoclast-activating cytokines (interleukin (IL)-1, IL-6, tumor necrosis factor α). As a consequence, young patients with CF fail to reach a normal peak bone mass (9), and adult patients with CF show an accelerated bone loss leading to early manifestation of osteoporosis (10).

Previous cross-sectional studies have shown partly contradicting results about correlated factors (3–6, 11). The aim of this study was to assess the prevalence of this disorder in a large group of adult patients with CF and to assess multiple risk factors and their impact on BMD. A second aim was to compare quantitative

ultrasound (QUS) of the calcaneus to dual-energy x-ray absorptiometry (DEXA) in patients with CF. QUS might be interesting as a screening tool, as it is easy to perform, quick, and free of radiation.

Materials and methods

Patients

The study was a cross-sectional, observational evaluation of adult CF patients. A total of 75 patients were recruited from the regional adult CF center without any particular selection criteria. Diagnosis was made by either genotyping or pathologic sweat tests and typical clinical presentation. The examinations were performed between July 1998 and December 2000 during routine visits at the outpatient center for CF of the Department of Pneumology, University Hospital of Freiburg, Germany.

Apart from DEXA osteodensitometry, calcaneus sonography and laboratory measurements, the following parameters were determined: BMI, history of fractures, vitamin D and calcium intake (oral supplements), and current use of oral or inhaled glucocorticoids. FEV₁ (forced expiratory volume in one second) was determined by spirometry and was expressed as a percentage of predicted value.

Bone density assessment

BMD was measured by DEXA using a Lunar DPX-IQ (Lunar Germany, Bad Nauheim, Germany). Scans were performed at two different sites: lumbar spine (L2-L4) and femoral neck. The coefficient of variation for BMD measurements in our department is 0.01–0.015 g/cm² (≈ 1 –1.5%). Results were expressed as BMD (g/cm²) and T scores representing standard deviations from young, healthy control subjects. T scores were classified according to the WHO criteria as normal (T score > -1), osteopenia ($-1 > \text{T score} > -2.5$) or osteoporosis (T score < -2.5). Simultaneously, QUS of the calcaneus was performed expressed as stiffness index (Achilles+, Ultrasound Densitometer, Lunar Germany). The stiffness index combines the parameters speed of sound (SOS) and broadband ultrasound attenuation (BUA). The coefficient of variation of the stiffness index is 1.7%. Results were expressed as T scores. The reference data were those provided by the DEXA and sonography manufacturers.

Laboratory measurements

Serum calcium was measured by a colorimetric assay (Hitachi Analyzer), 25-OH vitamin D was determined by a radioimmunoassay (Biosource Europe, Nivelles, Belgium), intact parathyroid hormone (PTH) by a chemiluminescence assay (Nichols Advantage, Nijmegen, The

Netherlands), free testosterone by a radioimmunoassay (DPC, Los Angeles, CA, USA), osteocalcin by a radioimmunoassay (Labor Limbach, Heidelberg, Germany), and bone-specific alkaline phosphatase (BAP) by an immunoassay (Metra Biosystems, Osnabrück, Germany). Pyridinoline and deoxypyridinoline were measured in morning urine samples by high-performance liquid chromatography (HPLC).

Statistical analysis

All statistical analyses were performed with Stat View 5 (SAS Institute Inc., Cary, NC, USA). Data are presented as means $\pm$ S.E.M. (standard error of the mean). BMD and T scores were correlated with the other variables using Spearman's rank correlation or linear regression analysis. Associations between BMD and nominal variables were assessed by *t*-tests. To determine which of the significantly associated variables predicted bone density after adjustment for covariables, multivariate analysis was performed by a stepwise regression analysis. A *P* value of less than 0.05 was considered significant.

Results

The main characteristics of the 75 adult patients with CF are summarized in Table 1. Twenty percent (11/55) had a positive fracture history (at various sites, not further differentiated). The prevalence of low BMD is shown in Table 2. The stiffness index showed moderate correlation with the lumbar spine BMD ($r = 0.49$, $P < 0.0001$) and the femoral neck BMD ($r = 0.62$, $P < 0.0001$). Using the femoral neck BMD to diagnose 'osteoporosis' (T score < -2.5) and calculating with a cut-off T score of the stiffness index of < -2.5 , we found a sensitivity of only 57%, a speci-

Table 1 Clinical and biochemical parameters of 75 adult patients with CF, indicated as mean levels $\pm$ S.E.M.

Parameter	Mean $\pm$ S.E.M.	Normal range
Age (years)	25.3 $\pm$ 0.8	
Gender (male/female)	38/37	
BMI (kg/m ²)	19.9 $\pm$ 0.3	
FEV ₁ (%)	54.3 $\pm$ 3.1	
Calcium (mmol/l)	2.38 $\pm$ 0.02	2.15–2.75
25-OH vitamin D (ng/ml)	27.7 $\pm$ 2.3	20–120
PTH (pg/ml)	45.9 $\pm$ 2.9	10–70
Osteocalcin (ng/ml)	4.6 $\pm$ 0.45	3–12
BAP (U/ml)	32.9 $\pm$ 2.7	10–23
Pyridinoline (nmol/mmol crea/h)	113.4 $\pm$ 9.3	40–100
Deoxypyridinoline (nmol/mmol crea/h)	16.9 $\pm$ 1.6	9–20
Free testosterone (pg/ml)	15.1 $\pm$ 1.2	9–47

Free testosterone was only determined in males.

Table 2 BMD of the lumbar spine and the femoral neck and QUS of the calcaneus (measured as stiffness index) in 75 adult patients with CF.

	Mean $\pm$ S.E.M.	Normal (T score > -1)	Osteopenia ($-1 > \text{T score} > -2.5$)	Osteoporosis (T score < -2.5)
T score LS	-1.40 ± 0.17	39%	34%	27%
BMD LS (g/cm ²)	1.057 ± 0.02			
T score FN	-0.54 ± 0.16	61%	28%	11%
BMD FN (g/cm ²)	0.953 ± 0.02			
Calcaneus stiffness	-0.83 ± 0.19	54%	46% (T score < -1)	

LS = lumbar spine, FN = femoral neck.

ficity of 89%, and a positive and negative predictive value of 36 and 95% respectively.

Among the potential predictors of bone status, FEV₁, BMI, and the use of oral steroids correlated with all three measured sites, the lumbar spine, the femoral neck, and the stiffness index (Table 3). FEV₁ in particular showed a strong correlation in a linear regression model (Fig. 1). All other factors, such as age, sex, serum calcium, 25-OH vitamin D, PTH, and free testosterone in males did not consistently correlate with all three sites. Stepwise multiple regression analysis with backward elimination of 11 variables showed that FEV₁ and the use of oral steroids were the only independent predictors of lumbar spine BMD, accounting for 63% of the variation. For the femoral neck BMD, BMI and the use of oral steroids were independent predictors, accounting for about 34% of the variation. Regarding the calcaneus, BMI and sex showed the strongest correlation with the stiffness index, accounting for 36% of the variation.

Fifteen (20%) patients received oral glucocorticoids and 44 (60%) patients inhaled glucocorticoids at the time of bone mineral assessment. The mean lumbar spine T score in the group receiving oral glucocorticoids was significantly lower compared with the groups with

inhaled or no glucocorticoids (Fig. 2). Identical results were found for the femoral neck and the calcaneus.

Seventy-seven percent of all patients received oral vitamin D (800–1200 IU/day in single or multiple vitamin preparations) and/or calcium (usually 1000 mg/day) at the time of bone mineral assessment. Twenty-three out of 73 patients (32%) had vitamin D deficiency (25-OH vitamin D < 15 ng/ml) (12), although 18 of them (78%) were taking oral vitamin D preparations. There was no significant association between 25-OH vitamin D levels and BMD.

Twenty-one percent of the male patients (7/34) had decreased free testosterone levels (< 9 ng/ml), but free testosterone levels in males did not correlate with BMD. The lumbar spine T score in male patients was significantly lower compared with female patients (-1.9 vs -0.9 , $P = 0.004$). The same difference was found at the femoral neck, but not at the calcaneus. The male patients also had a significantly lower FEV₁ (46.8% vs 63.3%, $P = 0.02$) and lower 25-OH vitamin D levels (18 ng/ml vs 31 ng/ml, $P = 0.01$). BMI and age were almost identical in males and females.

The mean levels of the biochemical bone markers are shown in Table 1. Thirty-nine patients (56%) had elevated BAP levels. Crosslinks (pyridinoline and deoxy-

Table 3 Correlates of lumbar spine and femoral neck BMD and calcaneal QUS (stiffness index) determined by Spearman's rank correlation.

Variable	Lumbar spine BMD		Femoral neck BMD		Stiffness index	
	Coefficient of correlation	P value	Coefficient of correlation	P value	Coefficient of correlation	P value
FEV ₁	0.62	< 0.0001	0.62	< 0.0001	0.47	0.0001
BMI	0.55	< 0.0001	0.56	< 0.0001	0.56	< 0.0001
Age	0.06	0.63	-0.2	0.09	0.04	0.74
Sex (M vs F)	0.28	0.02	-0.03	0.77	-0.2	0.09
Oral steroids	-0.44	0.0002	-0.42	0.0003	-0.35	0.004
25-OH vit D	0.18	0.13	0.09	0.44	-0.05	0.66
PTH	-0.19	0.13	-0.14	0.26	0.02	0.87
OC	-0.26	0.04	-0.04	0.79	-0.23	0.09
BAP	-0.40	0.001	-0.11	0.40	-0.16	0.22
Pyd	-0.23	0.08	-0.12	0.35	-0.24	0.07
Dpd	-0.20	0.12	-0.19	0.14	-0.17	0.20
Testosterone	0.15	0.39	0.06	0.75	0.23	0.21

M = male, F = female, Oral steroids = use of oral steroids (yes/no), 25-OH vit D = 25-hydroxyvitamin D, OC = osteocalcin, Pyd = pyridinoline, Dpd = deoxypyridinoline, Testosterone = free testosterone in males.

Figure 1 Coefficient of correlation between FEV₁ and lumbar spine BMD in a linear regression analysis ($r = 0.68$, $P < 0.0001$, $R^2 = 0.46$).

pyridinoline) were elevated in 44% and 25% respectively. Bone formation markers (osteocalcin and BAP), but not bone resorption markers (pyridinoline and deoxypyridinoline) were negatively correlated with the lumbar spine BMD.

Discussion

The first description of demineralization in children with CF dates back to 1979 (13). Other reports published since mostly confirmed this observation (14–16). Increasing evidence suggests that this is a problem of adolescence and delayed puberty as prepubertal children with CF appear to have a normal BMD (17–19).

This study confirms that low BMD is common in adult patients with CF. Sixty-one percent had osteopenia or preclinical osteoporosis at the lumbar spine measured by DEXA, and 39% at the femoral neck.

Figure 2 Distribution of mean lumbar spine T scores grouped by corticosteroid use.

These figures are comparable to previous reports with a similar group of unselected CF patients in an ambulatory setting (3, 4, 6, 11). Other studies reported an even higher prevalence of osteoporosis and an increased fracture rate (5, 7). This may be partly explained by a more advanced state of disease, as Aris *et al.* (7) and Donovan *et al.* (5) examined CF patients with end-stage disease awaiting lung transplantation.

The BMD measurement is an areal density not correcting for bone volume. With CF patients having a low body size, DEXA may lead to falsely low BMDs. Previous studies have already addressed this problem and corrected for bone size by using bone mineral apparent density (3, 20) or quantitative computed tomography (6), all leading to similar results. Therefore, we concluded that it was sufficient to use standard DEXA to measure BMD.

This study investigated, for the first time, the potential role of QUS in adult patients with CF. QUS has been shown to predict fracture risk in post-menopausal osteoporosis (21), even independently of DEXA (22). QUS is quicker and easier to perform than DEXA, and free of x-ray exposure. It might therefore be an ideal tool for screening. In this study, QUS correlated with DEXA in CF patients to a similar degree as in other populations with secondary osteoporosis (23). The negative predictive value of 95% suggests that QUS has a potential to screen out CF patients with a normal BMD. However, the low positive predictive value of 36% indicates that the patients diagnosed osteoporotic by QUS would require further investigation with, for example, DEXA to confirm the diagnosis of osteoporosis.

Some studies did not find a relationship between bone status and pulmonary function tests or disease severity (5, 7), but those reports investigated severely affected patients with end-stage CF and a narrow range of pulmonary function. In our study, the range of pulmonary function was wider (FEV₁: 11–123% of predicted) and FEV₁ was the strongest predictor of BMD. Similarly, BMI, reflecting nutritional status, was highly significantly correlated with BMD and QUS. Both these factors reflect disease severity, but are not specific for CF. There is strong evidence that disease severity has the greatest impact on BMD in CF (3, 4, 6, 11). Moreover, one report suggested that patients with CF and a normal nutritional status had a normal BMD (24). Apparently, the pathogenesis of CF-associated low bone mass is a secondary result of disease severity and hence physical activity, but no specific effect of cystic fibrosis transmembrane conductance regulator (CFTR) on bone mineral metabolism. To date, there is no known effect of genotype on bone phenotype in CF.

Glucocorticoid therapy for pulmonary function is common in CF patients. The influence of glucocorticoids on BMD is well known and has previously been described in patients with CF (7, 25). The patients

receiving oral glucocorticoids had a markedly lower BMD, either due to the glucocorticoids themselves or because this group represented the more severely affected patients. This observation strengthens the importance of preferring inhaled glucocorticoids over systemic use wherever possible.

As in previous studies reported (3, 6) we found a significantly lower BMD in men compared with women. Conway *et al.* (3) and Haworth *et al.* (6) demonstrated that their male patients had equal lung functions or were clinically even healthier than the female patients. There was no apparent reason for the difference. In our study, male patients had a significantly lower FEV₁, possibly contributing to the lower BMD. The lack of sex hormones might play a role as was shown in another cross-sectional study (11), although there was no significant correlation between free testosterone levels and BMD found in our study.

Patients with CF are known to have a reduced vitamin D absorption due to their pancreatic insufficiency (26). This may, therefore, represent an important risk factor for the development of demineralization in these patients. Surprisingly, only one study showed a direct association between low 25-OH vitamin D levels and low BMD (5), whereas our study and many others did not (6, 7, 20, 25, 27).

Increased levels of biochemical bone markers indicate a high bone turnover in these patients. Baroncelli *et al.* have already postulated an imbalance of bone formation and bone resorption in young patients with CF as the cause of reduced mineralization in these patients (28). There is data showing a reduced mineralization during adolescence (9), but also an accelerated bone loss in adults of more than 2% per year (10), consistent with the high turnover found in our study. Interestingly, Aris *et al.* described a relationship of bone markers and disease severity in patients with CF (29).

In conclusion, this study confirms that low BMD is a frequent observation in adults with CF. QUS of the calcaneus cannot replace DEXA as standard method, but it may help to screen out patients with a normal BMD. Low bone mass in CF depends greatly on disease severity (FEV₁ and BMI) and the use of oral steroids. First line therapeutic strategies should aim for amelioration of lung function and hence exercise capacity as well as nutritional status in these patients.

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