New insights into the pathophysiology and management of osteoporosis in patients with beta thalassaemia

Ersi Voskaridou1 and Evangelos Terpos2,3

1Thalassaemia Centre, Laikon General Hospital, Athens, Greece, 2Department of Haematology, General Air Force Hospital, Athens, Greece, and 3Department of Haematology, Faculty of Medicine Imperial College London, Hammersmith Hospital, London, UK

Summary

Osteoporosis represents an important cause of morbidity in adult patients with thalassaemia major (TM). The pathogenesis of osteoporosis in TM is multifactorial, and includes bone marrow expansion, endocrine dysfunction and iron overload. Additional genetic factors, such as the COLIA1 gene polymorphism, seem to play an important role in the development of low bone mass in these patients. However, the mechanisms through which these factors lead to bone loss have not been completely clarified. The diminished osteoblast function is accompanied by a comparable or even greater increase in osteoclast activity. The receptor activator of nuclear factor-kappa B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) pathway has been recently recognized as the final, dominant mediator of osteoclast proliferation and activation. There is increased evidence that this pathway interferes in the pathogenesis of thalassaemia-induced osteoporosis. Currently, bisphosphonates that are potent inhibitors of osteoclast function have been used in TM patients with encouraging results. This review attempts to summarize all the novel data for the biology of bone damage in TM. It also describes the results of all major studies that have investigated the effects of different treatment modalities for TM-induced osteoporosis, their mode of action, and the future implications of their use.

Keywords: beta thalassaemia, osteoporosis, receptor activator of nuclear factor-kappa B ligand, osteoprotegerin, bisphosphonates.

Thalassaemia major (TM) is a hereditary haemoglobinopathy caused by a defect in the ability of erythroblasts to synthesize the beta chain of adult haemoglobin. Cooley and Lee (1925) described the first series of splenomegaly in non-transfused children with anaemia and peculiar bone changes with mongoloid appearance caused by the enlargement of the cranial and facial bones. This enlargement was thought to be due to anaemia and the consequent counterbalance mechanism of ineffective erythropoiesis, thus resulting in the dramatic expansion of the bone marrow, almost 30–40 times more than the normal. Marrow expansion causes mechanical interruption of bone formation, leading to cortical thinning, and is hitherto considered as a main reason of distortion and fragility of the bones in thalassaemia patients.

Since then, other bone abnormalities have also been described in patients with thalassaemia, such as spinal deformities, scoliosis, nerve compression, spontaneous fractures, osteopenia and osteoporosis. As treatment with transfusion programmes and chelation therapy has significantly prolonged survival in thalassaemia patients, osteopenia and osteoporosis represent prominent causes of morbidity in young adults of both genders with TM or thalassaemia intermedia (Pootrakul et al, 1981; Michelson & Cohen, 1988; Johanson, 1990; Orvieto et al, 1992; Jensen et al, 1998; Vichinsky, 1998). However, even in the highly transfused group, patients continue to develop osteoporosis, partly due to incessant but unexpected marrow hyperplasia (Vichinsky, 1998). During the last decade, the presence of osteopenia or osteoporosis in well treated TM patients has been described in different studies with a frequency of approximately 40–50% in the studied population (Giardina et al, 1995; Jensen et al, 1998; Abdollah Shamshirsaz et al, 2003). Therefore, understanding the underlying mechanisms for bone destruction in these patients seems to be a necessity. The pathogenesis of osteoporosis in TM is very complicated and differs from the pathogenesis of bone deformities characteristically found in non-transfused patients (thalassaemia intermedia), who develop bone distortion mainly due to ineffective haemopoiesis and progressive marrow expansion. Several genetic and acquired factors are implicated in bone destruction in TM. The typical delay in sexual maturation, presence of diabetes and hypothyroidism, parathyroid gland dysfunction, ineffective haemopoiesis with progressive marrow expansion, direct iron toxicity on osteoblasts and deficiency of growth hormone (GH) or insulin growth factor I (IGF-I) have been indicated as possible causes for thalassaemia-induced osteoporosis (De Vernejoul et al, 1982; Diamond et al, 1989; Jensen et al, 1997; Garofalo et al,
Overview of bone function and remodelling

The skeleton provides the mechanical support of the body and a reservoir for normal mineral metabolism. Bone is an active tissue constantly being remodelled and changing metabolically through the balanced activity of osteoclasts and osteoblasts on trabecular surfaces. On a microscopic level, bone metabolism always occurs on the surface of the bone at focused sites, each of which is termed a bone metabolism unit (BMU). Osteoclasts and osteoblasts are the cells that carry out bone metabolism at the fundamental BMU site. Therefore, although these cells account for only a small fraction of bone volume, their function is essential (Mundy, 1999). Bone turnover is always initiated by osteoclasts eroding a mineralized surface. After their activation by different factors (mechanical load, growth factors, hormones and cytokines), osteoclasts are attracted to the new BMU site where they erode the bone matrix, forming a lacunae. Resorption is then halted, followed by the recruitment of osteoblast groups to the outer edge of the erosion cavity that secrete new bone matrix and gradually fill in the resorption cavity. When the lacunae is filled with osteoid, this newly formed matrix is mineralized with hydroxyapatite, giving the BMU tensile strength (Christenson, 1997). Figure 1 depicts the normal bone resorption and formation process.

Osteoclasts

Osteoclasts are multinucleated cells, formed from the fusion of mononuclear progenitors of the monocyte/macrophage family (Hodge et al, 2004). Osteoclasts resorb bone by secreting proteases that dissolve the matrix and producing acid that releases bone mineral into the extracellular space under the ruffled border of the plasma membrane of osteoclasts (Roodman, 2004). Osteoclastogenesis requires contact between osteoclast precursors and stromal cells or osteoblasts. The adherence of osteoclasts to the bone surface is critical for the bone resorptive process, since agents that interfere with osteoclast attachment, such as cathepsin K, block bone resorption (Goldring, 2003).

Bone marrow stromal cells express two molecules that are essential to promote osteoclastogenesis: macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-kappa B (RANK) ligand (RANKL), which is a member of the tumour necrosis factor family (Itoh et al, 2000). M-CSF expands the pool of osteoclast precursors and RANKL in turn stimulates it to commit to osteoclast phenotype. Stromal cells and osteoblasts are the target cells of most osteoclastogenic factors that exert their effect by enhancing RANKL expression. Such agents include parathyroid hormone (PTH), thyroxine, 1,25-dihydroxyvitamin D3, and cytokines that use gp130 as part of their receptor, like interleukin-6 (IL-6) and oncostatin M (Teitelbaum, 2000; Horowitz et al, 2001).

RANKL is a type II transmembrane glycoprotein, while a soluble form (sRANKL) can be also released from its membrane-bound state by metalloproteinases (Kong et al, 1999). RANKL binds the RANK receptor on osteoclast precursors and induces the formation of osteoclasts by signalling through the nuclear factor-kappa B and Jun N-terminal kinase pathways. Osteoprotegerin (OPG), which is a member of the tumour necrosis factor receptor family secreted by stromal cells, is the decoy receptor for RANKL. OPG blocks the RANK–RANKL interaction and thus inhibits osteoclast differentiation and activation (Simonet et al, 1997; Morinaga et al, 1998; Horowitz et al, 2001).

The importance of the RANK/RANKL/OPG pathway in the formation of osteoclasts has been clearly demonstrated in knockout mice. Mice that lack either RANKL or RANK or that over-express OPG develop osteopetrosis because of decreased osteoclast activity (Kong et al, 1999; Kim et al, 2000). Conversely, OPG knockout mice are osteoporotic, develop multiple fractures and have decreased trabecular bone volume and numerous osteoclasts, since OPG cannot inhibit RANKL activity (Bucay et al, 1998). Thus, it is the balance between the expression of RANKL and OPG that determines the extent of bone resorption. The ratio of RANKL to OPG regulates the formation and activity of osteoclasts. The importance of RANKL in bone destruction has led to the development of recombinant OPG and antibodies against RANKL as potential treatments for bone diseases with an increased bone resorptive phase (Body et al, 2003; Vanderkerken et al, 2003).

Protons seem also to be important for osteoclast activation as HCO3~ acidosis stimulates resorption by activating mature osteoclasts, providing support for the critical role of acid–base balance in controlling osteoclast function (Meghji et al, 2001; Mori et al, 2003).

Osteoblasts are the bone-forming cells. They arise from mesenchymal stem cells, through a series of progenitor stages to form mature matrix secreting osteoblasts. The differentiation of osteoblasts is less well understood than the differentiation of thalassaemia.
osteoclasts (Stein & Lian, 1993; Ahdjoudj et al, 2004). Bone morphogenetic proteins (BMPs) are critical factors that stimulate the growth and differentiation of osteoblasts. The role of several other factors involved in the activation, proliferation and control of osteoblast progenitor cells has been partly clarified. Basic fibroblast growth factor (bFGF) increases both osteoblast proliferation and collagen synthesis within bone, but its precise method of action remains unknown (Power et al, 2004). Insulin-like growth factors (IGFs, type I and II) increase the protein content of osteoid by promoting preosteoblastic proliferation, decreasing collagen degradation, and increasing protein synthesis (Cornish et al, 2004). Transforming growth factors (TGF, beta 1 and beta 2) and platelet-derived growth factor (PDGF) also stimulate the population of precursor cells committed to becoming osteoblasts (Ortiz et al, 2003; Chaudhary et al, 2004).

Finally, a number of hormones, such as PTH, thyroxine, oestrogen, cortisol, insulin, and calcitonin, as well as vitamin D, are involved in the regulation of bone metabolism, effecting both progenitors and mature osteoblastic cells and osteoclasts. High levels of PTH, thyroxine, cortisol, and reduced levels of oestrogen, testosterone, vitamin D, calcitonin, and insulin accelerate bone loss, stimulating osteoblastic and osteoclastic activity (Christenson, 1997; Fig 1).

Osteopenia/osteoporosis and bone mineral density

Bone mass is the result of a balance between bone formation gained during growth and the subsequent bone loss. Several sensitive techniques are available for the quantitative assessment of the degree of total bone mass. Bone density measurement by dual X-ray absorptiometry (DEXA) of the lumbar spine, femoral neck and forearm is recommended as one of the most reliable and non-invasive technique for the assessment of bone mass (Grampp et al, 1997; Cefalu, 2004).
According to the World Health Organization (WHO, 1994), osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequential increase in fracture risk. The WHO based the diagnosis of postmenopausal osteoporosis on the presence of a BMD T-score that is 2.5 SD or greater below the mean for young women (Bonjour et al, 1999; Nguyen et al, 2000). The International Society of Clinical Densitometry (ISCD) used the same BMD criteria (Writing Group for the ISCD Position Development Conference, 2004) for the definition of osteoporosis in males, premenopausal women and children (Binkley et al, 2002; Hajjar & Kamel, 2004).

Acquired factors contributing to reduced BMD in beta-thalassaemia

Previous studies have demonstrated that multiple acquired factors are involved in the pathogenesis of osteopenia/osteoporosis in TM. They include the primary disease, itself causing bone marrow expansion (De Sanctis et al, 1998; Mahachoklertwattana et al, 2003a), and several secondary factors, such as hormonal deficiency (Anapliotou et al, 1995; Jensen et al, 1998; Raiola et al, 2003), iron overload (Anapliotou et al, 1995; Wonke, 2001), desferrioxamine toxicity (Olivieri et al, 1992; De Sanctis et al, 1996; Lala et al, 1998; Chan et al, 2002; Di Stefano et al, 2004), calcium, zinc and vitamin D deficiencies, and inadequate physical activity (Wonke, 1998; Bekheirnia et al, 2004). Most of these factors act through the imbalance in bone remodelling; they inhibit osteoblast activation and/or increase osteoclast function, leading to bone loss and osteoporosis.

Bone marrow expansion

Bone marrow expansion due to ineffective erythropoiesis is a typical finding in patients with TM and has been considered as a major cause of bone destruction (De Sanctis et al, 1998; Mahachoklertwattana et al, 2003b). Marrow expansion causes mechanical disruption of bone formation, leading to cortical thinning, increased distortion and fragility of the bones. Transferrin receptor studies have demonstrated increased bone marrow activity even in patients with low reticulocyte count or marrow hypoplasia (Vichinsky, 1998; Ma et al, 2003). However, no direct correlation was found between serum levels of soluble transferrin receptor (sTFR) and the severity of osteoporosis (Wonke, 1998).

Endocrine complications

Hypothyroidism, hypoparathyroidism, diabetes mellitus and mainly hypogonadism (as delayed puberty and/or secondary hypogonadism) are the main causes of osteopenia/osteoporosis in TM (Anapliotou et al, 1995; Jensen et al, 1998; Wonke, 1998; Carmina et al, 2004). Haemosiderosis of the pituitary gonadotrophic cells and iron deposition in the testes and ovaries are involved in the pathogenesis of endocrine complications in TM (Berkovitch et al, 2000; Wonke, 2001). Hypogonadism is a well-recognized cause of osteoporosis and osteopenia, not only in patients with TM but also in the general population, and is characterized by high bone turnover with an enhanced resorptive phase (Riggs et al, 1998). Oestrogen and progesterone appear to inhibit osteoclast activity and promote bone formation (Riggs, 2000), while testosterone has a direct stimulatory effect on osteoblast proliferation and differentiation (Olszynski et al, 2004) (Fig 1).

As mentioned in bone physiology, IGFs play also an important role in bone remodelling. Low serum IGF levels decrease osteoblast proliferation and bone matrix formation and reduce the activation of osteoclasts (Geusens & Boonen, 2002). Several studies have demonstrated a positive correlation between the BMD of the lumbar spine and the IGF-I concentration (Mahachoklertwattana et al, 2003a; Rucker et al, 2004). It is well documented that the GH-IGF axis is defective in TM. Thalassaemia patients have significantly lower circulating levels of IGF-I and the corresponding binding protein (IGFBP-III) than normal individuals, thus leading to increased bone resorption, decreased bone formation and finally to bone loss (Soliman et al, 1998; Lasco et al, 2002; Morabito et al, 2004; Perifanis et al, 2004).

Iron overload and desferrioxamine

Although endocrine dysfunction has a major role in the development of osteoporosis in transfused and non-transfused thalassaemia patients, the transfusion volume and chelation dose also influence the bone mass. Iron deposition in the bone impairs osteoid maturation and inhibits mineralization locally, resulting in focal osteomalacia. The mechanism by which iron overload interferes in osteoid maturation and mineralization includes the incorporation of iron into crystals of calcium hydroxyapatite, which consequently affects the growth of hydroxyapatite crystals and reduces the BMU tensile strength (Mahachoklertwattana et al, 2003b). On the other hand, desferrioxamine inhibits DNA synthesis, osteoblast and fibroblast proliferation, osteoblast precursors differentiation, and collagen formation, while it enhances osteoblast apoptosis, especially in patients who receive inappropriately high doses of desferrioxamine (De Sanctis et al, 1996; Chan et al, 2002).

Vitamin and trace minerals deficiencies

Vitamin C deficiency in iron-overloaded patients with low levels of serum ascorbic acid induces the risk of osteoporotic fractures (Michelson & Cohen, 1988). Vitamin D deficiency is also implicated in the pathogenesis of osteoporosis in TM patients due to the regulatory effect of vitamin D in both
osteoclasts and osteoblasts. Adequate calcium intake and small amounts of vitamin D administration during skeletal development can increase bone mass in adolescents and decrease bone loss in adult life (Johnston et al., 1992). However, most studies have failed to show reduced serum levels of 25-hydroxyvitamin D in TM patients. There is adequate data indicating that thalassaemia patients have also zinc deficiency (De Virgiliis et al., 1988; Arcasoy et al., 2001), which may lower their BMD (Bekheirnia et al., 2004). It is well-known that zinc and copper deficiencies are associated with osteoporosis (Cohen & Roe, 2000); thus, zinc supplementation may be administered in thalassemia patients with this trace mineral deficiency.

Physical activity

Patients with TM have reduced physical activity due to the complications of the disease and their overprotective parents, who do not encourage muscle activity. The association between mechanical stress and bone mass was first recorded by Galileo in 1683, who noted the relationship between body weight and bone size. Wheldon (1984) reported that immobility or prolonged bed rest leads rapidly to hypercalcirea, negative calcium balance and bone loss. He also mentioned that the duration and force of the muscle activity on bone are important in maintaining bone mass. In athletes the positive osteogenic effect of exercise has been proven by several studies (Todd & Robinson, 2003). Lane et al. (1986) studied male and female athletes, over 50 years old, who had been long distance runners for many years and found that their lumbar bone mass was higher when compared with sedentary controls. The above data suggest that lack of physical activity is another predisposing factor for osteoporosis in TM patients and muscle activity has to be encouraged in these patients.

Despite the major role of the above acquired factors in the development of thalassaemia-induced bone loss, there are thalassaemia patients who continue to present osteopenia and/or osteoporosis despite adequate transfusion and chelation programmes, hormonal replacement, and absence of other factors that contribute to the development of reduced BMD. It seems that there are underlying genetic factors playing a significant role in the imbalance of bone remodelling.

Genetic variants that predispose to reduced BMD

Genetic factors seem to play an important role in the development of low bone mass and osteoporotic fractures. These factors have been implicated in the pathogenesis of postmenopausal osteoporosis, as regulator genes of BMD, but have not been studied thoroughly in thalassaemia-induced osteoporosis.

The polymorphism at the Sp1 site of the collagen type Iα1 (COLIA 1) gene (collagen type I is the major bone matrix protein) was studied by Wonke et al. (1998), who found that approximately 30% of the TM patients were heterozygotes (Ss) and 4% were homozygotes (SS) for the Sp1 polymorphism. Female to male ratio was 2:1. The authors concluded that male patients with TM carrying the Sp1 mutation may develop severe osteoporosis of the spine and the hip more frequently than patients who do not carry this mutation. The COLIA 1 polymorphism has been associated with reduced BMD in postmenopausal osteoporosis, and predisposes women to osteoporotic fractures (Uitterlinden et al., 2001). The genes encoding collagen types Iα1 and Iα2 (COLIA 1 and COLIA 2 respectively) are also important candidates for the genetic regulation of BMD, as mutations that affect the coding regimens of these genes are implicated in the pathogenesis of osteogenesis imperfecta and osteoarthritis (Byers & Steiner, 1992; Uitterlinden et al., 2000). The study of the COLIA 1 polymorphism may help to identify those thalassaemia patients who are at a higher risk to develop osteoporosis and pathologic fractures (Perrotta et al., 2000).

Dresner Pollack et al. (2000) have reported that the vitamin D receptor (VDR) BsmI BB polymorphism represents a risk factor for bone mineral damage in adult patients with beta thalassaemia. A relationship between reduced growth with exon 2 (FokI) or intron 8 (BsmI) of VDR polymorphisms has also been described in healthy populations (Uitterlinden et al., 2002). Ferrara et al. (2002) examined the effect of FokI and Bsm1 polymorphisms of VDR on the stature and BMD of femoral neck and lumbar spine, in 108 prepubertal and pubertal beta-thalassaemia patients. The authors found significantly shorter stature and lower BMD in both sites of all patients with CC VDR genotype and significant shorter height and lower BMD of the lumbar spine in prepubertal and pubertal female patients with BB VDR genotype. However, there are thalassaemia patients with severe bone destruction who do not carry the above alleles, as well as patients who carry these alleles without impaired bone remodelling. Therefore, these polymorphisms may be considered only as indicators for the additional risk of low bone mass in beta-thalassaemia. Loss-of-function mutations in the gene of the VDR were also responsible for the bone disease that characterizes 1α,25-dihydroxyvitamin-D-resistant rickets (Malloy et al., 1994) and were associated with impaired bone density (Morrison et al., 1994; Cooper & Umbach, 1996).

The sequence variation of TGF-β1, 713–8delC, has also been associated with very low bone mass in osteoporotic and normal women (Langdahl et al., 1997). Bertoldo et al. (2000) also confirmed that the 713–8delC of TGF-β1 was related to severe bone loss and increased bone turnover in both normal and osteoporotic women. Perrotta et al. (2000) studied the association between BMD, COLIA 1 gene polymorphisms and 713–8delC of TGF-β1 in 135 beta-thalassaemia patients. A remarkable incidence of osteopenia and osteoporosis (90%) among regularly transfused patients was observed in this study; bone mass was lower in men than in women while male patients developed more prevalent osteopenia/osteoporosis of the spine than female patients. TGF-β1 polymorphism failed to
demonstrate a statistical correlation with BMD, while subjects who were heterozygous or homozygous for COLIA 1 gene polymorphism showed a lower BMD than subjects without the sequence variation. TGF-β1 polymorphism appears to influence bone mass mainly by increasing bone turnover (Langdahl et al., 1997; Bertoldo et al., 2000). On the contrary, COLIA 1 polymorphism seems to affect bone mass by influencing other aspects of bone metabolism, i.e. collagen structure. Therefore, as mentioned above, COLIA-1 polymorphism appears to be an independent risk factor for genetic susceptibility to osteoporosis (Perrotta et al., 2000; Uitterlinden et al., 2001).

The presence of restriction fragment length polymorphisms (RFLPs) for the vitamin D receptor (VDR) gene was studied by Masi et al. (1998). The authors identified a polymorphic (Tt) site at the VDR gene locus using the Taq I restriction fragment enzyme. Women carrying the tt genotype had significantly lower lumbar BMD compared with women carrying the Tt genotype. Oestrogen receptor and IL-6 gene loci have also been correlated with bone mass. The oestrogen receptor gene (ERG) has been found to be a regulator gene of bone mass but has not been studied in the thalassaemia population (Smith et al., 1994; Han et al., 1997).

All genetic factors that seem to be involved in the pathogenesis of thalassaemia-induced osteoporosis and their possible underlying mechanisms of action are depicted in Table I. However, genetic factors have rather a potential than practical role to-date. Further studies are needed to exact a final conclusion for the association between gene polymorphisms and bone mass in TM patients, although COLIA 1 gene polymorphisms seem to be of importance in the pathogenesis of thalassaemia-induced osteoporosis.

**Bone remodelling in thalassaemia-induced osteoporosis**

Most of the acquired factors described earlier act mainly through the inhibition of osteoblastic activity. There is evidence of reduced osteoblast function in TM. Morabito et al. (2004) have shown decreased levels of serum osteocalcin, a protein produced by osteoblasts, in patients with TM. Although osteoblast dysfunction is hitherto thought to be the major pathogenetic mechanism for osteoporosis in TM, there is also evidence of increased osteoclast activation in these patients. Both Dresner Pollack et al. (2000) and our group have shown that patients with TM and osteoporosis have elevated markers of bone resorption, such as urinary levels of N-telopeptides of collagen type I (NTX), which is a specific marker of bone resorption, and increased serum levels of tartrate resistant acid phosphatase isof orm 5b (TRACP-5b), an enzyme that is produced only by activated osteoclasts (Voskaridou et al., 2001, 2003). Furthermore, both NTX and TRACP-5b levels correlated with BMD of the lumbar spine in these patients (Dresner Pollack et al., 2000; Voskaridou et al., 2003). In accordance with these data, Lasco et al. (2002) and Morabito et al. (2004) have reported that pyridinoline and deoxypyridinoline, other markers of bone resorption, are increased in patients with TM and osteoporosis compared with normal controls. But what is the responsible mechanism for this osteoclast activation in thalassaemia patients?

The RANK/RANKL/OPG system seems to be of great importance for the activation and proliferation of osteoclast precursors. We have previously shown, in 13 patients with TM and osteoporosis, that serum levels of sRANKL were slightly increased, compared with controls (5.8 ± 4.2 pmol/l vs. 4.6 ± 1.4 pmol/l; P = 0.071), while the OPG serum levels were significantly reduced in this cohort of patients (2.6 ± 1.7 pmol/l vs. 4.0 ± 0.4 pmol/l in TM patients and controls, respectively; P < 0.001); thus the ratio of sRANKL/OPG was increased in TM patients (Voskaridou et al., 2003). In accordance with these results, Morabito et al. (2004) has shown, in 30 patients with TM and osteoporosis, using the same methods as our group, that sRANKL serum levels were significantly increased (8.1 ± 2.8 pmol/l vs. 4.5 ± 1.2 pmol/l in TM patients and controls, respectively; P < 0.0001), while OPG serum levels were reduced, but not significantly compared with controls (3.0 ± 1.3 pmol/l vs. 3.6 ± 1.4 pmol/l). This study also

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<thead>
<tr>
<th>Gene and polymorphism</th>
<th>Possible underlying mechanism of action</th>
<th>Number of thalassaemia patients</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLIA 1* (Sp1 polymorphism)</td>
<td>Down-regulation of structure and synthesis of procollagen type-I</td>
<td>188</td>
<td>Wonke et al., 1998</td>
</tr>
<tr>
<td>VDR** (FokI and BsmI polymorphisms)</td>
<td>Down-regulation of gender-related growth factors</td>
<td>108</td>
<td>Ferrara et al., 2002</td>
</tr>
<tr>
<td>TGF-β*** (713-8delC) and COLIA 1 (Sp1 polymorphism)</td>
<td>Reduced osteoblast function and proliferation</td>
<td>135</td>
<td>Perrotta et al., 2000</td>
</tr>
</tbody>
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*Collagen type Ia1 gene.
**Vitamin D receptor.
***Transforming growth factor-beta.
confirmed that the ratio of sRANKL/OPG is increased in patients with TM and osteoporosis, providing evidence for the role of RANKL/OPG system in the pathogenesis of osteoporosis in thalassaemia. The increase of RANKL, followed by unmodified OPG levels, with the consequent increase of RANKL/OPG ratio may represent the cause of uncoupling on bone turnover observed in thalassaemia patients. In both studies, there was no correlation between sRANKL or OPG levels with BMD of the lumbar spine or the femoral neck. However, Morabito et al (2004) have shown a negative correlation between RANKL and free testosterone in male thalassaemia patients and with 17-β oestradiol in female thalassaemia patients, which suggests that the RANKL/OPG system may be involved in mediating the action of sex steroids on bone. Furthermore, a correlation between the sRANKL/OPG ratio and erythropoietin levels that has also been reported recently, represents a mechanism through which anaemia, by continuously stimulating the erythropoietin synthesis and determining bone marrow hyperplasia, may increase bone resorption through enhanced RANKL levels (Morabito et al, 2004). All these data support the complex pathogenesis of osteoporosis in TM and reflect the difficulties in the management of this complication of thalassaemia.

Management of thalassaemia-induced osteoporosis

Prevention and general principles

The prevention and treatment of early bone loss is the best policy. An annual check of the BMD, starting in adolescence, is considered crucial. Physical activity must always be encouraged. Moderate and high impact activities are to be supported. Exercise has additional benefits: it improves the cardiovascular system, reduces the risk of diabetes and prevents depression. Smoking should be discouraged. Adequate calcium and zinc intake during skeletal development can increase bone mass in adult life and, in combination with the administration of low doses of vitamin D, may prevent bone loss and fractures (Lindsay, 1993; Soliman et al, 1998; Lasco et al, 2001). Early diagnosis and treatment of diabetes mellitus is also important, as the association between diabetes and low bone mass in TM patients has been well documented (Jensen et al, 1998). Furthermore, adequate iron chelation may prevent iron toxicity in the bone and sufficient blood transfusions may inhibit uncontrolled bone marrow expansion.

Hormonal replacement

Prevention of hypogonadism seems to be the most effective way for preventing osteoporosis and other bone deformities in thalassaemia patients (Lindsay, 1993; Jensen et al, 1998). Anapliotou et al (1995) recommended that continuous hormonal replacement therapy with transdermal oestrogen for females or human chorionic gonadotrophin for males improves bone density parameters. However, despite hormonal replacement, calcium and vitamin D administration, effective iron chelation, and normalization of haemoglobin levels, patients with TM continue to lose bone mass (Lasco et al, 2001; Carmina et al, 2004).

Calcitonin

Canatan et al (1995) evaluated the effect of calcitonin (CT), a potent inhibitor of osteoclasts, on bone mass in 14 patients with TM. One hundred IU of CT were administered, three times a week, for 1 year in combination with the daily administration of 250 mg of calcium. At the end of treatment period, bone pain had disappeared, radiological findings of osteoporosis had been improved and the number of fractures had decreased in the treatment group but not in controls. CT had no important side effects. Both parenteral and intranasal instillations are available.

Hydroxyurea

Ten patients with TM were given hydroxyurea, at 1.5 g per o.s. daily, in an attempt to reduce marrow hyperplasia diagnosed by magnetic resonance imaging (MRI). Hydroxyurea improved bone pain and MRI findings (Angastiniotis et al, 1998). However, these results have not been confirmed by other studies.

Bisphosphonates

The increased bone resorption observed in patients with thalassaemia-induced osteoporosis has led to the use of bisphosphonates in the management of osteoporosis in this cohort of patients. Bisphosphonates are potent inhibitors of osteoclastic bone resorption. They act by inhibiting osteoclastic recruitment and maturation, preventing the development of monocyte precursors into osteoclasts, inducing osteoclast apoptosis and interrupting their attachment to the bone (Suda et al, 1997; Fleisch, 1998).

All bisphosphonates have a high affinity for bone minerals and are preferentially delivered to sites of increased bone resorption or formation. In view of the accumulation of the bisphosphonates in bone, it is of great clinical interest that the inhibition of bone resorption reaches a certain steady level even when the compounds are given continuously, suggesting that, at the therapeutic dosage, there is no danger of a continuous decrease in bone turnover in the long run, leading to increased bone fragility, as seen in osteoporosis (Lin, 1996; Body, 1998). The decrease of bone resorption due to bisphosphonates is accompanied by an increase in calcium balance and mineral content of bone (Fleisch, 1998).

Bisphosphonates in patients with postmenopausal osteoporosis increase BMD and prevent bone fractures (Meunier et al, 1999). In thalassaemia osteoporosis, almost all generations of
Bisphosphonates have been used in an attempt to increase the BMD and improve the abnormal bone remodelling. Morabito et al. (2002) scheduled a randomized, placebo-controlled study to investigate the effects of 2 years daily oral administration of alendronate or intramuscular administration of clodronate on BMD, bone turnover markers, safety and tolerability in 25 thalassaemia patients with osteoporosis. Patients were randomized to receive placebo (eight patients) or 100 mg of clodronate, i.m., every 10 d (eight patients) or 10 mg alendronate per o.s. daily (nine patients). All patients also received 500 mg/d elemental calcium and 400 IU/d cholecalciferol. After 2 years of follow-up, the lumbar spine and femoral neck BMD had decreased significantly in the placebo group. Clodronate reduced bone resorption markers, deoxypyridinoline and pyridinoline, and inhibited bone loss but it was unable to increase BMD at all studied sites. Daily treatment with alendronate normalized the rate of bone turnover, and resulted in a rise in BMD of the spine and the hip. This increment was statistically significant at the femoral neck, whereas at the lumbar spine the gain was less marked. Alendronate caused few adverse effects, including upper gastrointestinal symptoms, but no patient discontinued the study.

The ineffectiveness of clodronate was established in another randomized, placebo-controlled trial (Pennisi et al., 2003), in which 30 male patients with TM and osteoporosis were randomized to receive clodronate at a dose of 300 mg i.v. every 3 weeks for 2 years or active placebo (calcium and vitamin D). In calcium and vitamin D-treated patients, a significant decline in spine, femoral, and total body areal bone density was observed at the end of the study. In the patients given intravenous clodronate a substantial stability of bone mass was reported, which was not significantly changed at the end of the study. The urinary excretion of deoxypyridinoline showed a progressive significant decline throughout the study period in clodronate-treated patients. However, no significant change was observed in broadband ultrasound attenuation (BUA) values of the lumbar spine in both groups of patients (Pennisi et al., 2003).

Pamidronate, a second generation aminobisphosphonate, has been given intravenously in patients with TM and osteoporosis. Firstly, Wonke (2001) evaluated the effect of pamidronate on the BMD of 39 TM patients. Pamidronate i.v. was given at doses of 15–60 mg, in a 40 min infusion, at monthly intervals. A significant improvement in BMD was observed in most patients.

Our group compared the effects of two different doses of pamidronate, 30 mg vs. 60 mg, on BMD of the lumbar spine, the femoral neck and the forearm and on markers of bone remodelling and osteoclast function in 26 patients with thalassaemia and osteoporosis. Thirteen patients with TM and five patients with thalassaemia intermedia were given pamidronate at a dose of 30 mg in a 2-h i.v. infusion, once a month for 12 months; another eight patients (four with TM and four with thalassaemia intermedia) received a dose of 60 mg/month, in an attempt to explore whether increasing the dose of pamidronate might have any additional effect. The i.v. route was preferred to oral administration in order to override the problem of gastrointestinal malabsorption of oral bisphosphonates, which is less than 10%, and it is further reduced by food containing milk or iron (Fleisch, 1998). Both groups included patients with comparable degrees of osteoporosis and hypogonadism. All patients were also receiving calcium, and vitamin D supplement prior to, and during the 12-month follow-up period of the study. Administration of 30 mg of pamidronate resulted in a significant increase of the BMD of the lumbar spine in all patients, but not the BMD of the femoral neck and the forearm. The 60 mg of pamidronate group showed a similarly significant increase in the BMD of the lumbar spine in both transfusion-dependent and transfusion-independent patients. Administration of both doses of pamidronate was also followed by a clear decrease in the markers of bone resorption (NTX, and TRACP-5b), OPG, and osteocalcin that was similar in patients of both treatment groups. Furthermore, most patients who complained of severe bone pain at the onset of the study had a significant reduction of pain after treatment period. No severe adverse-events were reported in this study (Voskaridou et al., 2003).

In another recent study (Perifanis et al., 2004), 29 patients with transfusion-dependent beta-thalassaemia and severe osteoporosis were given zoledronic acid, the most potent third generation bisphosphonate to-date, at a dose of 1 mg intravenously every 3 months over a 12-month period. All patients were also receiving calcium and vitamin D supplement prior to and during the study. Administration of zoledronic acid was followed by a clear increase in the BMD of the lumbar spine, as well as by a significant decrease in IGF-1 and a significant increase in OPG serum levels. No treatment-related side-effects were observed in this study (Perifanis et al., 2004).

These studies confirm the effectiveness of bisphosphonates in the treatment of thalassaemia-induced osteoporosis. Alendronate, pamidronate and zoledronic acid seem to have the greatest efficacy. However, more trials must be conducted in order to clarify the exact role of each bisphosphonate, the long-term benefit and side-effects as well as the effects of the combination of bisphosphonates with other effective agents, such as hormonal replacement, in thalassaemia-induced osteoporosis. Table II summarizes all the above data for the agents used in the management of thalassaemia-induced osteoporosis.

**Conclusion and future perspectives**

Thalassaemia-induced osteoporosis is multifactorial and therefore, very difficult to manage. Osteoporosis is a progressive disease; thus prevention and early diagnosis are very important. Adequate hormonal replacement, effective iron chelation, improvement of haemoglobin levels, calcium and vitamin D administration, physical activity, and no smoking, are currently the main measures for the management of the disease. However, novel pathogenetic data suggest that the reduced
osteoblastic activity, which is believed to be the basic mechanism of bone loss in TM, is accompanied by a comparable or even greater increase in bone resorption, through the RANK/RANKL/OPG pathway. Therefore, the role of bisphosphonates, that are potent inhibitors of osteoclast activation, arises as a major factor in the management of osteoporosis in these patients. However, many aspects need to be clarified before the broad use of bisphosphonates can be introduced in TM-induced osteoporosis: Which one? How long for? At what dose? The combination of bisphosphonates with other effective agents also needs to be evaluated in randomized trials. Other novel agents that stimulate bone formation, such as teriparatide, a recombinant peptide fragment of PTH (Jiang et al., 2003), strontium ranelate, a second anabolic agent (Meunier et al., 2004), which seem to prevent osteoporotic fractures in postmenopausal women, are under study but their effects in TM-induced osteoporosis remains to be proven. Finally, recombinant OPG, which reverses osteopenia in ovariectomized mice (Kostenuik et al., 2004) and reduces osteoclast activation in humans with myeloma and breast cancer bone disease (Body et al., 2003) may be another future agent that may help in the management of this difficult complication of thalassaemia.

References


Table II. Therapeutic regimens used for thalassaemia-induced osteoporosis.

<table>
<thead>
<tr>
<th>Therapeutic agent</th>
<th>Dosage</th>
<th>Number of patients</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormonal replacement Males: long-acting testosterone esterase</td>
<td>For 16–32 months 250 mg, i.m., every 4 weeks 0.625 mg/d; 5 mg/d for 10 d monthly</td>
<td>67</td>
<td>Anapliotou et al., 1995</td>
</tr>
<tr>
<td>Females: equine oestrogen + medroxyprogesterone</td>
<td>100 IU, intranasal, three times weekly, for 1 year</td>
<td>14</td>
<td>Canatan et al., 1995*</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>1.5 g/d for 1 year</td>
<td>10</td>
<td>Angastiniotis et al., 1998</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>10 mg/d, p.o., for 2 years 100 mg every 10 d, i.m., for 2 years</td>
<td>25</td>
<td>Morabito et al, 2002*</td>
</tr>
<tr>
<td>Bisphosphonates Clodronate</td>
<td>300 mg, i.v., every 3 weeks for 2 years</td>
<td>30</td>
<td>Pennisi et al, 2003*</td>
</tr>
<tr>
<td>Pamidronate</td>
<td>15–60 mg, i.v., monthly, for 1 year</td>
<td>39</td>
<td>Wonke, 2001</td>
</tr>
<tr>
<td>Pamidronate</td>
<td>30 mg, i.v., monthly for 1 year 60 mg, i.v., monthly for 1 year</td>
<td>18</td>
<td>Voskaridou et al, 2003</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zoledronic acid</td>
<td>1 mg, i.v., every 3 months for 1 year</td>
<td>29</td>
<td>Perifanis et al, 2004</td>
</tr>
</tbody>
</table>

*Randomized, placebo-controlled trial.


with beta-thalassemia: correlation with growth and hormonal data. *Metabolism, 47*, 541–548.


