Bone Disease in Thalassemia: A Molecular and Clinical Overview

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Thalassemia bone disease is a common and severe complication of thalassemia—an inherited blood disorder due to mutations in the α or β hemoglobin gene. In its more severe form, severe anemia is present, and treatment with frequent red blood cell transfusion is necessary. Because the body has limited capacity to excrete iron, concomitant iron chelation is required to prevent the complications of iron overload. The effects of chronic anemia and iron overload can lead to multiple end-organ complications such as cardiomyopathy, increased risks of blood-borne diseases, and liver, pituitary, and bone disease. However, our understanding of thalassemia bone disease is incomplete and is composed of a complex piecemeal of risk factors that include genetic factors, hormonal deficiency, marrow expansion, skeletal dysmorphism, iron toxicity, chelators, and increased bone turnover. The high prevalence of bone disease in transfusion-dependent thalassemia is seen in both younger and older patients as life expectancy continues to improve. Indeed, hypogonadism and GH deficiency contribute to a failure to achieve peak bone mass in this group. The contribution of kidney dysfunction to bone disease in thalassemia is a new and significant complication. This coincides with studies confirming an increase in kidney stones and associated accelerated bone loss in the thalassemia cohort. However, multiple factors are also associated with reduced bone mineral density and include marrow expansion, iron toxicity, iron chelators, increased bone turnover, GH deficiency, and hypogonadism. Thalassemia bone disease is a composite of not only multiple hormonal deficiencies but also multiorgan diseases. This review will address the molecular mechanisms and clinical risk factors associated with thalassemia bone disease and the clinical implications for monitoring and treating this disorder. (Endocrine Reviews 37: 320–346, 2016)
I. Introduction

Thalassemia is derived from the Greek words thalassa meaning “sea” and haema meaning “of blood.” Indeed, it was first described in people who lived around the Mediterranean. The condition is highly prevalent in Mediterranean countries, the Middle East, and Southeast Asia. However, due to widespread migration, thalassemia can now be found across the globe (Figure 1).

The clinical spectrum of thalassemia encompasses the asymptomatic carriers to the severe thalassemia major patients who require lifelong blood transfusions and iron chelation. The optimal control of iron overload improves mortality, but this increased longevity is associated with a higher burden of complications. Thalassemia is associated with multiple endocrine complications involving the pituitary, thyroid, pancreas, gonads, parathyroid, and bone (1).

Thalassemia bone disease is a common but poorly understood disease that affects both younger and relatively older patient cohorts. The multiple contributing factors to bone loss present diagnostic and therapeutic challenges in thalassemia but also emphasize the importance of an integrated approach to management between the hematologist and other specialty units.

II. Thalassemia

Thalassemia is an autosomal recessive disorder and is the most common monogenic disorder worldwide (2). The mutation occurs in either the \( \alpha \) or \( \beta \) globin gene, resulting in phenotypes with varying severities of anemia. The major clinical distinction between the thalassemias is the need for red blood cell transfusion. The need for transfusion therapy is highly associated with mortality and treatment-related complications in thalassemia (3).

The common feature of all thalassemia syndromes derives from an imbalanced production of either the \( \alpha \) or \( \beta \) globin chain (4). When produced in excess, damage to red blood cell precursors and mature red blood cells leads to anemia. This leads to a compensatory increase in the expansion of the ineffective marrow with deleterious effects on bone formation and growth. The need for regular red blood cell transfusions can be associated with severe iron overload (5). The major cause of morbidity and mortality is the effect of iron deposition in the endocrine organs, liver, and heart, which results not only from transfusion therapy but also from increased intestinal absorption of iron (5, 6).

A. Classification and diagnosis of thalassemia

The hemoglobinopathies describe a broad range of diseases of the red blood cell and include: structural hemoglobinopathies (hemoglobins with altered abnormal amino acid sequences), hereditary persistence of high levels of fetal hemoglobin, acquired hemoglobinopathies (eg, met- or sulfhemoglobin due to toxic exposures), and the thalassemias (7). The thalassemias are broadly separated into mutations of either the \( \alpha \) or \( \beta \) globin chain, ie, \( \alpha \) or \( \beta \) or thalassemic variants, where structurally abnormal hemoglobin is associated with coinherited thalassemic phenotypes, eg, hemoglobin E (HbE) (4).

1. \( \alpha \)-Thalassemia

The \( \alpha \)-thalassemia syndromes are primarily caused by gene deletions on chromosome 16 of the \( \alpha \) chain and are usually inherited in an autosomal recessive manner (4). The four clinical subtypes of \( \alpha \)-thalassemia syndromes are classified according to the number of gene deletions, and this corresponds to their clinical severity (8).

Silent \( \alpha \)-thalassemia carriers or trait (deletion of one or two genes) may have a slight reduction in mean cell hemoglobin or mean cell volume. Hydrops fetalis, which represents the most severe form of \( \alpha \)-thalassemia (four gene deletions) is not compatible with life. The Hemoglobin H shows ragged inclusion...
bodies due to precipitation of hemoglobin H along with hypochromia and anisopoikilocytosis on blood film.

2. **β-Thalassemia**

The β-thalassemia is characterized by a defect in production of the β-globin chain, which is found on chromosome 11 (4, 9). The major and intermedia forms are inherited in an autosomal recessive pattern. β-Thalassemia major is characterized by the inheritance of two abnormal β-globin genes leading to severe anemia and a need for red cell transfusion in the first few years of life (3, 10). Thalassemia intermedia is less clearly defined because patients may tolerate anemia reasonably well with signs and symptoms of anemia appearing in later childhood or early adult life. β-Thalassemia minor results from the inheritance of only one defective β-globin gene and leads to a mild anemia. The clinical heterogeneity of β-thalassemia therefore encompasses the major form, which is characterized by severe anemia and dependence on chronic transfusion therapy, and the relatively asymptomatic β-thalassemia trait (9).

β-Thalassemia major can be diagnosed clinically on the basis of severe anemia and clinical signs of severe erythropoiesis, ie, hepatosplenomegaly and bone deformity. However, the noninvasive prenatal diagnosis of thalassemia major using fetal DNA in maternal plasma is highly accurate (11). The laboratory abnormalities are characterized by a severe hypochromic microcytic anemia and elevated levels of fetal hemoglobin, Hemoglobin A2, or both.

### III. Iron Overload

The body has limited capacity to excrete iron, and iron in excess can lead to long-term damage to tissue. Iron is required for many essential and basic cellular functions, but its major role in mammals is to carry oxygen as part of hemoglobin. Normal body iron stores are 3–4 g; an excess of iron of 20 g or more can lead to organ damage (5). Iron overload is a universal complication of transfusion-dependent thalassemia (3, 12). Increased intestinal absorption of iron occurs in response to ineffective erythropoiesis and chronic anemia. Each unit of transfused red blood cells contains 200–250 mg of iron, and because the body has no mechanism for excreting excess iron, iron overload readily occurs in patients after 10 to 20 transfusions (13). Excessive body iron can lead to increased free iron, which is highly toxic to cells (14).

Iron is tightly bound to various proteins and enzymes in the body. Ferritin, and to a lesser extent hemosiderin, is the major storage form of iron, and transferrin binds iron for transportation into cells (15). The labile iron pool describes the reservoir of free iron available for cellular iron exchange and is localized primarily in the cytosol (5, 16). Under conditions of iron overload, the labile iron pool is increased, whereby free iron is able to promote the production of free radicals through the Fenton reaction (14, 16). Free radicals are highly unstable and reactive due to the presence of unpaired electrons. They can react with and modify proteins, nucleic acids, and fatty acids in cell membranes and plasma lipoproteins (14, 17). Without adequate treatment, the deposition of excessive iron leads to multiple end-organ damage including cardiomyopathy, liver dysfunction, anterior pituitary hormonal deficits, and parathyroid, pancreatic, and bone disease (3).

 Whereas cardiac, liver, and pituitary failure is easily recognizable, assessing the degree of iron overload is more complicated. There are different validated and standardized methods for assessing iron overload that include serum ferritin, monitoring transfusion quantity, quantifying liver iron using biopsy, and magnetic resonance imaging (MRI) assessment of cardiac and liver iron (18). The serum ferritin level is the simplest and most widely available measure of iron overload. It correlates with total body iron, is a useful measure of iron chelation treatment adequacy, and is an accurate prognostic indicator of survival in transfusion-dependent thalassemia (19). Assessment of iron overload in the liver and particularly the heart is important in the management of thalassemia because iron-mediated cardiac toxicity remains one of the leading causes of death in transfusion-dependent thalassemia (5, 20–22). MRI is increasingly used for the assessment of liver and cardiac iron because MRI images darken at a rate proportional to the iron concentration, with the half-life of this darkening defined as T2*. The change in MRI image darkening can be accurately quantified to reflect tissue iron concentration (18).

The life expectancy of patients with transfusion-dependent thalassemia is inversely proportional to iron levels and has improved dramatically since the introduction of iron chelation therapy (22, 23). The major causes of mortality are cardiac disease (22), followed by infection, liver disease, and malignancy (24). The prognosis for survival without cardiac disease is excellent when serum ferritin concentrations remain below 2500 ng/L with chelation therapy (19). Indeed, survival has improved dramatically over the past half century. In a large Italian cohort of thalassemia major, at the age of 15 years, the Kaplan-Meier estimate of survival after the first decade of life was 80.6% for subjects born in 1960–64, 84.2% for those born in 1965–69, and 96.9% for those born in 1970–74 (24). However, mortality has decreased and survival has improved not only due to improvements in iron chelation...
but also due to the development and implementation of dedicated, integrated, multidisciplinary, skilled centers worldwide and the development of evidence-based standardized protocols for the management of anemia and iron overload.

A. Iron chelators

Chronic transfusion leads to iron overload and the necessary use of iron chelators to reduce the toxicity of iron on tissues (25). There are three major types of iron chelators available (Table 1 and Figure 2).

Deferoxamine is the most studied of the available iron chelators. The effective use of deferoxamine in reducing total body iron stores leads to improved survival and morbidity from cardiac, liver, and endocrine complications of iron overload (23). Deferoxamine is characterized by poor oral bioavailability and is rapidly cleared by the kidney. The deferoxamine-iron complex in serum is eliminated chiefly by the kidneys and to a lesser extent by the liver, where it is excreted into the bile (26). Rapid clearance by the kidney requires continuous sc or iv administration. The invasive route of administration and discomfort at the site of sc infusion contributes to the poor treatment compliance associated with deferoxamine therapy (27).

The oral iron chelator deferasirox is well absorbed from the gastrointestinal tract and was a much welcome alternative to deferoxamine. The phase III trials, completed in 2006, confirmed the efficacy of deferasirox in the treatment of iron overload in thalassemia major (28). The deferasirox-iron complexes are excreted predominantly through the hepatobiliary system into the bile (29). The most common side effects include rash, gastrointestinal disturbances, and mild nonprogressive increases in serum creatinine (28, 30). However, testing for renal tubulopathy, and in particular hypercalciuria, was not routinely performed in these early pivotal studies. Increasing and worrying reports of renal tubular dysfunction including hypercalciuria have emerged with deferasirox (31, 32), but this has not been examined systematically. Recently, studies have confirmed that deferasirox, when used in therapeutic doses, leads to dose-dependent hypercalciuria (33).

Deferiprone is an alternative oral iron chelator excreted predominantly through the kidney; it is the only iron chelator that crosses the blood–brain barrier (34). Deferiprone is mainly absorbed from the stomach and is susceptible to food-drug interactions or other gastric factors that affect absorption (30). Wide variation in the metabolism and clearance of deferiprone among patients has been observed and is dependent on the degree of iron overload and availability of chelatable iron (30). The utility of this medication has been limited by its narrow therapeutic index and safety concerns, including a risk of agranulocytosis (35).

IV. Epidemiology of Thalassemia Bone Disease

Thalassemia bone disease includes a heterogeneous group of conditions that includes bone deformity, pain, marrow expansion, reduced bone density, and fractures (36). The characteristics of thalassemia bone disease are unique compared to those of the more typical idiopathic
osteoporosis seen in the general community. The difference lies in the relatively young age of patients (predominantly in their 20s to 30s), the multitude of risk factors for bone loss (many of which are unique to patients with thalassemia), the epidemiology of fractures, and the response to treatment with bone-preserving agents. These factors contribute to the complex pathophysiology and management of thalassemia bone disease.

Our understanding of thalassemia bone disease is incomplete, given the complex piecemeal collection of risk factors, which includes hormonal deficiency, marrow expansion, iron toxicity, chelator toxicity, and increased bone turnover (37–40). These risk factors also impact on pubertal growth, nutrition, and exercise and can lead to important changes in body composition and the inter-relationship between bone, fat, and muscle (41).

The prevalence of osteoporosis in transfusion-dependent thalassemia has been well characterized, but studies examining the longitudinal change in bone mineral density (BMD) have been small and of relative short duration (42, 43). Similarly, data on fracture burden have been mainly self-reported in most studies and cross-sectional in design (44, 45). The relationship between BMD and fracture is a well-established concept in osteoporosis (46, 47). However, fracture risk is a composite of multiple factors, many of which will lead to significant deterioration in bone microarchitecture, without a concomitant reduction in areal BMD as measured by dual-energy x-ray absorptiometry (DXA) (48). The difficulty, of course, lies in assessing the relative contribution of multiple risk factors for osteoporosis when deciding on an appropriate therapeutic strategy for patients.

A. Low bone mass and strength

The morbidity associated with low bone mass and fragility fracture is of increasing importance as survival rates for transfusion-dependent thalassemia improve. Multiple risk factors are implicated in bone loss and have been previously described (49, 50). There have been many studies describing the prevalence of reduced bone mass in thalassemia, but direct comparison of individual studies is difficult (49, 51–53). The problem lies in the inconsistent use of the DXA Z score, T score, and areal BMD to describe patient cohorts. Moreover, it is necessary to make appropriate adjustments when using DXA in the relatively young (age, 20–30 years) thalassemic patient population. In general, the BMD Z score is the most appropriate descriptive parameter of bone density in the young thalassemia cohort. However, apart from age and gender, changes to body size and bone size also need to be considered (54).

In a small study of 18 children (age, 5.8 ± 1.5 years) with β-thalassemia major who received hypertransfusion and chelation therapy, Z scores were noted to be <−2.5 in 22.2% of children, between −1 and −2.5 in 38.8%, and >1 in 38.8% (55). Adolescent patients with transfusion-dependent thalassemia had suboptimal BMD, with 61.3% of patients demonstrating a Z score of <−2 and 22.6% having a Z score between −1 and −2 (56). In contrast, in a study of children and adolescents (age, 5–20 years) with β-thalassemia major who received regular transfusion and chelation therapy, Z scores were within the normal range for all subjects, with a mean Z score of 0.42 for females and 0.41 for males (P = .018) (57).

Adolescence is a critical period of bone accrual when clinical and lifestyle factors including nutrition, exercise, and endocrinopathies (principally sex hormones and GH) play an integral role in optimizing bone health and achieving peak bone mass (58). The need to adjust for bone size when employing DXA in a skeleton undergoing rapid growth can also make comparisons of bone density in adolescent patients difficult (59). The major determinant of BMD in young adults and beyond is dependent on the peak bone mass achieved and the rate of bone loss thereafter. The ability to achieve peak bone mass is compromised in patients with transfusion-dependent thalassemia (49, 60). The different transfusion and chelation treatment protocols in thalassemia...
units worldwide account for differences not only in mortality but also in skeletal health.

The impact of gender on bone mass in thalassemia is dependent on gonadal hormone sufficiency, but other factors are likely to be implicated (61). The age at which peak bone mass is reached has been reported to occur at 22.4 years (95% confidence interval [CI], 19.0–31.8) in females and 29.8 years (95% CI, 23.5–34.0) in males (49). The contribution of hypogonadism to BMD in severe thalassemia in both genders is important (61), with hypogonadism present in 34% of females and 38% of males (49). However, even in eugonadal subjects with β-thalassemia major, males had lower Z scores than females (Z score, −3.0 in males, −2.0 in females; *P* = .004) (62). Furthermore, in a risk model of bone density where potential confounders of gender, endocrinopathies, and iron overload were taken into account, male subjects with thalassemia still had reduced BMD compared to their female counterparts (62). The implication that male patients appeared to be at greater risk of bone loss is clinically significant. Young males are also more likely to engage in higher risk activities and thereby increase their risk of traumatic fractures.

### B. Fractures

Fractures were highly prevalent (up to 50%), multiple, and frequently healed with resultant deformity in patients with β-thalassemia major before the introduction of effective transfusion and chelation therapy (44, 63, 64). In a study of 62 patients between 10 and 32 years of age, one in three had sustained fractures and one in five had multiple or recurrent fractures (63). Moreover, deformities were often caused by the premature fusion of the epiphyses of the long bones in patients and occurred at sites involving the lower tibia and fibula, upper humeral, and lower femoral epiphyses (63).

The introduction of red blood cell transfusion and concomitant iron chelation therapy has led to improved bone health through various mechanisms. It leads to a reduction in medullary expansion and cortical bone thinning, the reduced incidence of hypogonadism, and a reduction in other endocrine complications such as hypoparathyroidism and metabolic disorders that predispose to low bone density and fractures (65, 66). In a retrospective study of 702 patients with thalassemia, the overall prevalence of fracture was 12.1% with near equal distribution in males (12.7%) and females (11.5%) (67). Fractures occurred more frequently in thalassemia major (16.6%) and thalassemia intermedia (12.2%) compared to hemoglobin E-β thalassemia (7.4%) and α-thalassemia (2.3%) (67). Other studies have reported the prevalence of fractures to be roughly 36% (49, 68). Fractures have been described to occur more frequently in the upper extremities (53.3% of all fractures), whereas spine and pelvic fractures occurred in 10.6% (45). A clear gradation of fracture risk with worsening BMD as measured by DXA is less clear in pediatric metabolic bone disease compared to osteoporosis in postmenopausal women (54). In a retrospective study, the average BMD Z and T scores were 0.85 SD lower among patients with a history of fractures where the mean Z/T score was −2.78 vs −1.93 (95% CI, −0.49 to −1.22; *P* = .02) (67).

The major limitation in the assessment and optimal management of thalassemia bone disease is the lack of large longitudinal studies examining fracture incidence. The studies reporting fracture incidence are few in number, and they have small sample sizes and short follow-up duration. In a 2-year prospective study of 105 patients, 14 (13.3%) patients sustained a total of 28 fractures, seven patients sustained more than one fracture, and two-thirds of these fractures were caused by trivial trauma (69). In a smaller study of 38 patients over a 5-year period, the incidence of fractures was 21% (70).

The incidence of fractures in the general community is partly dependent on gender and age. The incidence is uniform in women until the menopause when fracture incidence accelerates; in males, the incidence is high in young men and falls until the age of 60, when it increases again (71). Studies examining fracture prevalence in thalassemia patients younger than 20 years of age report relatively reduced fracture rates, presumably due to decreased physical activity associated with chronic disease (45, 67). The prevalence of fractures is often derived from self-reported cases (49, 67, 68) and therefore lacks the integrity and objectivity of radiologically confirmed fractures. Because spinal x-rays were not routinely performed, this may also account for the relatively reduced rate of vertebral fractures. Risk factors for fracture in thalassemia include the need for regular transfusion as in β-thalassemia major (49, 67, 68), increasing age (45, 67), male gender (45, 68), low body mass index (68), a history of sex hormone replacement (67), and lower BMD (67) (Figure 3).

### V. Mechanisms of Bone Loss in Thalassemia

The major mechanisms that account for bone loss in the general community are also relevant to those with thalassemia. In particular, deficiencies in sex and growth hormones are important contributing factors to bone loss, in addition to risk factors that are unique to the thalassemia cohort. These risk factors are related to the long-term complications of anemia and its treatments, resulting in narrow expansion and skeletal deformity together with iron...
and chelator toxicity. The contribution of kidney dysfunction to bone disease in thalassemia also requires further study because increasing reports of renal tubular dysfunction including hypercalciuria have emerged with the use of the oral iron chelator, deferasirox (Figure 4).

A. Genetic factors

Genetic factors account for up to 70% of the variance in BMD in the general population (72).

A polymorphism in the vitamin D receptor (VDR) has been found to be associated with skeletal and nonskeletal parameters in thalassemia major. It has been associated with short stature (73) and BMD in thalassemia major (74, 75). Moreover, an association with an increased risk of renal tubular dysfunction has also been demonstrated (76).

The collagen type I α1 gene locus (77) as well as polymorphism of the VDR gene (78) are promising determinants of bone mass. In a small study of thalassemia major, VDR and calcitonin receptor gene polymorphisms were not found to be associated with osteoporosis (75). In contrast, another study examining VDR polymorphisms (FokI, TaqI, and BsmI) related to low BMD were shown to be significantly correlated with lower BMD at the lumbar spine (74). The relationship between VDR polymorphism and low BMD had also been demonstrated in another study among patients with the BB VDR genotype (79).

The question of whether polymorphism in the VDR influences the response to treatment in thalassemia bone disease has been examined (80, 81). In a small study of 40 patients with thalassemia major, BsmI polymorphism was associated with an improved response in BMD to treatment with alendronate therapy (81).

The identification of multiple polymorphic gene markers in the VDR underlies the complex and multifactorial contribution and interaction between the gene and environmental factors in thalassemia bone disease. However, larger epidemiological studies followed by appropriate interventional studies are required before a causal relationship between VDR, BMD, and the response to antiresorptive medications can be established.

B. Bone biology (RANK-ligand and WNT/β-catenin signaling)

The receptor activator of nuclear factor κ (RANK)/RANK ligand (RANKL) osteoprotegerin (OPG) system is a key mediator of osteoclast differentiation and formation (82). OPG acts as a natural decoy receptor to RANK by binding to RANKL and leading to reduced osteoclast formation and differentiation (83). Thalassemic patients show no differences in plasma levels of OPG compared with controls and significantly higher plasma levels of RANKL, with a consequent significantly lower OPG/RANKL ratio (84). This may partly account for the enhanced osteoclastic bone resorption and bone loss characteristics of these patients (84). Moreover, RANKL had a significant negative correla-
tion with T and estradiol levels in male and female thalassemic subjects (84). This suggests that increasing sex hormone treatment may reduce RANKL and reduce the increase in bone resorption. There was no relationship between RANKL and OPG levels with BMD in thalassemia (84, 85), although this relationship has also been found to be inconsistent in the normal population (86, 87). Iron toxicity can also lead to altered bone remodeling. In a mouse model of iron overload, reactive oxygen species generated from the Fenton reaction was associated with increased markers of bone resorption and deterioration in bone microarchitecture, which was reversed after treatment with antioxidants (88).

Sclerostin, a Wnt signaling inhibitor that is produced by osteocytes and inhibits osteoblast function, was found to be elevated in subjects with thalassemia (89) (Figure 5). Moreover, sclerostin levels were significantly correlated with BMD at the lumbar spine, femoral neck, and radius (89). These findings suggest that a high sclerostin level may serve as a marker of increased osteocyte activity in thalassemia patients.

Dickkopf-1 is an inhibitor of Wnt signaling (90) and plays an important role in osteoblast differentiation. In 66 patients with thalassemia major who received therapy with zoledronic acid in a placebo-controlled, randomized trial, baseline levels of Dickkopf-1 were increased and correlated with increased bone turnover markers and reduced BMD at the lumbar spine and the distal radius (91). Interestingly, treatment with zoledronic acid did not reduce the high baseline levels of sclerostin (89) but did lead to a reduction in levels of Dickkopf-1 (91). Teriparatide does not lead to a reduction in sclerostin levels in postmenopausal women with osteoporosis (92) but leads to further increases to baseline levels of Dickkopf-1 (93). The use of teriparatide after bisphosphonate therapy in thalassemia major has been shown to increase BMD at the total hip and femoral neck in case reports (94).

C. Iron overload in vitro and in vivo studies

The osteoblast is derived from mesenchymal stem cells and is the principal cell responsible for bone formation. It is primarily responsible for the laying down of bone matrix and mineralization (95). In cell studies, ferritin leads to down-regulation of osteoblast specific genes (96–98) and is attributed to the ferroxidase activity of ferritin (96).

Chronic iron overload increases the total labile iron pool in the body. Free iron that is not chelated may promote the production of free radicals through the Fenton reaction (Figure 6) in patients with severe thalassemia. In a murine model, reactive oxygen species had been shown to accumulate in the bone marrow as a consequence of ovarietomy and lead to bone loss (99). Increased oxidative stress in MC3T3-E1 cells (mouse cell line for the study of in vitro osteoblast differentiation) has been shown to inhibit the differentiation of osteoblastic cells (100). Iron also promotes RANKL-induced osteoclast formation via oxidative stress in RAW 264.7 cells (mouse leukemic monocyte macrophage cell line), and this effect was attenuated by the administration of an antioxidant (101). In an iron-overloaded mouse model, increased reactive oxidative stress led to increased bone resorption and trabecular and cortical thinning of bone (88). In contrast, bone histomorphometry in iron-loaded pigs showed reduced bone apposition and formation consistent with reduced osteoblast recruitment but intact osteoclast resorption surfaces (102).

D. Iron overload clinical studies

Iron overload can lead to loss of bone mass through direct toxicity on bone, pituitary and/or gonadal dysfunction leading to hypogonadism, and chronic liver disease (103). In patients with hemochromatosis, osteoporosis was detected in 25% and osteopenia in 41%, but BMD was also independently associated with alkaline phosphatase (a marker of liver function), hypogonadism, and serum ferritin (104). Indeed, higher ferritin levels have also
been shown to correlate with lower BMD and accelerated bone loss in the general community (105).

The relationship between serum ferritin and BMD is less clear in subjects with transfusion-dependent thalassemia. In cross-sectional studies of thalassemia bone disease, serum ferritin levels were not found to be significantly associated with BMD (49, 106). Similarly, there was no significant association between serum ferritin or BMD in a large 19-year longitudinal study of thalassemia bone disease (107). The failure to show a statistically significant association between serum ferritin and BMD in thalassemia may be due to several reasons. Serum ferritin is one of many competing risk factors for bone loss in thalassemia, making it difficult to demonstrate statistical significance in univariate or multivariate analysis even after adjusting for confounding factors. Moreover, ferritin as a measure of iron overload changes dynamically in response to chelation therapy and blood transfusion. Chronic iron overload is associated with deficits in pituitary function leading to hypogonadism and GH deficiency; hypoparathyroidism is found in up to 14.6% of thalassemia subjects receiving transfusion (108, 109). Therefore, quantitating the effect of iron on BMD is a composite of multiple risk factors.

**E. Bone marrow expansion**

Blood transfusion not only provides a source of normal red blood cells for oxygen transport but also reduces the degree of marrow expansion (66). The vertebrae is an important site for marrow expansion, and significant cortical thinning and disruption of trabecular bone can occur (66, 110). In contrast, the relative absence of bony deformity, red marrow activity, and overlying soft tissue artifact at the femoral neck enables a more accurate assessment of BMD using DXA in longitudinal analysis (111).

Supporting the concept that marrow expansion can lead to reduced BMD, a longitudinal study showed a significant positive correlation between hemoglobin levels and BMD (111). In a cross-sectional analysis, hemoglobin was found to be positively correlated with BMD in β-thalassemia (66). A recent study...
had confirmed increased erythropoiesis in males compared to females with β-thalassemia major after adjusting for hemoglobin levels (112). This may account for the greater loss of BMD seen with male thalassemia even after adjusting for confounding factors (107). Moreover, it raises the important clinical question of whether transfusing patients (particularly males) to a higher hemoglobin concentration might lead to reduced bone loss (111).

F. Hypogonadism

Hypogonadism in transfusion-dependent thalassemia results primarily from pituitary failure (113–115) and to a lesser extent gonadal failure (115). It is secondary to iron overload and is the most common endocrine complication (115). The prevalence of hypogonadism in thalassemia has been reported to be between 22.9 and 54.7% (22, 116, 117). The difference between the thalassemia cohort and the general population is the higher prevalence and younger age of onset of hypogonadism in thalassemia. This has longer term implications for growth, fertility, and osteoporosis, particularly because most patients will not have achieved peak bone mass (49).

The decline in BMD due to estrogen deficiency is dramatic and characterizes the transition from the eugonadal to menopausal state (118). Indeed, whereas males do not experience a similar precipitous drop in BMD at midlife, the critical role of estradiol in regulating bone resorption in males is well established (119). Hypogonadism is associated with lower BMD in patients with thalassemia (61). In a cross-sectional study of thalassemia, hypogonadal subjects receiving regular transfusion therapy had the most severe reduction in BMD; this was associated with an increase in markers of bone resorption whereas bone formation markers remained normal (38).

In adequately treated hypogonadal subjects with thalassemia, there was no significant reduction in BMD based on cross-sectional (37, 61) and preserved BMD was also seen in the non-thalassemia population (107). However, treatment of hypogonadism in thalassemia does not completely address the deficiency in BMD (120), and in eugonadal thalassemia patients, GH may have an important role in the maintenance of bone health (121, 122).

G. GH and IGF-1

GH is important in the regulation of bone growth (123, 124). It increases bone formation in two ways: via a direct interaction with GH receptors on osteoblasts, and via an induction of endocrine and autocrine/paracrine IGF-1 (123). The anabolic effect of intermittent PTH administration in bone appears to be mediated through IGF-1-dependent mechanisms (125). GH treatment also results in increased osteoclast formation (126). Thus, the action of GH on bone metabolism in GH-deficient adults is 2-fold: it stimulates bone resorption (126, 127) and bone formation (127, 128). GH initially increases bone resorption with a concomitant bone loss that is followed by a phase of increased bone formation, with a net increase in bone mass occurring after 6 months and lasting for up to 12–18 months of GH treatment (123). Vitamin D has also been shown to have a positive correlation with circulating IGF-1 and IGF binding protein-3 in the normal population (129), although this has not been consistently demonstrated in the thalassemia cohort (60).

The true prevalence of GH deficiency in thalassemia is uncertain due to the variability of the diagnostic criteria employed, ie, clinical, baseline, and provocative hormonal testing. In a multicenter questionnaire of 3817 β-thalassemia major patients, GH deficiency was documented in 7.9% of males and 8.8% of females (1). In a study of 28 adult patients with thalassemia major, GH deficiency was present in 32.1% of subjects as demonstrated using an arginine plus GHRH stimulation test (130). In a cross-sectional study, increased ferritin concentrations, male gender, and the presence of hypogonadism were all associated with lower serum IGF-1 and IGF binding protein-3 concentrations (60).

In patients with β-thalassemia major, growth retardation is a common complication, and treatment with recombinant human GH for 2 years resulted in an increase in height growth velocity (131). Although the results of short-term GH therapy are encouraging, the impact of treatment on the final height of non-GH-deficient short thalassemic children remains uncertain (132). In addition, growth failure in adolescent thalassemic patients can be due to poor nutrition, iron overload, the toxic effects of deferoxamine, or hypogonadism (133).

In patients with transfusion-dependent thalassemia, subjects with GH deficiency had reduced BMD (134, 135) but showed an unbalanced bone turnover with increased bone resorption relative to bone formation indices compared to normal controls (135, 136). The presence of increased bone turnover and reduced BMD in thalassemia, despite adequately treated hypogonadism, suggests that reduced IGF-1 in these patients may play an important role in bone loss (135). In prepubertal GH-deficient children with thalassemia major, GH treatment for 1 year was able to increase but not normalize bone turnover (134). However, it is likely that longer treatments with GH may lead to larger and more sustained increases in BMD.

H. Hypoparathyroidism

Hypoparathyroidism is a condition of reduced PTH secretion. The biochemical abnormalities include hypocalcemia, hyperphosphatemia, and reduced PTH levels...
The effect of PTH on bone is complex, but hyperparathyroidism is a clinical entity of high bone turnover as opposed to hypoparathyroidism, which is one of low bone turnover. Therefore, the effect of hypoparathyroidism on the skeleton is one of relative increase in BMD Z scores, particularly at the lumbar spine (138, 139). Patients with sporadic idiopathic hypoparathyroidism have increased BMD at the lumbar spine and hip but not in the forearm, compared to normal, healthy matched controls (139). The increase in BMD appears to be related to the duration of the disease rather than the serum calcium levels (139). The nonskeletal manifestations of hypoparathyroidism are secondary to the deposition of calcium-phosphate deposits in the basal ganglia and other tissues (140).

Hypoparathyroidism is a recognized complication of chronic iron overload in patients with transfusion-dependent thalassemia. The prevalence of hypoparathyroidism has been reported to be between 13.5 and 14.6% (108, 109) in severe thalassemia. Consistent with its effect on BMD in general, BMD was greater in the thalassemic patients with hypoparathyroidism compared to normal thalassemic patients ($Z$ score = $-1.975 \pm 0.89$ vs $2.246 \pm 0.97$), although the difference was not statistically significant (109). Despite the tendency for higher BMD, its impact on longer term fracture risk has not been established in thalassemia. Indeed, in a group of non-thalassemic patients with surgical hypoparathyroidism, there was an increased frequency of morphometric vertebral fractures (141).

Several nonskeletal manifestations of hypoparathyroidism have been reported. Exacerbation of cardiac failure in the setting of hypoparathyroidism-induced hypocalcemia has been reported in thalassemia major (142, 143). Several case series have also reported extensive cerebral calcification (144, 145), which may increase the risk of seizures and subsequent fracture in thalassemia.

I. Renal-bone dysfunction

The intimate relationship between bone structure and kidney dysfunction is evident in renal osteodystrophy (146). The kidney plays an essential role in regulating calcium and phosphate directly and indirectly through PTH, 1,25-dihydroxyvitamin D, and fibroblast growth factor 23 (FGF-23), an important regulator of phosphate homeostasis (147). FGF-23 is secreted by osteocytes and osteoblasts in response to oral phosphate loading or increased serum 1,25-dihydroxyvitamin D levels (147). Iron infusion has been shown to induce hypophosphatemia through elevation in FGF-23 (148). This is particularly pertinent in the context of iron overload and the increasing number of reports of urine phosphate wasting in thalassemia (149). More recently, therapeutic doses of deferasirox have been shown to cause dose-dependent hypercalciuria in thalassemia major (33). Increased urine phosphate and calcium loss in association with deferasirox may therefore be an important risk factor for the development of osteomalacia in thalassemia. Multiple other factors are also likely to contribute to the renal-bone dysfunction seen in thalassemia (Figure 7).

The presence of kidney dysfunction is a relatively recent but potentially concerning complication associated with thalassemia. Deferasirox, the oral iron-chelating agent, is known to cause a reversible increase to serum creatinine (28); other abnormalities of renal function have been described in thalassemia subjects, mainly through case reports (150). Abnormal elevated levels of renal tubular markers have been detected in subjects with thalassemia major on deferoxamine. These markers include urinary N-acetyl β-D-glucosaminidase (35.9%), fractional excretion (FE)-Na (29.1%), FE-K+ (7.8%), FE-uric acid (52.4%), urine protein creatinine ratio (0.3%), and urine calcium to creatinine ratio (22.3%) (151). These renal disorders occurred in greater frequency with age, increased duration of transfusion, and deferoxamine usage (151). In particular, an elevated urine phosphate and calcium loss in association with deferasirox may therefore be an impor-
tant risk factor for the development of osteomalacia in thalassemia. Multiple other factors are also likely to contribute to the renal-bone dysfunction seen in thalassemia. Urine calcium to creatinine ratio was found to be present in 32.2% of transfusion-dependent thalassemia patients, with most of these patients on deferroxamine or deferriprone (32).

Cases of renal failure and Fanconi syndrome have also been described with deferasirox (152). Fanconi syndrome leads to reduced absorption of glucose, amino acids, phosphate, and bicarbonate by the proximal renal tubule. The Fanconi syndrome secondary to deferasirox is generally reversible on cessation of therapy but can occur on rechallenge of the drug (153). The loss of urine phosphate can lead to impaired bone mineralization and osteomalacia. Osteomalacia secondary to renal phosphate wasting with deferasirox therapy, which was reversible on cessation of deferasirox, has also been reported (149).

The pathogenic mechanism(s) leading to renal impairment with deferasirox has not been fully elucidated but is likely to be multifactorial. The kidney tubule is likely to be already compromised by the long-term effects of chronic iron overload and anemia, which are present in all patients with transfusion-dependent thalassemia (154). Iron can catalyze the Fenton reaction, leading to the formation of free oxygen radicals, and this can cause oxidative stress and damage to cells. The effect of oxidative stress can also be exacerbated by anemia. Deferasirox is particularly lipophilic and can enter cells freely and form complexes with potentially toxic elements such as iron (152). These intracellular complexes are less lipophilic and remain trapped within the cytoplasm, leading to toxicity. This insult can lead to lipid peroxidation, cell damage, and dysfunction.

Kidney stones are a recognized complication of hypercalciuria and are associated with reduced BMD and increased fracture risk in the general community (155). Apart from case reports and anecdotal experience, the prevalence of kidney stones and its associated effect on the skeleton was largely unappreciated in thalassemia. In a retrospective study of subjects with transfusion-dependent thalassemia, the prevalence of asymptomatic kidney stones was found to be 18.1% (111). These stones were more prevalent in male subjects and associated with reduced femoral neck BMD and an increased risk of fractures among male stone formers. Of significance, stone formers in this study cohort were found to have higher serum creatinine and lower ferritin levels, suggesting a possible causative role for deferasirox (111). The recent finding that deferasirox leads to dose-dependent hypercalciuria provides a biological mechanism for both increased kidney stones and accelerated bone loss in thalassemia major (33); this is an area deserving of further research.

J. Calcium and vitamin D

Vitamin D plays an important role in calcium homeostasis and musculoskeletal health. The definition of what constitutes optimal 25-hydroxyvitamin D [25(OH) vitamin D] levels is not clear (156). The circulating 25(OH) vitamin D level needed to suppress serum PTH concentration had been proposed, but levels have varied widely between 30 and 99 nmol/L (157, 158). Expert opinion suggests that the optimal serum 25(OH) vitamin D level for bone health is between 50 and 80 nmol/L (156). The prevalence of suboptimal 25(OH) vitamin D has been well described worldwide, and in an Australian cohort, 25(OH) vitamin D levels <28 nmol/L were present in 11.3% of subjects, and 43.2% of the subjects had levels <50 nmol/L (159).

In thalassemia patients, 12.8% had 25(OH) vitamin D concentrations <27 nmol/L, and 82% had levels <75 nmol/L, with 25(OH) vitamin D levels being lowest in adolescence (60). Other studies have shown 25(OH) vitamin D levels to be inversely proportional to age (160). In thalassemia major patients, the 25(OH) vitamin D levels were negatively correlated with age (P < .05) and with serum ferritin (P < .05). Thalassemia major patients who had lower 25(OH) vitamin D levels <17.8 ng/mL had higher serum ferritin levels (P < .01) and higher PTH (P < .05) compared to those with normal 25(OH) vitamin D (160). Despite the high prevalence of low vitamin D status among thalassemia major patients, the association between low 25(OH) vitamin D levels and reduced BMD has been inconsistent (161, 162). Moreover, no studies have been able to demonstrate that vitamin D supplementation independently increases BMD in thalassemia. In the absence of interventional studies of vitamin D therapy on BMD or fractures, adoption of appropriate vitamin D targets as in the general population is most appropriate in the thalassemia cohort.

K. Bone turnover markers

Biochemical markers of bone turnover are a noninvasive way of assessing whole-body bone remodeling (163). These markers can include products produced by osteoclasts and osteoblasts or osteoclast-generated degradation products of bone matrix. In clinical practice, changes in bone turnover are an independent risk factor for fracture and are also useful in monitoring the effect of osteoporosis therapy (163, 164).

Patients with thalassemia bone disease have evidence of increased bone resorption, but normal levels of bone formation markers have also been reported (38, 136, 165–
 résultant in a study of 17 children (mean age, 13.4 years) with secondary mineralization of osteoid seams (171). Interest-
many thalassemia major patients may show defects in sec-
function, and in particular hypercalciuria (32, 150), in
defects (170).
severe with cortical bone fissures and focal mineralization
of other studies where changes in cortical bone were most
was seen. The lack of osteomalacia was also characteristic
levels, although no evidence of abnormal osteoid volume
and low 25 (OH) vitamin D and 1,25 (OH) vitamin D
(122). These patients had normal bone turnover markers
IGF-1 and BMD at the total hip but not the lumbar spine
volume over total volume was positively correlated with
served measures of other bone parameters on biopsy. Bone
volume over total volume was positively correlated with
IGF-1 and BMD at the total hip but not the lumbar spine
(122). These patients had normal bone turnover markers
and low 25 (OH) vitamin D and 1,25 (OH) vitamin D
levels, although no evidence of abnormal osteoid volume
was seen. The lack of osteomalacia was also characteristic
of other studies where changes in cortical bone were most
severe with cortical bone fissures and focal mineralization
defects (170).
However, the increased prevalence of renal tubular dys-
function, and in particular hypercalciuría (32, 150), in
many thalassemia major patients may show defects in sec-
ondary mineralization of osteoid seams (171). Interest-
ally, in a study of 17 children (mean age, 13.4 years) with
β-thalassemia/HbE and thalassemia major who were sub-
optimally transfused, focal osteomalacia was the predom-
inant bone abnormality.
The contribution of iron toxicity on bone is unclear.
In vitro models have shown that iron toxicity can pro-
VII. The Assessment of Bone Structure,
Bone Mineral Density, and Body Composition
in Thalassemia
A. Bone biopsy
Bone histomorphometry provides an important visual
overview of the complex and competing factors involved
in thalassemia bone disease. In a study of 18 eugonadal
subjects with β-thalassemia/HbE, Domrongkitchapaorn et al (122) examined bone histomorphometry after tetra-
cycline double labeling. The most striking abnormality
was the reduction in trabecular bone volume over total
volume in these subjects in the presence of relatively pre-
served measures of other bone parameters on biopsy. Bone
volume over total volume was positively correlated with
IGF-1 and BMD at the total hip but not the lumbar spine
(122). These patients had normal bone turnover markers
and low 25 (OH) vitamin D and 1,25 (OH) vitamin D
levels, although no evidence of abnormal osteoid volume
was seen. The lack of osteomalacia was also characteristic
of other studies where changes in cortical bone were most
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optimally transfused, focal osteomalacia was the predom-
inant bone abnormality.
The contribution of iron toxicity on bone is unclear.
In vitro models have shown that iron toxicity can pro-
omite osteoclast activity (102) and reduce osteoblast
function (97); animal models of iron overload have
demonstrated a reduction in parameters of bone for-
mation (102). In patients with thalassemia, stainable
iron was negatively correlated with trabecular bone vol-
ume. However, iron overload showed no changes to
osteoid volume, thickness, or osteoblast surface (172).
On balance, iron toxicity in thalassemia may exert its
effect on bone indirectly through an increased risk of
hypogonadism and IGF-1.
The variability seen on bone histomorphometry in
transfusion-dependent thalassemia is not entirely surpris-
ing; the many competing factors associated with deterio-
ration in bone quality and different treatment protocols
associated with each thalassemia unit most likely account
for this (Figure 8).
B. Dual-energy x-ray absorptiometry
DXA is the most widely used modality for the as-
essment of bone mineralization in clinical practice. It
serves to identify those patients at greatest risk of fra-
gility fractures, to guide decisions regarding treatment,
and to monitor the response to therapy (46). The rela-
tively low cost per study and radiation exposure, in the
range of 2–5 mrem (173), has made it a safe and acces-
sible tool for use in the management of osteoporosis.
The precision of DXA, expressed as a coefficient of vari-
ation, has been reported to be as low as 0.48% for the
lumbar spine and 0.85% for the femoral neck (174). This
enables reliable long-term follow-up of patients
with reduced BMD.
Multiple large prospective studies have confirmed the
accuracy of DXA in predicting fracture risk. In postmeno-
pausal women, for each standard deviation reduction in
femoral neck bone density, the age-adjusted risk of hip
fracture was increased 2.6-fold (175). BMD at the femoral
neck was also a better predictor of hip fracture than mea-
surements of BMD at the spine or radius (175). However,
non-BMD risk factors for fractures such as gender, age,
family history of osteoporosis, glucocorticoid use, and
hypogonadism are important factors in both the general and
thalassemia population (36, 50, 176). This is in addition
to the thalassemia-specific risk factors such as marrow
expansion, iron overload, and kidney stones that are
known to reduce BMD and are associated with fracture
risk (111).
There are several limitations to the use of DXA that
are particularly relevant to the thalassemia population.
BMD, as measured by DXA, corrects BMD for the area
(height and width) but not for the volume (height,
width, and thickness) of bone. Therefore, if two people
with identical “true” volumetric bone density are com-
pared, the shorter person will have a lower BMD than the taller one (59, 177). Controversy persists about the optimal method for adjusting for variations in bone size, body composition, and maturity; ideally, the adjustment method should prove to be a stronger predictor of fracture (177). Thalassemia bone disease is still very much a problem of young adults. The high prevalence of GH deficiency and hypogonadism, compounded with the significant impact of anemia and the need for chronic transfusion, contributes to reduced skeletal size and a failure to reach peak bone mass in severe thalassemia (49). Indeed, nutrition, physical activity, pubertal stage, disease severity, patient and family fracture history, and medication exposure need to be considered in the assessment of pediatric bone disease.

The lumbar spine and femoral neck are the two most common sites for measurement of BMD using DXA. The presence of multiple degenerative changes at the spine can impact on the accuracy of DXA. In a study of women between 40 and 84 years of age, numerous pathologies were demonstrated that could impact on BMD. Osteophytes were present in 45.8%, osteochondrosis in 21.5%, vascular calcification in 24.3%, and scoliosis in 22.2% (178). The spine in patients with thalassemia is also a site of significant bone deformity secondary to significant marrow expansion and deferoxamine-induced bone dysplasia (179, 180). Therefore, the precision of DXA in monitoring BMD changes at the spine in transfusion-dependent thalassemia may be significantly affected by these morphological changes. The femoral neck is also the most accurate site for monitoring the longitudinal changes in BMD in patients with thalassemia (107).

An assessment of bone quality can be derived through the use of the trabecular bone score (TBS), which is derived from the spine through DXA and is a predictor of fracture risk (181, 182). TBS has been shown to be lower in patients with thalassemia compared to controls and to also correlate with BMD, but an association between TBS and fracture is yet to be established (183).

C. Peripheral quantitative computer tomography

The bone loss that occurs in thalassemia is unlikely to affect cortical and trabecular bone in equal measure. This presents obvious limitations for DXA, which is a two-dimensional measure of bone density and therefore encapsulates cortical and trabecular components of bone into a single parameter.

Quantitative computed tomography (QCT) is a three-dimensional technique for quantifying BMD at the spine, proximal femur, forearm, and tibia. This diagnostic modality has a number of advantages over conventional...
DXA: cortical and trabecular bone can be separated, trabecular volumes of interest are largely independent of degenerative changes in the spine, and three-dimensional geometric parameters can be determined (184).

A number of small studies have investigated skeletal status in thalassemia employing QCT. In a study of 48 patients, the correlation coefficient between DXA BMD and QCT trabecular BMD was 0.545 (P < .001). The classification of patients into normal, osteopenia, and osteoporotic categories, using QCT Z scores, was in better agreement with the assignment based on trabecular number (k = 0.209; P = .053) than the classification using DXA Z scores (k = 0.145; P = .12) (185). Another study employing peripheral QCT showed lower tibial trabecular volumetric BMD, cortical area, cortical bone mineral content, cortical thickness, periosteal circumference, and section modulus Z scores in thalassemic patients compared to controls (52).

Although there have been relatively few studies employing QCT techniques to assess thalassemia bone disease, this technique appears to have significant advantages over conventional DXA in assessing bone status in the pediatric cohort and at the spine. The question of whether QCT parameters are superior to DXA measures in predicting fracture risk remains to be answered.

D. Body composition using dual-energy x-ray absorptiometry

The study of body composition is concerned with the percentage and distribution of fat and lean tissue in the body. This can be derived from most DXA machines after a total body density study, but despite its availability, the assessment of body composition using DXA is an underutilized tool.

The study of body composition is particularly relevant in thalassemia with its impaired growth, poor nutritional status, and delayed skeletal and sexual maturation. However, the nature of associated body composition deficits has been poorly studied. In the general population, body composition is influenced by many factors including age, gender, gonadal status, nutrition, exercise, and hormonal factors (186). Lean tissue mass has been shown to be highly correlated with BMD up to middle age, and fat mass begins to account for a larger variance in BMD thereafter (187–189).

The need for chronic transfusion in severe thalassemia may have significant long-term consequences on nutrition, physical activity, and growth (41). Hepatosplenomegaly is an important complication of ineffective erythropoiesis and will lead to an overestimation of total lean tissue mass if measured by DXA. The need to account for multiple confounding factors in such an analysis is challenging. These factors and others may be implicated in the sexual dimorphism of body composition and bone density in patients with transfusion-dependent thalassemia.

 Whereas the effect of hypogonadism on BMD and body composition has been well described in the general population, the influence of hypogonadism on body composition in adult transfusion-dependent thalassemia is uncertain. In a pediatric cohort, hypogonadism was not correlated with measures of body composition in subjects with transfusion-dependent thalassemia (41). In a cross-sectional study of transfusion-dependent thalassemia, hypogonadism attenuates the strength of the muscle-bone relationship in males but strengthens the positive correlation of skeletal muscle mass and fat mass in female subjects (190).

The effect of reduced lean muscle mass on falls risk, BMD, and fractures has been described in the general population (191). Low grip strength is associated with reduced BMD (192), falls, and fractures in the general population (193). In a cross-sectional study of patients with thalassemia, the plasma level of klotho protein was found to be lower in patients with thalassemia major compared to healthy controls and was correlated with handgrip strength, and these patients had a higher probability of fragility fractures (194).

Exploring the inter-relationship between fat mass, lean tissue mass, and BMD in the hypogonadal thalassemic cohort may provide insights into the effect of sex hormone replacement, nutrition, and exercise on BMD. GH therapy results in increased muscle mass and reduced fat mass (195). There have been no studies examining the effect of hypogonadism or GH deficiency on body composition in subjects with transfusion-dependent thalassemia.

Many studies have reported inconsistent findings in describing the relationship between fat, muscle, and bone. One reason may be the failure to account for the multicollinearity between body weight, fat, lean tissue, and other covariates with BMD (196). Collinearity is a common but important statistical problem in multiple regression analysis and can be seen in many analyses of body composition (196). It can occur when the correlations among the independent variables, ie, weight, fat, and lean tissue, are highly correlated in a multiple regression analysis where BMD is the dependent variable. When two or more variables are highly correlated, they convey essentially the same information from a statistical perspective. This can lead to the paradoxical situation where the overall statistical model is significant, but none of the independent variables that were adjusted in the multiple regression model are significant (197). Therefore, it makes some variables statistically insignificant where they otherwise should be, and vice versa.
E. Bone deformity

The cellular hypoxia that results from the more severe forms of thalassemia results in increased erythropoietin production (198). High levels of erythropoietin lead to significant marrow expansion in sites of extramedullary erythropoiesis. This leads to deformities of the skull, face, vertebrae, and long bones with skeletal consequences of disfigurement and increased risk of fracture (199) (Figure 9, A and B). Indeed the BMD at the total body, femoral neck, and lumbar spine was found to be negatively correlated with serum transferrin receptor levels, a marker of erythropoietic activity (66).

Chronic back pain is common in transfusion-dependent thalassemia. In those with thalassemia major and intermedia, extensive, severe, and multilevel lumbar disc degeneration has been described (200). Scoliosis is also more common and is associated with lower hematocrit levels (201). Moreover, the incidence, evolution, and etiology of scoliosis in β-thalassemia differs from that of idiopathic scoliosis, suggesting that scoliosis in β-thalassemia represents a distinct entity (202). The vertebrae are also characterized by cortical thickening and have a striated appearance resulting from preservation and thickening of the vertical trabeculae (203) (Figure 10).

Deferoxamine use is also associated with bone dysplasia, leading to reduced final height (199, 204), widening of the growth plate, biconvex contours of vertebral end-plates, and platyspondyly with decreased vertebral body height (179, 199). The age at which defer-oxamine therapy is initiated appears to influence the risk of bone deformity. In a retrospective study, defer-oxamine therapy initiated after 3 years of age, and only after iron overload has been established, did not lead to compromised longitudinal growth (205). Patients with thalassemia major have short stature, which can be secondary to disproportionate truncal shortening; the causes are multifactorial, but hypogonadism and iron chelation therapy may be implicated (206).

The presence of spinal dysmorphism may compromise the accuracy of measuring lumbar spine BMD using DXA. This is an important factor that has yet to be investigated in clinical studies of thalassemia bone disease.

VII. Treatment

A. Overview

The severe anemia and the complications related to its treatment underlie the unique set of risk factors that characterize thalassemia bone disease. Optimal transfusion and chelation therapy undoubtedly reduce the severity of bone fragility and deformity (207). Skeletal morbidity associated with hormonal complications such as hypogonadism further contributes to reduced peak bone mass and long-term reduction in BMD. Randomized control studies investigating the effect of sex steroids on BMD are lacking; this adds to the uncertainty regarding what constitutes the most appropriate dose and route for sex hormone replacement in thalassemia bone disease.

Calcium and vitamin D replacement is common in thalassemia, but evidence of improvement in BMD and/or fractures is lacking, as is the case in the normal population. The experience with pharmacological treatment of osteoporosis in thalassemia is mainly confined to the bisphosphonates. There is minimal experience with teriparatide, denosumab, and strontium ranelate showing improvements in BMD, but fracture endpoints are lacking.

B. Bisphosphonates

Bisphosphonates are potent inhibitors of bone resorption and are widely used in the treatment of osteoporosis, myeloma, and metastatic bone disease. The newer generation bisphosphonates interfere with the mevalonate pathway by inhibiting farnesyl diphosphate synthase, thereby blocking prenylation of GTP-binding proteins (208). The effect is decreased osteoclast recruitment, differentiation, activity, and survival, resulting in reduced bone resorption. This can be seen through a reduction in bone turnover markers and an increase in BMD, with a reduction in the risk of fracture (208).

Table 2 summarizes the published randomized control studies of bisphosphonates in thalassemia to date (209–213). These studies were between 12 and 24 months in duration, with the largest study enrolling 118 subjects (213). The randomized design of these studies is their major strength, but there are several limitations. The small number of patients and the short duration of treatment mean there is insignificant power to detect fracture incidence. Despite the mean age of study participants being <50 years, only one study reported DXA Z scores (209); the other studies reported areal BMD and T scores (210–213). It is also important to stress that the zoledronic acid used in these trials was given at a dose of 4 mg every 3 to 6 months for up to 2 years (211, 212), in contrast to the more common dose of 5 mg once a year in the non-thalassemia population. A recent Cochrane review of randomized control studies of thalassemia major patients treated with bisphosphate therapy concluded that treatment resulted in an increase to BMD (214).

Treatment with zoledronic acid, alendronate, and neridronate was associated with a significant increase in BMD, but clodronate was not associated with a significant increase in BMD despite achieving a significant reduction in bone turnover markers (210). A direct comparison be-
between BMD is not possible in these trials, given the different treatment agents and baseline risk of osteoporosis in each study. A significant increase in BMD was associated with a concomitant significant reduction in bone resorption markers in these studies, except for the study by Gilfillan et al (212). The iv bisphosphonates have the advantage of being able to be administered after the patient’s regular blood transfusion. Furthermore, treatment with iv zoledronic acid overcomes problems of compliance because bone effects last for at least 12 months (215). The effect of zoledronic acid on BMD in thalassemia major is enduring, with improvements seen 24 months after discontinuation of treatment (216). This also raises the question of whether a yearly dose of zoledronic acid at 5 mg, rather than a 3- to 6-monthly regimen of zoledronic acid as used in some trials (211, 212), is effective in improving BMD in thalassemia major.

Incident fractures, particularly of the vertebrae, are key efficacy endpoints in bisphosphonate trials. Despite demonstrating an increase in BMD and a reduction in turnover markers, antifracture efficacy data are lacking from bisphosphonate trials in patients with thalassemia. The lack of fracture efficacy data in this cohort is due mainly to small study size and short study duration. In addition, fractures may be dependent on extrinsic factors such as high risk-taking activity in young subjects. Back pain in transfusion-dependent thalassemia is common and may be due to spinal deformity or, more frequently, to bone marrow expansion (36). The effect on bone pain with bisphosphonate treatment was assessed objectively in two randomized trials in thalassemia and showed a significant improvement as early as 3 months after treatment with zoledronic acid (211, 213).

The use of zoledronic acid was associated with a “flu-like” reaction in a small proportion of thalassemia subjects with the first infusion, but this was absent with subsequent treatments (211, 212). The clinical concerns related to bisphosphonate-associated osteonecrosis of the jaw and atypical femoral fractures (AFFs) have not been described in clinical trials in thalassemia patients. However, a recent case series has confirmed three cases of bisphosphonate-associated osteonecrosis of the jaw in thalassemia major patients taking alendronate (217). Dental examination should be considered before the use of potent antiresorptive medications. There have been four reported cases of AFFs in patients with thalassemia who have received bisphosphonates (218–219). The relationship between bisphosphonate use and AFF cannot be considered to be causal yet, but alterations in the material properties of bone resulting from low bone turnover may be
an important pathogenic factor (220, 221). Increased clinical vigilance for both osteonecrosis of the jaw and AFF is required of clinicians treating bone disease in thalassemia.

The other clinical concern relates to the use of very potent bisphosphonates in a patient cohort that is relatively young, typically in their third or fourth decade. The decision to commence bisphosphonate therapy in patients with thalassemia must therefore include a careful risk assessment that accounts for the severity of bone disease, history of fracture, plans for pregnancy, and other skeletal comorbidities. This highlights the need for experienced clinical judgment and for treatment to be considered on a case-by-case basis. Future studies will need to address the optimal frequency and duration of bisphosphonate treatment. The concept of bisphosphonate holidays has been well described in postmenopausal osteoporosis, but whether this can be extrapolated to the thalassemia major population is uncertain and requires further investigation (222).

C. Sex steroid therapy

The relationship between hypogonadism and sex hormone replacement with BMD and fractures is clearly defined in the non-thalassemic population (223). The paucity of longitudinal studies and the variable dose and route of administration of sex hormone replacement in thalassemia has made it difficult to quantitate the effect of hypogonadism and its treatment on BMD. In a prospective study of 67 patients with thalassemia major, the effect of hypogonadism and sex hormone replacement regimens on BMD was assessed; lumbar spine BMD was 30% lower than controls, those with untreated hypogonadism had the lowest BMD, and females on continuous topical hormone replacement therapy (HRT) achieved the highest BMD (61). The optimal dose of HRT for skeletal health in thalassemia remains a matter of debate. The inconsistency between sex hormone replacement and improvement in BMD in some published studies (37) reflects the retrospective nature of these studies with limited numbers and heterogeneous hormone preparations. Chatterjee et al (120) conducted a prospective study of treated hypogonadal females that included individuals with thalassemia major and non-thalassemia. Although BMD improved in both groups, the non-thala-
semic group had a greater increase in spinal T scores in contrast to the thalassemia major patients (120). There are currently no studies investigating the effect of hypogonadism and its treatment with HRT on fracture outcomes in thalassemia.

The current literature supports the benefits of sex hormone replacement in optimizing skeletal health. However, important clinical questions pertaining to dose, route of administration, and the impact of treatment on fracture endpoints in thalassemia remain unanswered.

D. Others (zinc, teriparatide, strontium ranelate, denosumab, and romosozumab)

The use of zinc in patients with thalassemia was associated with an increase in total body bone mineral content but no significant change in bone mass or BMD at the hip or spine (214, 224). Zinc appears to be an important nutrient for growth and development in thalassemia, and zinc deficiency is widespread in this cohort (225, 226).

Teriparatide leads to an increase in BMD and reduction in fractures in men (227) and postmenopausal women (228). The clinical use of teriparatide in thalassemia major leads to an increase in BMD, although the experience is limited to case reports, and therefore its use should be considered strictly on an individual basis (94, 229). The use of teriparatide as a follow-on treatment after prolonged or failed bisphosphonate therapy appears clinically justified and sound in thalassemia major. However, severe extramedullary erythropoiesis can lead to spinal cord compression and treatment with radiation therapy in transfusion-dependent thalassemia; teriparatide therapy would be contraindicated in these cases given the potential concerns of bone malignancy.

Strontium ranelate leads to an increase in BMD and a reduction in fractures (230), although concerns regarding

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<th>Table 2. Summary of Randomized Control Studies of Bisphosphonates in Thalassemia Published to Date</th>
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<td><strong>First Author (Ref.)</strong></td>
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<td>Study design</td>
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<td>Active treatment I</td>
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<td>Baseline BMD (placebo)</td>
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<td>Lumbar spine</td>
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<td>Femoral neck</td>
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<td>Baseline BMD (active treatment)</td>
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<td>Lumbar spine</td>
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<td>Percentage change in BMD in active group</td>
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<td>Femoral neck</td>
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<tr>
<td>Placebo, −7.4</td>
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<td>Alendronate, 5.64a</td>
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Abbreviations: TM, thalassemia major; TI, thalassemia intermedia; ZA, zoledronic acid.

a Statistically significant compared to placebo (P < .05).
increased cardiovascular risks have surfaced more recently (231). In a small study of 24 patients with thalassemia major, treatment with strontium ranelate improved BMD and normalized bone turnover markers, as well as lowering serum sclerostin levels (232). However, the use of strontium ranelate will require caution, given the increased baseline risk of thromboembolism (233) and cardiac iron overload in transfusion-dependent thalassemia.

Denosumab is a fully human monoclonal antibody to RANKL and a potent antiresorptive used in the treatment of osteoporosis. In a small study of 30 subjects with thalassemia major, denosumab administered every 6 months for 1 year was associated with a 9.2% and 6.0% increase in BMD at the lumbar spine and femoral neck, respectively (234). The most common side effect was pain in the back and extremities (12%) and nausea (10%), whereas hypocalcemia occurred in 7% (234). The risk of hypocalcemia is a potential concern in thalassemia, given the predilection for low serum calcium secondary to hypoparathyroidism (108, 109) and renal impairment associated with the use of deferasirox (28). Thus, the use of denosumab in thalassemia major will require increased vigilance for associated hypocalcemia.

Romosozumab is a monoclonal antibody that binds and inhibits sclerostin. Romosozumab increases bone formation and has been shown to increase BMD in postmenopausal women (235). Thalassemia patients have been found to have higher baseline levels of sclerostin (89). The corollary is that the use of a specific antisclerostin antibody, such as romosozumab, may be beneficial in treating reduced BMD in patients with thalassemia. However, the efficacy and safety profile of this drug in the treatment of low BMD and fractures in thalassemia remains to be established.

Moreover, the optimal frequency and duration of bisphosphonate administration is not known. The role of anabolic therapies such as teriparatide and, in the near future, the antisclerostin antibody romosozumab needs to be studied. The optimal dose and route of application of sex hormone therapy for bone preservation also remain to be established.

Important questions remain regarding the mechanism underlying the increased prevalence of kidney stones and associated bone loss in thalassemia. Whether or not this defect in renal tubular dysfunction is secondary to the underlying thalassemia condition, the role of iron overload and iron chelators needs to be clarified. Moreover, the clinical sequelae of renal tubular dysfunction such as hypercalcemia or phosphate wasting leading to accelerated bone loss and kidney stones also need to be confirmed in longitudinal studies.

VIII. Perspectives and Conclusion

The current management of thalassemia major with regular transfusion therapy and concomitant iron chelation has significantly improved life expectancy for patients over the past four decades. Thalassemia bone disease has emerged as the major challenge in addressing the increasing burden of morbidity associated with transfusion-dependent thalassemia.

Despite its high burden of disease, several basic but important knowledge gaps remain in our understanding of thalassemia bone disease. Prospective longitudinal studies examining fracture incidence are required. Bisphosphonates have been used in the treatment of low BMD and fractures, but these studies have not been adequately powered to detect a difference in fracture reduction.

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