CHAPTER 11

Diagnosis and management of cystic fibrosis related low bone mineral density

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In 1938, Anderson [1] described the clinical and pathological features of cystic fibrosis (CF). Anderson [1] related the clinical syndrome to a lesion in the pancreas and observed that bronchiectasis, fatty degeneration of the liver, vitamin A deficiency and osteoporosis were common. Since Anderson's initial description of the disease there has been a dramatic accumulation of knowledge about the underlying pathophysiology of CF, and in parallel with this, improved treatment has transformed a disease characterised by childhood death into one with a mean actuarial survival extending into the fourth decade. With this improved survival, clinically relevant extrapulmonary complications of the disease have emerged, such as low bone mineral density (BMD) and fragility fracture.

Low BMD was first reported in patients with CF in 1979 [2, 3] and since that time research has concentrated on documenting the prevalence, natural history, prevention and treatment of CF related low BMD. This chapter will summarise current knowledge, outline the author's thoughts on management and discuss current areas of uncertainty that require further research.

Definition

Osteoporosis is a systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture [4]. Osteoporosis is a disorder in which there is a reduction of bone mass in the absence of a change in the ratio of mineralised and nonmineralised bone, and osteomalacia is a disorder where there is an increase in the proportion of nonmineralised bone. As a result of pancreatic insufficiency and the malabsorption of fat-soluble vitamins, patients with CF may develop low BMD from either osteoporosis or osteomalacia.

Fractures

Fragility fracture is the major clinical consequence of low BMD and unless bone densitometry becomes part of routine practice, the diagnosis of osteoporosis will continue to be made after a fragility fracture has occurred. Disordered bone metabolism is an asymptomatic process and once low BMD has declared itself through fragility

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fracture, the opportunity to prevent the development of osteoporosis and its long-term sequelae have been lost.

The prevalence of fragility fracture in children and adults with CF remains unclear. To date, there have been no published reports of fracture rates in large unselected CF populations with robust control data. There have been a number of case reports of fragility fractures that had devastating clinical consequences [5–7] (figs 1 and 2) and a number of studies reporting population-based fracture rates. Henderson and Specter [8] reported that females with CF aged 6–16 yrs had higher fracture rates than controls or male CF patients. Elkin *et al*. [9] reported that 35% of adults had a history of fracture, 9% of which were rib fractures. Aris *et al*. [10] reported fracture rates two-fold higher in females aged 16–34 yrs and in males aged 25–45 yrs. Rib and vertebral fractures were particularly prevalent, being 10- and 100-fold more common than in the general population. This study reported a particularly high vertebral fracture rate, possibly as a consequence of the participants having end-stage lung disease and being considered for lung transplantation. In this series the majority of the vertebral wedge fractures were relatively mild with 60% of the fractures showing anterior height loss of 10–25%, but 6% fractures showed anterior height loss of >40%.

Rib and vertebral fractures are particularly relevant in patients with CF because they can be associated with a rapid decline in lung function, either through causing a pneumothorax or by inhibiting effective sputum clearance. Vertebral fracture, vertebral deformity and kyphosis can lead to a reduction in forced vital capacity and reduced

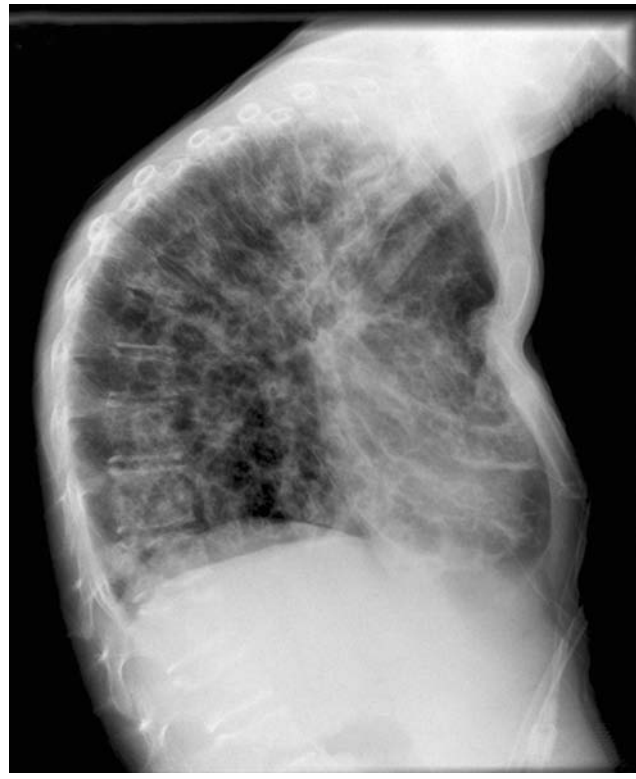

Fig. 1. – Lateral chest radiograph of a 16-yr-old female with cystic fibrosis and severe lung disease and osteoporotic fractures of the sternum and the vertebral bodies of B6 and B7. Reproduced with permission from [6].

Fig. 2. – Magnetic resonance imaging scan showing bilateral fractured neck of the femur sustained during grand mal seizures in a 34-yr-old male with cystic fibrosis and osteoporosis. Reproduced with permission from [7].

ventilatory efficiency. Some centres also view osteoporosis as a relative contraindication to lung transplantation. Thus established osteoporosis may result in reduced longevity in CF patients.

It is unclear if the increased fracture rate in current adult cohorts is due to the less advanced nutritional and pulmonary care available in the previous two decades. Advances in all aspects of CF care may now allow normal bone development and lead to a reduced incidence of fracture in the future. Alternatively, if the pathogenesis of CF-related bone disease is a cystic fibrosis transmembrane regulator (CFTR)-related process, bone health may not benefit from these advances in treatment. However, for the current generation of adults, fracture rates are likely to increase as they get older due to normal age-related bone loss unless bone-sparing treatments are instituted.

Diagnosis

BMD is most commonly measured through dual energy X-ray absorptiometry (DXA). This technique measures areal BMD ($g \cdot cm^{-2}$) of the total body, lumbar spine, proximal femur and distal forearm. BMD results are compared with reference populations and reported as standard deviation scores from the mean. The Z score is the standard deviation score from the mean BMD of an age- and sex-matched control population. The T score is the standard deviation score from the mean BMD of a control population of sex-matched young adults at peak bone mass. Peak bone mass is normally achieved during the third decade of life, at which time Z and T scores are similar.

The World Health Organization (WHO) working definition of osteoporosis, based on bone densitometry, is summarised in table 1. This system is validated in postmenopausal females but the relationship between BMD and fracture risk has not been established in other groups. For this reason the authors recommend that in the context of CF: 1) BMD values should be expressed as Z scores in premenopausal females and in males <50 yrs and as T scores thereafter. 2) The term osteoporosis should be confined to those with a history of fraglity fracture. 3) The term low BMD be applied to children or adults with a

Table 1. – The World Health Organization (WHO) working definition of osteoporosis

Normal	BMD not >1 SD below the average value for young adults.
Low bone mass	BMD >1 SD below the young adult average but no more than 2.5 SD below.
Osteoporosis	BMD >2.5 SD below the young adult average value.
Severe osteoporosis (established osteoporosis)	BMD >2.5 SD below the young adult average value and the presence of one or more fragility fractures.

BMD: bone mineral density; SD: standard deviation.

BMD Z score <-2, with the caveat that Z scores may be unreliable in individuals of small body size.

DXA results must be interpreted with caution in children and in individuals with small stature [11]. DXA is a two- rather than a three-dimensional measurement and can lead to erroneous assessments of the depth of the bone, resulting in falsely low BMD results in patients with small bones. Various corrections for body size can be performed, but these have not been validated in CF and do not necessarily accurately predict future fracture risk. Additional adjustments can be made for pubertal status, skeletal maturity and body composition, but at present there is no consensus on the optimum method to use. There is also a paucity of robust normative BMD data for children. Thus Z scores must be interpreted in the light of the best available paediatric reference databases and the reference database should be sited in the report.

Cross-sectional bone mineral data

Early studies investigating bone mass in CF used single photon absorptiometry, a technique that only evaluates the appendicular skeleton that is composed mainly of cortical bone. Hahn *et al.* [3] reported a 14% decrease in bone mass of the distal forearm and Mischler *et al.* [2] assessed bone mass in a group aged 5–24 yrs and found demineralisation in 37% of young males and 63% of young females aged >15 yrs.

As technology advanced, dual energy ray absorptiometry and quantitative computed tomography (QCT) were used to measure BMD in the lumbar spine and proximal femur, sites rich in trabecular bone. Gibbens *et al.* [12] reported a 10% reduction in lumbar spine BMD compared with local controls using QCT in 57 patients with a mean age of 12 yrs. Three specialist CF centres in the UK performed detailed adult prevalence studies. The Manchester CF Centre (Manchester, UK) reported that 34% (48 out of 143) of adults had a BMD Z score of <-2 in the lumbar spine, proximal femur or distal forearm [13]. The Leeds CF Centre (Leeds, UK) reported that 66% of 114 patients had osteopenia or osteoporosis defined by WHO criteria using T scores [14]. The Royal Brompton Hospital reported that 38% of 107 patients had Z scores of <-1, with 13% having Z scores <-2 [9]. Numerous studies have followed, the larger of which are summarised in table 2. These studies show that 15–25% of CF adults have a BMD Z score <-2 in the lumbar spine or proximal femur.

Change in BMD with age

Buntain *et al.* [16] performed an outstanding controlled cross-sectional study involving 153 subjects with CF and 149 local healthy controls. They found normal BMD in children with CF, normal axial BMD (after adjustment for height) in adolescents with CF and a significant reduction in axial and total body BMD in adults with CF. Bhudhikanok *et al.* [15] studied 41 CF patients aged 9–50 yrs. BMD increased by 1–2%

Table 2. – Summary of cross-sectional studies of bone mineral density (BMD) in adults with cystic fibrosis

First author [Ref.]	Subjects M/F	Age yrs	Method of measurement	Site	Low BMD
HAHN [3]	21 (12/9)	12–36	SPA	Distal radius	14% reduction
GIBBENS [12]	57 (29/28)	3–21	QCT	LS	10% mean reduction
BHUDHIKANOK [15]	49 (19/30)	10–48	DEXA	LS, FN, Total body	53% Z<-2
ARIS [10]	70 (35/35)	>18	DEXA	LS, FN, Total body	39% T<-1, 57% T<-2.5
HAWORTH [13]	151 (83/68)	15–52	DEXA QCT SXA	LS, FN, Distal radius	34% Z<-2
CONWAY [14]	114 (53/61)	16–34	DEXA	LS, FN Total body	55% males and 43% females have T score <-1
ELKIN [9]	107 (58/49)	18–60	DEXA	LS, FN Total body	38% Z score <-1, 13% Z score <-2
BUNTAIN [16]	153 (84/59)	5–56	DEXA	LS, FN Total body	Adolescents reduced TB Adults reduced at all sites
KING [17]	88 (53/35)	Mean 30	DEXA	LS, FN	17% Z<-2

M: male; F: female; SPA: single photon absorptiometry; QCT: quantitative computed tomography; LS: lumbar spine; DEXA: dual energy X-ray absorptiometry; SXA: single energy X-ray absorptiometry; FN: femoral neck.

per year in axial sites, except in adult males (n=6) who experienced a 1.2% reduction in the femoral neck. The BMD Z scores of the younger patients reduced as they aged, suggesting that increases were less substantial than those expected in normal age-matched controls. HAWORTH *et al.* [18] prospectively measured the BMD of 114 CF adults. In 55 patients with a mean age of 19 yrs, in whom BMD would normally be expected to increase annually before reaching peak bone mass, BMD was stable in the lumbar spine but reduced by >2% per year in the proximal femur. In 59 patients with a mean age of 30 yrs, in whom BMD would normally be expected to remain stable having achieved peak bone mass, there were no significant changes in the lumbar spine but there were significant annual reductions in the proximal femur (-1.5%) and distal forearm (-0.8%). Collectively, these studies suggest BMD is satisfactory in well nourished children with preserved lung function, following which many patients experience premature bone loss in adolescence/early adult life.

Bone histomorphometry

Bone histomorphometry can detect alterations in bone structure and remodelling that are not detected with qualitative methods. Interactive computerised methods are used to perform complex measurements involving two-dimensional images and applications of formulae enable the extrapolation of data to three-dimensional quantities. Bone histomorphometry has helped to characterise the bone disease in CF and has shown that although osteomalacia does occur in this population [19], osteoporosis is the predominant pathology [20].

In 2000, HAWORTH *et al.* [21] published data from an analysis of autopsy bone samples from 15 CF patients and 15 young adult controls. Eleven patients had undergone transplants and as a consequence had received immunosuppressive medications. The histomorphometrical data were indicative of decreased osteoblastic and increased osteoclastic activity. The reduction in osteoblastic activity was due to a reduction in osteoblastic number and a decrease in the biosynthetic potential of osteoblasts, as proven by osteoid production. The osteoclastic changes were due to an increase in the number of osteoclasts and an increase in resorptive activity. However, in the absence of tetracycline labelling, dynamic indices of bone formation and resorption could not be assessed.

A detailed analysis of iliac crest bone biopsies after double tetracycline labelling was published in 2001 [20]. Data were from 20 CF adults with recent DXA Z scores of <1.5 at

the lumbar spine or femoral neck. Patients were excluded if they had undergone transplantation. Control data were gained from 18 age- and sex-matched healthy subjects. One patient had osteomalacia as defined by strict histomorphometric criteria. This patient had a low serum 25-hydroxyvitamin D (25OHD) of 20 $nmol \cdot L^{-1}$ and a raised parathyroid hormone (PTH) of 199 $pg \cdot mL^{-1}$. Other significant findings were a significantly lower cancellous bone area in the CF group, a decrease in bone formation rate, wall width and mineral apposition rate and an increased in mineralisation lag time when compared with control (fig. 3). Structural analysis revealed a trend towards decreased connectivity. Resorption cavities were significantly reduced when compared with controls and osteoclast numbers were not increased. There were no significant differences between male and female patients in any indices of remodelling. A subgroup analysis was performed on those prescribed (n=8) and not prescribed (n=12) glucocorticoids. This revealed significant reductions in bone formation rate, mineralisation perimeter and activation frequency in the steroid-treated group, but there was no difference in activity of osteoblasts represented by mean wall width and the mineral apposition rate.

These histomorphometry studies confirm that the low BMD identified by densitometry in CF adults is real. Although rare, some patients have osteomalacia and measuring the 25OHD and PTH concentrations will probably identify these individuals without having to perform a biopsy. Reduced bone formation appears to be the predominant problem, possibly due to inhibition of mature osteoblasts or increased osteoblast apoptosis. However, it is likely that increased bone resorption occurs during periods of ill health/infection.

Bone turnover markers

Biochemical markers of bone turnover are products of bone cells or products released during the degradation of type 1 collagen. They show high variability with peak concentrations occurring in the morning, which makes the timing of samples important. In general, studies using bone turnover markers in CF agree with the histomorphometrical findings. Measurements performed in adult CF populations show uncoupling of bone balance with decreased bone formation markers [22, 23] and increased markers of bone resorption when compared with control [22, 24]. The routine measurement of biochemical markers of bone turnover in the clinical care of individuals with CF is not recommended, although the markers may be used to predict gain in BMD following therapy with antiresorptive agents.

Aetiology and risk factors for low BMD

Influence of mutant CFTR

It is currently unclear if the disordered bone metabolism in CF patients is directly related to abnormal CFTR function [25]. It is not known if CFTR is expressed in osteoblasts or osteoclasts and other organs where mutant CFTR could potentially affect bone metabolism include the gut, kidney and the parathyroid glands. King *et al.* [17] reported lower BMD in patients with the DF508 genotype and Haworth *et al.* [13] reported high levels of bone turnover in DF508 homozygotes compared with heterozygotes. However, the cross-sectional and longitudinal BMD studies indicate that bone health deteriorates in adolescence/early adult life, suggesting that the disordered bone metabolism in CF is most likely due to the secondary complications of CFTR dysfunction.

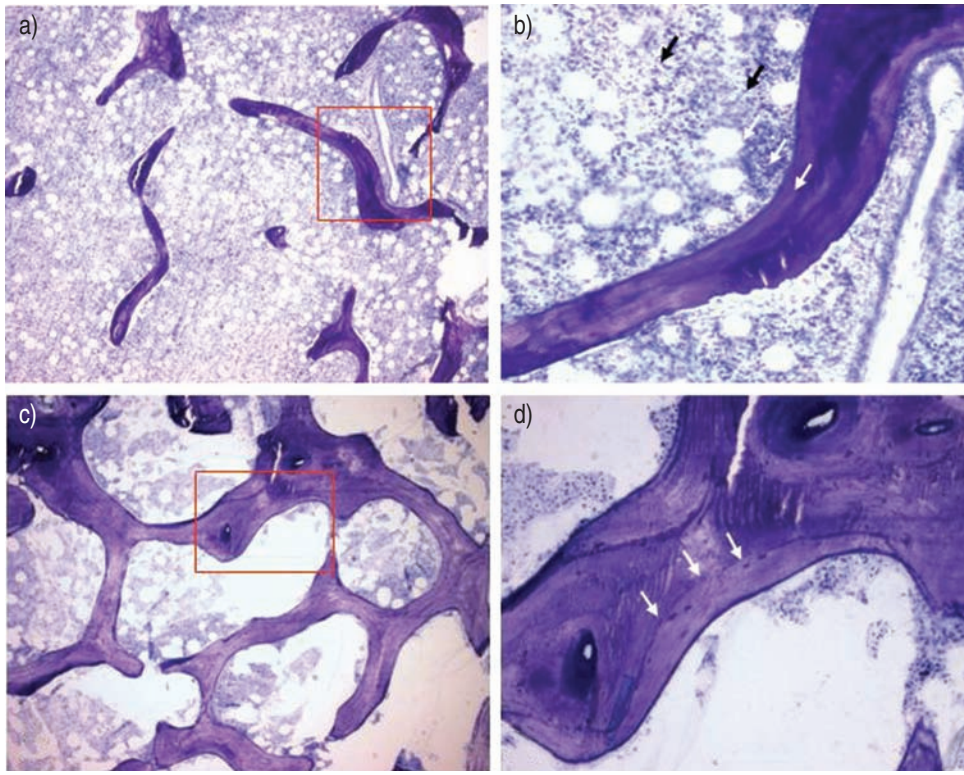

Fig. 3. – A representative cystic fibrosis (CF) bone biopsy alongside a normal control biopsy. (a) CF subject: Toluidine blue stained section of iliac crest bone biopsy showing decreased cancellous bone connectivity and decreased cancellous bone area. b) CF subject: Higher power magnification of boxed area demonstrating thin wall width as compared with control. c) Normal subject: Toluidine blue stained section of iliac crest bone biopsy from a normal patient demonstrating good connectivity of cancellous bone. d) Normal subject: Higher power magnification of boxed area showing normal wall width of two complete packets. White arrows: wall width of bone packets; black arrows: crenated surface of a shallow resorption cavity. Reprinted from Elkin *et al.* [20] with permission.

Disease severity and pro-inflammatory cytokines

Numerous cross-sectional studies have been performed in CF populations and there seems little doubt that low BMD is associated with disease severity as represented by forced expiratory volume in one second (FEV1), body mass index (BMI), number of *i.v.* antibiotics days and exercise tolerance [9, 10, 12–16, 18]. Those awaiting transplantation invariably have low BMD [26], whilst patients with a good BMI and lung function appear to have near-normal bone density [27, 28].

Acute pulmonary infection in CF is associated with increased circulating levels of interleukin (IL)-6, IL-1 and tumour necrosis factor (TNF)-α. These cytokines are also known to increase osteoclast formation and activity [29]. The first evidence that systemic inflammation may play a role in bone loss was in 1999 when Haworth *et al.* [13] reported serum C reactive protein to show an inverse correlation with DXA Z scores. Ionescu *et al.* [30] showed that BMD was related to FEV1 and to levels of IL-6 and TNF-α-soluble receptors in 22 CF adults. Aris *et al.* [31] demonstrated urinary N-telopeptides and serum IL-1, IL-6 and TNF-α levels fell and osteocalcin levels rose in CF patients

following antibiotic treatment for infective exacerbations (fig. 4). It is important to state that these studies do not show cause and effect but should lead to more laboratory research to confirm the hypothesis. Further evidence suggesting a pathophysiological role of cytokines in CF bone loss is supplied by a 1-yr longitudinal study of 100 CF adult demonstrating serum IL-6 levels to be an independent predictor of change in bone mineral content [32]. The IL-6 levels in this study were also correlated with urinary N-telopeptide cross-links.

Vitamin D

Vitamin D deficiency can lead to osteomalacia whilst subclinical deficiency or insufficiency can lead to osteoporosis by increasing bone turnover *via* secondary hyperparathyroidism. The two main forms of vitamin D are ergocalciferol (D2) and cholecalciferol (D3). Ergocalciderol is obtained from dietary sources and cholecalciferol is formed following sun exposure to the skin when the precursor 7-dehydrocholesterol

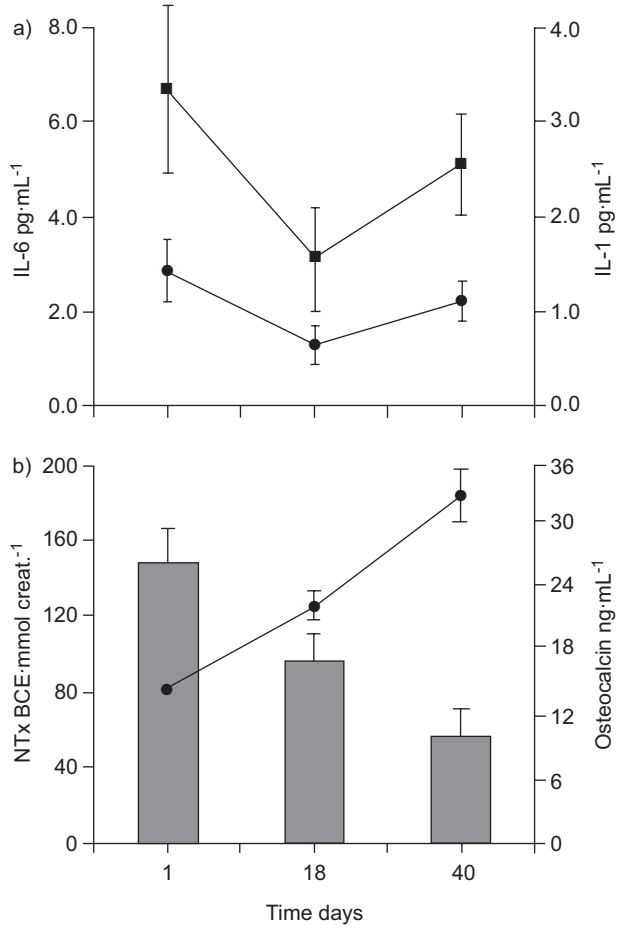

Fig. 4. – Response of a) interleukin (IL)-1 (●) and IL-6 (■) and b) bone turnover markers (■: cross-linked N-telopeptides (NTx); ●: osteocalcin) to antibiotic therapy. BCE·mmol creat.$^{-1}$: bone collagen equivalents per millimole of creatinine. Printed from Aris *et al*. [31] with permission.

Table 3. – Vitamin D levels in patients with cystic fibrosis

First author [Ref.]	Patients n	Age yrs	Location	25OHD ng·mL^{-1}	% Low ng·mL^{-1}	On multivitamins %
BUNTAIN [16]	141	5.3–55.8	Brisbane, Australia	24	25%<20	82% (200 IU)
CHAVASSE [33]	290	5–17	London, UK	26		6%<10
CONWAY [14]	98	18–29	Leeds, UK	18	40%<15	
ELKIN [9]	104	18–60	London, UK	15±11#	83%<20	>90%
HAWORTH [13]	151	15–52	Manchester, UK	19±11.1#	38%<15	100% (900 IU)
OTT [34]	71	18–57	Seattle, Canada	19±10#	63% <20	87%
ARIS [10]	70	>18	Chapel Hill, NC, USA	21±11#	20%	>90%
HENDERSON [35]	50	4.9–19	Chapel Hill	26±1.8#	24%<18	79%
BHUDHIKANOK [36]	49	8–48	CA, USA		52%<18	100% (400 IU)
SALAMONI [27]	14	<21	Switzerland	31±17#	14%<18	
SHANE [37]	11	>18	New York, USA	17±13#	36%<10	
STEAD [38]	31	17–52	London, UK	10±6#	26%<15	77%
HANLY [39]	20	14–25	Dublin, Ireland	10	75%<13	None
HAHN [3]	21	12–36	MO, USA	12±4#	29%<10	100%

#: Data are presented as ±SD. 25OHD: 25-hydroxyvitamin D.

absorbs ultraviolet (UV)B light. Both decreased sunlight exposure (due to ill health and the potential for increased photosensitivity with commonly prescribed antibiotics) and the malabsorption of fat-soluble vitamins place individuals with CF at high risk of developing hypovitaminosis D.

Many studies have reported low 25OHD levels in patients with CF (table 3); however most have not found a correlation between serum 25OHD levels and measured BMD [9, 10, 13, 16, 18, 39, 40]. Recently, BUNTAIN *et al.* [16] reported a significant BMD deficit in the presence of vitamin D sufficiency in a large Australian cohort, suggesting that low BMD in CF can occur independently of vitamin D insufficiency. The role of vitamin D in the pathogenesis of low BMD in CF therefore remains unclear; however, it is highly probable that low serum 25OHD levels contribute to the loss of bone by causing intermittent secondary hyperparathyroidism and a consequent increase in bone turnover. This may well occur during the winter and spring months when serum levels will be at the lowest and, consequently, PTH at its highest. HAWORTH *et al.* [13] reported a negative relationship between mean BMD Z score and PTH in 139 adults with CF, which supports this hypothesis. A further study reported significantly lower 25OHD levels and a trend towards a higher mean PTH in a CF group compared with age- and sex-matched controls [24]. These data further suggest that some patients with CF have mild secondary hyperparathyroidism, which can lead to uncoupled bone turnover.

Vitamin D absorption appears to vary considerably amongst individuals with CF. LARK *et al.* [41] reported that CF adults absorbed less than one-half of the total amount of oral vitamin D2 given as a one-off dose in comparison with non-CF controls. The 25OHD levels in the CF group as a whole did not rise in response to the vitamin D2, contrary to the control group who showed a doubling of serum levels. However, there was a wide variability in the individual absorption curves of the CF subjects with three subjects actually exceeding the average of the controls.

Recent data confirms that the absorption of vitamin D is variable in CF patients, with many individuals failing to respond to high-dose treatment. BOYLE *et al.* [42] demonstrated that only 8% of patients with serum 25OHD levels <30 ng·mL^{-1} increased their serum level with high-dose ergocalciferol (50,000 IU·week^{-1} for 8 weeks). No clinical characteristics predicted a response. Thirty-three patients went on to receive 50,000 IU twice weekly for 8 weeks, following which the mean change in serum 25OHD or PTH was -1.2 ng·mL^{-1}. It therefore appears that a subpopulation of patients with CF has difficulty in

absorbing adequate vitamin D and, if these patients are also not getting adequate sunlight exposure, they are highly likely to develop hypovitaminosis D and hyperparathyroidism.

Calcium

CF patients are at risk of a negative calcium balance from malabsorption, low vitamin D and increased gastrointestinal excretion. Aris *et al*. [43] found CF subjects to absorb less calcium that controls; this appeared to improve but was not completely reversed by pancreatic supplements. Hahn *et al*. [3] found 24-h urinary excretion of calcium to be reduced in CF subjects and hypothesised that this was due to reduced calcium absorption and consequent PTH effects on the proximal renal tubule causing calcium retention. There is no current evidence that low calcium intake or increased loss leads to decreased BMD in CF. However, adequate calcium intakes are important to help achieve optimal peak bone mass both before and during puberty and it is clearly sensible to ensure that patients are receiving the recommended daily intake. The effect of calcium and vitamin D supplementation on bone metabolism and bone density was assessed in a 1-yr randomised, double-blind, placebo-controlled trial [44]. Patients were randomised to receive calcium (1,000 $mg \cdot day^{-1}$) and colecalciferol (800 $IU \cdot day^{-1}$) or placebo, in addition to their standard multivitamin supplements (ergocalciferol 900 $IU \cdot day^{-1}$). Although no change in 25OHD or PTH levels was demonstrated between groups, the rate of decline in lumbar spine, total hip and distal forearm bone density appeared reduced in the treatment group compared with the control group, though these changes did not reach statistical significance.

Vitamin K

Vitamin K was once the forgotten fat-soluble vitamin but recently there has been much more interest from clinicians and researchers to investigate serum levels in CF patients and hypothesise about the role of hypovitaminosis K in CF bone disease. There is growing evidence that many patients with CF may have inadequate levels of vitamin K. In 1999, Rashid *et al*. [45] performed a prospective study investigating 98 patients and 62 healthy controls. They reported that 78% of pancreatic insufficient patients and all those with CF liver disease had raised PIVKA-II (proteins induced by vitamin K antagonism) levels. Osteocalcin, the major noncollagenous protein of the bone matrix, undergoes a vitamin K-dependent, post-transcriptional carboxylation of the glutamic acid residues, which results in a higher mineral binding coefficient with the calcium ions in the new bone-forming matrix. It is therefore feasible that vitamin K deficiency may in part be causing the abnormal bone formation reported in histomorphometry studies. Aris *et al*. [46] measured total and carboxylated osteocalcin in 52 CF patients and found the carboxylated levels to be lower in CF when compared with non-CF controls. Although there was a moderate association between carboxylated osteocalcin and both prothrombin time and T score of the lumbar spine, no direct measurement of vitamin K was performed and therefore no real cause and effect proven. Conway *et al*. [47] found similar results in a group of 93 children with CF. Serum vitamin K levels showed a significant negative correlation with undercarboxylated osteocalcin levels, but showed no correlation with any marker of bone turnover. Undercarboxylated osteocalcin levels were correlated significantly with bone turnover markers, which themselves showed a significant negative correlation with measurements of BMD and bone mineral content. However, there were no significant correlations between carboxylated or undercarboxylated osteocalcin levels and bone density measurements. The authors concluded that through its role in the carboxylation of osteocalcin, vitamin K deficiency might be associated with an uncoupling

of the balance between bone resorption and bone formation. To date, a cause–effect relationship between vitamin K deficiency and low bone mass has not been proven.

Glucocorticoids

Most of the larger cross-sectional studies have detected an association between oral glucocorticoid usage and low BMD. ARIS *et al.* [10] found a cumulative dose of glucocorticoids to be a predictor of BMD in CF patients awaiting transplantation. BHUDHIKANOK *et al.* [15] reported glucocorticoid use to be associated with significantly lower BMD Z scores at the femoral neck and lumbar spine. Both studies investigated populations with high steroid usage. ELKIN *et al.* [9] investigated a cohort with less steroid use and found a negative correlation between oral use and femoral neck Z score supporting the findings of CONWAY *et al.* [14]. Some authors have suggested that young adults who have low use of oral glucocorticoids are less likely to have low bone mass. This is probably due to the cohort investigated being in good health with good nutritional status and relatively good lung function. In the longitudinal study by HAWORTH *et al.* [18], the oral glucocorticoid dose was significantly and inversely related to change in BMD in the lumbar spine and proximal femur. The histomorphometry study by ELKIN *et al.* [20], which revealed changes not dissimilar to those observed in glucocorticoid-induced bone loss (decreased bone formation rate and decreased trabecular wall thickness), add weight to the argument for the role of glucocorticoids in the pathogenesis of bone loss in CF. It is therefore most probable that steroids contribute, but are not the sole cause of, decreased BMD in adults with CF.

Physical activity

There is no direct evidence that lack of weight-bearing exercise leads to low BMD in this patient group. Again, lack of evidence does not rule out a causal effect and a number of studies have found a correlation between the amount of exercise performed and BMD, particularly in the axial skeleton [9, 13, 14, 15, 18]. At present it is unknown in patients with CF if weight-bearing exercise can lead to increases in peak bone mass, preserve BMD or increase BMD in those with low BMD.

Hypogonadism

Hypogonadism is likely to be detrimental to bone health, as androgens have a protective effect on the skeleton. The data concerning secondary hypogonadism in adult males with CF is conflicting with some groups reporting normal testosterone levels and others low. This is most likely to be due to different methods of measurement. ELKIN *et al.* [9] found 31 out of 58 males to have low total serum testosterone, 18% having low free testosterone. The latter significantly correlated with total body BMD. This finding was corroborated by ROSSINI *et al.* [48], who investigated 191 adults (100 males) and found serum oestradiol levels to be below the normal range in 23% of the females and 27% of the males. Serum oestradiol was significantly related to femur BMD values in both females and males. Interestingly, significantly lower serum oestradiol and free testosterone levels were observed in males with vertebral fractures. HARDIN *et al.* [49] recently reported beneficial effects of growth hormone treatment in prepubertal children in terms of height, weight, bone mineral content and lean tissue mass.

Screening

Bone densitometry

Due to the uncertainties surrounding interpretation of BMD results in children, the optimal age for commencing DXA screening is not known. Some authorities suggest performing DXA from 8 yrs of age, if risk factors for low BMD have been identified [50], while an alternative view is that DXA might only be appropriate when individuals have stopped growing [16]. Most clinicians suggest that DXA should be performed before puberty and repeated every 1–3 yrs depending on the results to ensure that bone accrual is occurring at a satisfactory rate. Serial measures also allow the identification of peak bone mass, after which bone-sparing treatments can be considered if premature bone loss occurs.

Screening for fractures

It is important to identify fragility fractures. Chest radiographs should be examined specifically for evidence of vertebral wedge fractures. In the present authors' view, lateral thoracic and lumbar spine radiographs should not be performed regularly in every patient due to the relatively high radiation dose involved, but should be performed in patients at risk of fragility fracture based on clinical and DXA findings.

Management

As problems with bone health in adults are likely to have their origins in childhood, it is extremely important for paediatric care workers to optimise factors that are likely to affect bone health in the future and for adult care workers to continue these management principals after transition. Vitamin D levels, vitamin K intake, calcium intake and lean body mass should be optimised. Outdoor weight-bearing physical activity should be encouraged and glucocorticoid treatment kept to a minimum. Pubertal delay or hypogonadism should be recognised and treated. Where appropriate, advice should be given about the deleterious effects of smoking and alcohol on BMD. Treatments to prevent the progression of lung disease should be optimised due to the probable negative impact of pulmonary infection/systemic inflammation on bone health.

Vitamin D supplementation

The published Cystic Fibrosis Foundation (CFF) guidelines [50] recommend that vitamin D supplementation is used to maintain a serum 25OHD >30 $ng \cdot mL^{-1}$. This figure was selected because, in non-CF individuals, serum PTH levels rise when serum 25OHD levels fall below this threshold. Recently, Boyle *et al.* [51] investigated a group of 111 patients and found 65.8% had levels <30 $ng \cdot mL^{-1}$. These individuals had significantly higher serum PTH levels (>50 $pg \cdot mL^{-1}$) than those with higher serum 25OHD. These data suggest that maintaining 25OHD levels >30 $ng \cdot mL^{-1}$ is an appropriate goal in CF to stop secondary rises in serum PTH.

Vitamin D supplementation needs to be individualised according to levels. The CFF Consensus Panel recommended a minimum of 400 and 800 IU ergocalciferol be taken by infants and individuals aged >1 yr, respectively [50]. The UK Cystic Fibrosis Trust (CFT) advises 800–2,000 IU daily in pancreatic insufficient patients aged >12 yrs [52]. Serum 25OHD should be measured at least annually and the dosage of supplementation adjusted accordingly. Autumn is the optimal time to measure the serum

25-hydroxyvitamin D, on the assumption that solar exposure is maximal over the summer months. If levels are low in the autumn it is reasonable to assume that levels will reduce further during the winter and that additional supplementation is required. These high serum levels may be difficult to achieve in northern latitudes and further research is needed to determine the optimal form and dose for vitamin D replenishment. It is likely that in certain individuals oral doses will have to be very high [42] and it might be that in these resistant cases treatment with UVB might be beneficial [53]. Alternatively, more polar vitamin D analogues, such as calcitriol, may be indicated [54].

Calcium intake

The degree of gastrointestinal calcium malabsorption in individual CF patients is difficult to quantify, but it is important to ensure that the minimum recommended intake is met. The CFF consensus panel recommended intakes of 1,300–1,500 $mg \cdot day^{-1}$ after the age of 18 yrs to optimise bone health [50].

Vitamin K supplementation

As vitamin K is metabolised within 24 h [55], it is likely that a daily dose will be required. The optimum dosage of vitamin K is unknown and different recommendations have been published, for example, the CFF Consensus Panel have suggested 0.3–0.5 $mg \cdot day^{-1}$ and the UK CFT have suggested 10 $mg \cdot day^{-1}$ [50, 52]. While there is no evidence supporting the routine supplementation of vitamin K, it is the authors' practice to supplement vitamin K with either two ADEK (Axcan Scandipharm Inc, Birmingham, AL, USA) *q.d.* or menadiol 10 mg *q.d.*

Endocrine issues

Tanner staging should be performed in children and adolescents to ensure normal pubertal development. Likewise, it is recommended that morning serum testosterone levels are measured annually in adolescent/adult males and a menstrual history should be taken annually in adolescent/adult females to screen for hypogonadism. An endocrinology referral may be appropriate for patients with pubertal delay, hypogonadism or poor growth. An oral glucose tolerance test should be performed annually in adolescents and adults to screen for CF-related diabetes.

Bisphosphonates

Bisphosphonates are potent inhibitors of osteoclastic bone resorption and are also thought to inhibit osteoblast apoptosis. Alendronate is licensed in the UK for the prevention and treatment of postmenopausal osteoporosis, treatment of male osteoporosis and the prevention and treatment of corticosteroid induced osteoporosis. The licensed indications for risedronate are not quite so extensive and, for both drugs, the licensed indications for the weekly preparations are limited to the treatment of postmenopausal osteoporosis. Intravenous pamidronate is not licensed for the treatment of osteoporosis in the UK, although it is often used for this indication in selected patient groups.

The efficacy of *i.v.* pamidronate was assessed in a randomised, controlled trial involving 28 CF adults with low bone density [56]. The pamidronate-treated patients received pamidronate 30 mg every 3 months and both groups received calcium (1 $g \cdot day^{-1}$) and vitamin D (800 $IU \cdot day^{-1}$). After 6 months of treatment, the pamidronate group (n=13) showed a significant increase in absolute bone density compared with the

control group (n=15) in the lumbar spine (mean difference: 5.8%, 95% confidence interval: 2.7–8.9%) and total hip (3.0%, 0.3–5.6%). Several patients experienced severe bone pain after each pamidronate infusion but patients taking oral glucocorticoids and patients who had recently completed *i.v.* antibiotic treatment were asymptomatic, suggesting that these interventions had a protective effect [57]. Severe musculoskeletal pain was also experienced by three out of five participants enrolled in a study to investigate the efficacy of annual *i.v* zoledronic acid [58]. Although the study was consequently stopped, it is noteworthy that the zoledronic acid-treated patients demonstrated a significant increase in lumbar spine BMD compared with placebo at 6 months.

The efficacy of alendronate (10 mg·daily^{-1}) was assessed in a 1-yr randomised, double-blind, placebo-controlled trial in 48 CF adults with low bone density [59]. All patients received cholecalciferol 800 IU·day^{-1} and calcium 1,000 mg·day^{-1}. In the alendronate group compared with the control group, the mean±SD change in bone density was 4.9±3.0 *versus* -1.8±4.0% in the lumbar spine ($p<0.001$) and 2.8±3.2 *versus* -0.7±4.7% in the femur ($p=0.003$).

In a nonrandomised study reporting change in bone density in CF adults either prescribed or not prescribed oral bisphosphonates (alendronate or etidronate) for a mean duration of >2 yrs, bisphosphonate-treated patients experienced more favourable changes in bone density in the lumbar spine, femoral neck and total body than nonbisphosphonate-treated patients [60].

The efficacy of *i.v.* pamidronate (30 mg every 3 months) was assessed in a 2-yr randomised, controlled study in 34 CF patients after lung transplantation. All patients received colecalciferol (800 IU·day^{-1}) and calcium (1,000 mg·day^{-1}). The patients treated with pamidronate gained 8.8±2.5 and 8.2±3.8% in the lumbar spine and femur after 2 yrs compared with controls who gained 2.6±3.2 and 0.3±2.2%, respectively ($p \leq 0.015$ for both) [61]. None of the patients in this study developed bone pain, reinforcing the suggestion that glucocorticoids reduce the risk of pamidronate-associated bone pain in people with CF.

In the authors' opinion, bisphosphonate treatment in CF adults should be considered when the following occur: 1) The lumbar spine or total hip Z score is <-2 and the patient has sustained significant bone loss despite implementation of the general measures to optimise bone health. The significance of bone loss is determined by the coefficient of variation of the measurement and this varies according to the type of densitometer used, the skeletal site and the experience of the operator. 2) The patient has sustained a fragility fracture. 3) The patient is starting a prolonged (>3 months) course of oral glucocorticoid treatment and has a BMD Z score of <-1.5. 4) The patient is listed for or has received a solid organ transplant and has a BMD Z score of <-1.5.

There are no published data reporting the outcome of bisphosphonate use in CF children. Bisphosphonates may be beneficial in children with a history of fragility fracture and those either listed for transplantation or post-transplantation. Some authorities suggest bisphosphonates for children who have documented bone loss despite implementing the general measures for optimising bone health, but the current authors would strongly recommend seeking the advice of a paediatric bone specialist before commencing treatment.

The choice of bisphosphonate depends on clinical circumstance and patient preference. Intravenous bisphosphonates circumvent some of the problems associated with the oral bisphosphonates, such as poor oral bioavailability, upper gastrointestinal intolerance and adherence, but are associated with severe bone pain. However, in the authors' experience, oral alendronate and risedronate can also cause bone discomfort when first used in CF patients, but these effects usually wane with subsequent dosing. The

prescription of a 3- to 5-day course of prednisone before administering *i.v* pamidronate may help to prevent or minimise the bone pain associated with *i.v* bisphosphonates [50].

If oral preparations are used it is particularly important that patients are advised not to take calcium supplements at the same time as the bisphosphonate, as the two bind and are excreted from the gut. To facilitate the gastrointestinal absorption and minimise the risk of oesophageal reactions, bisphosphonates must be taken while fasted and patients must remain upright for a period after taking the drug. This is likely to affect adherence in CF patients, many of whom already have complex treatment regimens. Fasting might also be difficult for diabetics or for patients who feed overnight, and remaining upright may interfere with chest clearance techniques. The weekly dosing regimens available with alendronate and risedronate are therefore attractive for patients with CF; trials in CF are ongoing at the present time.

Bisphosphonates should be used with great caution in premenopausal females, as they cross the placenta and some infants born to mothers taking bisphosphonates have been found to be hypocalcaemic. Animal studies have shown foetal skeletal abnormalities, but the relevance of this to human pregnancies is not known. Female patients who may wish to become pregnant presently or in the future should be counselled about this and informed consent obtained. Even if the drug has been discontinued prior to conception, there is a theoretical risk of the drug being released from bone during pregnancy. Females should use adequate contraception while being prescribed bisphosphonates.

Monitoring response to treatment is important and the present authors would suggest repeating bone density measurements initially annually. If patients continue to lose bone mass while taking oral bisphosphonates and poor adherence or poor gut absorption are thought likely, these can be overcome by changing to *i.v.* pamidronate. Vitamin D insufficiency and osteomalacia should be excluded before commencing bisphosphonates through the measurement of serum corrected calcium, 25OHD and PTH levels, and annual levels should be measured thereafter. Bisphosphonates should not be prescribed to patients with renal impairment.

Conclusion

Despite the enormous amount of research published over the last decade, many questions remain about the pathogenesis and optimal management of CF-related low BMD. Further research is required to elucidate whether CFTR dysfunction plays a primary role in the development of low BMD or whether it is a secondary phenomenon. It remains to be seen if future generations of CF patients will reach normal levels of peak bone mass following improvements in nutritional and pulmonary care over the last decade. There continues to be much uncertainty as to interpretation of DXA measurements in children and adolescents, particularly if they are of small stature and as they grow. There also continues to be a paucity of robust local normative BMD data in children. Further research is required to clarify optimal levels of calcium intake, particularly in childhood and during the pubertal growth spurt. The optimal dosing regimen for vitamin K and the best method for normalising 25OHD concentrations also require urgent attention. Despite all of these unanswered questions, much can now be done to improve the bone health of CF patients to ensure that their skeleton remains sufficiently robust to support them as their mean age of survival increases.

Summary

Low bone mineral density (BMD) was first reported in patients with cystic fibrosis (CF) over 25 yrs ago and, since that time, research has concentrated on documenting the prevalence, natural history, prevention and treatment of CF-related low BMD. These studies show that 15–25% of CF adults have a BMD Z score below -2 in the lumbar spine or proximal femur when measured by dual energy X-ray absorptiometry. Histomorphometry confirms that the low BMD identified by densitometry in CF adults is real. A few patients have osteomalacia, and measuring the 25-hydroxyvitamin D (25OHD) and parathyroid hormone concentrations should identify these individuals. Reduced bone formation appears to be the predominant problem possibly due to inhibition of mature osteoblasts or increased osteoblast apoptosis. It is likely that increased bone resorption occurs during periods of ill health/infection and as disease severity worsens. This is supported by data from bone turnover markers. The aetiology of the documented low BMD appears complex. Numerous studies have found associations with low BMD and forced expiratory volume in one second, body mass index and intravenous antibiotic use. These factors are likely surrogates for disease severity and more recent data suggest that cytokines may play a role in bone loss. Many studies document low levels of 25OHD; however few have found low 25OH vitamin D to correlate with low BMD. It is highly probable that low serum 25OHD levels contribute to the loss of bone by causing intermittent secondary hyperparathyroidism and a consequent increase in bone turnover. Recent data demonstrate difficulty in achieving adequate serum levels of 25OHD with wide variability in vitamin absorption. More interest has also being shown in elucidating the role of low serum vitamin K and decreased bone formation.

In this chapter, current knowledge is summarised, the authors' thoughts on management are outlined, and current areas of uncertainty that require further research are discussed.

Keywords: aetiology, bone, cystic fibrosis, management.

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