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Issue: *Cooley's Anemia: Ninth Symposium***Treatment options for thalassemia patients with osteoporosis**Evangelos Terpos<sup>1</sup> and Ersi Voskaridou<sup>2</sup><sup>1</sup>Department of Clinical Therapeutics, University of Athens School of Medicine, Athens, Greece. <sup>2</sup>Thalassemia Center, Laikon General Hospital, Athens, Greece

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Osteoporosis represents a prominent cause of morbidity in patients with thalassemia. The delay in sexual maturation, the presence of diabetes and hypothyroidism, the parathyroid gland dysfunction, the progressive marrow expansion, the iron toxicity on osteoblasts, the iron chelators, and the deficiency of growth hormone or insulin growth factors have been identified as major causes of osteoporosis in thalassemia. Adequate hormonal replacement, effective iron chelation, improvement of hemoglobin levels, calcium and vitamin D administration, physical activity, and smoking cessation are the main to-date measures for the management of the disease. During the last decade, novel pathogenetic data suggest that the reduced osteoblastic activity, which is believed to be the basic mechanism of bone loss in thalassemia, is accompanied by a comparable or even greater increase in bone resorption. Therefore, the role of bisphosphonates, potent inhibitors of osteoclast activation, arises as a major factor in the management of osteoporosis in thalassemia patients.

**Keywords:** thalassemia; osteoporosis; bisphosphonates; therapy; pathogenesis

**Introduction**

Thalassemia major (TM) is a hereditary hemolytic anemia caused by a defect in the ability of erythroblasts to synthesize the  $\beta$  chain of adult hemoglobin. Several bone abnormalities are present in patients with TM, including the enlargement of the cranial and facial bones, spinal deformities, scoliosis, nerve compression, spontaneous fractures, and bone loss. The incidence of osteopenia or osteoporosis in well-treated TM patients has been found to be approximately 40–50%, and therefore osteoporosis represents a prominent cause of morbidity in TM patients of both genders.<sup>1</sup> The pathogenesis of osteoporosis in TM is very complicated and differs from the pathogenesis of bone deformities characteristically found in nontransfused patients (thalassemia intermedia; TI), who develop bone distortion mainly due to accelerated hemopoiesis and progressive marrow expansion. Several genetic and acquired factors are implicated in bone destruction in TM. The typical delay in sexual maturation, the presence of diabetes and hypothyroidism, the parathyroid gland

dysfunction, the accelerated hemopoiesis with progressive marrow expansion, the direct iron toxicity on osteoblasts and the deficiency of growth hormone (GH) or insulin growth factor I (IGF-I) have been indicated as possible causes for thalassemia-induced osteoporosis.<sup>1–3</sup> Furthermore, iron chelation has correlated with growth failure and bone abnormalities, and high desferrioxamine dosage has been associated with cartilage alterations.<sup>4,5</sup> More puzzling, however, is the observation that, despite the normalization of hemoglobin levels, adequate hormone replacement, and effective iron chelation, patients continue to show an unbalanced bone turnover with an increased resorptive phase resulting in seriously diminished bone mineral density (BMD).<sup>6,7</sup>

**Pathogenesis of osteoporosis in thalassemia**

According to the World Health Organization, 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.<sup>8</sup> The cut-off of 2.5 standard deviations below the normal mean in BMD for the respective age is used for the definition of osteoporosis, whereas the decrease of BMD between 1.5 and 2.5 standard deviations below the normal mean for the respective age is defined as osteopenia.<sup>9</sup> The most important factors that are implicated in the pathogenesis of bone loss in thalassemia patients are described later.

### Genetic factors

Genetic factors seem to play a 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 thalassemia-induced osteoporosis. The polymorphism at the Sp1 site of the collagen type Ia1 (COLIA 1) gene (collagen type I is the major bone matrix protein) was studied by Wonke and colleagues,<sup>10</sup> who found that approximately 30% of the TM patients were heterozygotes (Ss) and 4% were homozygotes (SS) for the Sp1 polymorphism. The authors have 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.<sup>11</sup> The genes encoding collagen types Ia1 and Ia2 (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.<sup>12</sup> The study of COLIA 1 polymorphism may help in identifying thalassemia patients who are at higher risk to develop osteoporosis and pathologic fractures.<sup>13</sup>

Other genetic factors that have been reported to correlate with bone mineral damage in adult patients with  $\beta$ -thalassemia include the vitamin D receptor (VDR) BsmI BB polymorphism, the loss-of-function mutations in the gene of the vitamin D receptor, the sequence variation of 713-8delC of transforming growth factor- $\beta$ 1, the presence of restriction fragment length polymorphisms for the calcitonin (CT) receptor gene, estrogen receptor and interleukin-6 gene loci.<sup>1,13</sup> Although further studies

are needed to exact final conclusion for the association between gene polymorphisms and bone mass in TM patients, COLIA 1 gene polymorphisms seem to be of importance in the pathogenesis of thalassemia-induced osteoporosis.

### Acquired factors

**Endocrine complications.** Hypothyroidism, hypoparathyroidism, diabetes mellitus, and mainly hypogonadism (as delayed puberty and/or secondary hypogonadism) are considered as major causes of osteopenia/osteoporosis in TM.<sup>1-3,5</sup> Hemosiderosis of the pituitary gonadotrophic cells and iron deposition in the testes and ovaries are involved in the pathogenesis of endocrine complications in TM.<sup>14</sup> 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 enhanced resorptive phase.<sup>15</sup> Estrogen and progesterone appear to inhibit osteoclast activity and promote bone formation, whereas testosterone has a direct stimulatory effect on osteoblast proliferation and differentiation.<sup>3</sup> IGFs play also an important role in bone remodeling. Low-serum IGF levels decrease osteoblast proliferation and bone matrix formation and reduce the activation of osteoclasts.<sup>16</sup> Several studies have demonstrated a positive correlation between the BMD of the lumbar spine and the IGF-I concentration.<sup>17</sup> It is well documented that the GH-IGF axis is defective in TM. Thalassemia 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.<sup>18,19</sup>

**Iron overload and desferrioxamine.** Iron deposition in the bone impairs osteoid maturation and inhibits mineralization locally, resulting in focal osteomalakia. 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 bone metabolism unit tensile strength.<sup>20</sup> Furthermore, desferrioxamine inhibits DNA synthesis, osteoblast, and fibroblast proliferation, osteoblast precursors differentiation, and collagen formation, although enhances osteoblast apoptosis, especially

in patients who receive inappropriately high doses of desferrioxamine.<sup>21</sup>

**Bone marrow expansion.** Bone marrow expansion, which is a typical finding in patients with TM, has been considered as a major cause of bone destruction.<sup>20</sup> Transferrin receptor studies have demonstrated increased bone marrow activity even in patients with low reticulocyte count or marrow hypoplasia.<sup>22</sup> However, there was found no direct correlation between serum levels of soluble transferrin receptor and the severity of osteoporosis.<sup>10</sup>

**Vitamin deficiencies.** Vitamin C deficiency in iron-overload patients with low levels of serum ascorbic acid induces the risk of osteoporotic fractures.<sup>23</sup> 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 skeleton development can increase bone mass in adolescents and decrease bone loss in adult life. However, most studies have failed to show reduced serum levels of 25-hydroxyvitamin D in TM patients.

**Physical activity.** Patients with TM have reduced physical activity due to the complications of the disease and the overprotection by their parents who do not encourage muscle activity. Thus, the lack of physical activity is another predisposing factor for osteoporosis in TM patients and muscle activity has to be encouraged in these patients.<sup>1-3</sup>

These factors can lead to the destruction of bone in thalassemia patients by increasing the osteoclast function and/or reducing the osteoblast activity.

**Increased osteoclast function in thalassemic patients with osteoporosis.** During the last decade, there was sufficient data supporting that increased osteoclast activation is present in TM patients. Patients with TM and osteoporosis have elevated markers of bone resorption, such as N-terminal cross-linking telopeptide of collagen type-I (NTX) and tartrate-resistant acid phosphatase type 5b (TRACP-5b)<sup>24,25</sup> that correlated with BMD of the lumbar spine in these patients.<sup>25,26</sup> This increased osteoclast activity seems to be at least partially due to an imbalance in the receptor-activator of nuclear factor-kappa B ligand (RANKL)/osteoprotegerin (OPG) system and the overproduction of cytokines that are involved in the osteoclast differentiation and

function.<sup>26</sup> The RANK/RANKL/OPG pathway is of great importance for the activation and proliferation of osteoclast precursors. We and others have shown that the ratio of sRANKL/OPG in the serum was increased in thalassemia patients with osteopenia/osteoporosis, providing evidence for the role of RANKL/OPG system in the pathogenesis of osteoporosis in thalassemia.<sup>26-28</sup> Serum levels of IL-1 $\alpha$ , TNF- $\alpha$ , IL-6, and TGF- $\beta$ , that are able to increase osteoclast function, were elevated in TM and correlated with bone resorption and lumbar BMD,<sup>29</sup> suggesting their involvement in the pathogenesis of TM osteoporosis and supporting the role of the immune system in the bone loss of TM.

**Reduced osteoblast function in thalassemic patients with osteoporosis.** There is evidence of reduced osteoblast function in TM. Histomorphometry studies have revealed that increased osteoid thickness, increased osteoid maturation and mineralization lag time, which indicate impaired bone matrix maturation, and defective mineralization is present in children and adolescents with TM.<sup>20</sup> In addition, iron deposits appeared along mineralization fronts and osteoid surfaces, whereas focal thickened osteoid seams were found together with focal iron deposits.<sup>20,30</sup> Finally, dynamic bone formation histomorphometry studies established reduced bone formation rate in TM patients.<sup>20</sup> This reduced bone formation is thought to-date to be mainly the result of iron poisoning in osteoblasts and/or the result of reduced function of GH and IGF-1 axis in TM patients.<sup>1</sup> However, novel molecules seem to be implicated in osteoblast dysfunction in TM. Dickkopf-1 (Dkk-1) is a Wnt signaling inhibitor, which inhibits the osteoblast differentiation and function. We have recently shown that serum levels of Dkk-1 were increased in TM patients with osteoporosis and correlated with lumbar spine and wrist BMD. Interestingly, when zoledronic acid (ZA) was given in these patients there was a reduction in Dkk-1 levels, which was not observed in the placebo group of this randomized trial.<sup>31</sup>

## Management of thalassemia-associated osteoporosis

**Prevention and general principles.** Prevention and treatment of early bone loss make the best policy. Annual checking of BMD starting in adolescence is considered indispensable. Physical activity

must always be encouraged. Moderate and high impact activities are to be supported. Exercise has additional benefits: it improves cardiovascular system, reduces the risk of diabetes, and prevents depression. Smoking should be discouraged. Adequate calcium intake during skeleton development can increase bone mass in adult life and in combination with administration of low doses of vitamin D may prevent bone loss and fractures.<sup>32</sup> 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.<sup>1</sup> 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 thalassemia patients.<sup>1-3,5,32</sup> Anapliotou and colleagues recommended that continuous hormonal replacement therapy with transdermal estrogen for females or human chorionic gonadotrophin for males improves bone density parameters.<sup>33</sup> However, despite hormonal replacement, calcium and vitamin D administration, effective iron chelation, and normalization of hemoglobin levels, patients with TM continue to lose bone mass.<sup>6,7</sup>

**Calcitonin.** Canatan and colleagues have 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 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 been decreased in the treatment group but not in controls. CT had no important side effects.<sup>34</sup> Both parenteral and intranasal instillations are available.

**Hydroxyurea.** Ten patients with TM were given hydroxyurea (HU), at a dose of 1.5 g p.o. daily, in an attempt to reduce marrow hyperplasia diagnosed by MRI. HU improved bone pain and MRI findings.<sup>35</sup> However, in another study, the administration of HU for at least 2 years did not manage to show any improvement of the BMD compared with patients who did not receive HU.<sup>36</sup>

**Bisphosphonates.** The increased bone resorption observed in patients with thalassemia-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. In thalassemia osteoporosis, almost all generations of bisphosphonates have been used in an attempt to increase the BMD and improve the abnormal bone remodeling. Morabito and colleagues 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 thalassemia patients with osteoporosis.<sup>37</sup> Patients were randomized to receive placebo (eight patients) or 100 mg of clodronate, i.m., every 10 days (eight patients) or 10 mg of alendronate per os daily (nine patients). All patients also received 500 mg of elemental calcium and 400 IU cholecalciferol daily. 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.<sup>37</sup> The ineffectiveness of clodronate was established in another randomized, placebo-controlled trial.<sup>38</sup>

Pamidronate, a second-generation aminobisphosphonate, has been given intravenously in patients with TM and osteoporosis. First, Wonke evaluated the effect of 15 mg of pamidronate on BMD. Pamidronate was given in a 40 min infusion, at monthly intervals. A significant improvement in BMD was observed in most patients.<sup>10</sup> Our group compared the effects of two different doses of pamidronate, 30 mg versus 60 mg, on BMD of the lumbar spine, femoral neck, and forearm and on markers of bone remodeling and osteoclast function

in 26 patients with thalassemia and osteoporosis. Thirteen patients with TM and five patients with TI 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 TI) received a dose of 60 mg/month, in an attempt to explore whether increasing the dose of pamidronate might have any additional effect. Both groups included patients with comparable degrees of osteoporosis and hypogonadism. All patients were also receiving calcium and vitamin D supplement prior 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 of markers of bone resorption (NTX and TRACP-5b), OPG, and osteocalcin that was similar in patients of both treatment groups. Furthermore, most patients complaining for 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.<sup>25</sup>

Zoledronic acid is the most potent third generation bisphosphonate to-date and has been found to be extremely efficacious in increasing BMD in TM patients. We reported the results of a randomized, placebo-controlled trial of ZA in 66 thalassemia patients with osteoporosis. The patients were randomized to receive 4 mg ZA intravenously every 6 months (23 patients; group A) or every 3 months (21 patients; group B), or to receive placebo every 3 months (22 patients; group C), for a period of 1 year. Patients of group B had a significant increase in their lumbar spine BMD, which was accompanied by dramatic reductions in bone pain, and bone markers. Patients in placebo group showed no alteration in BMD of any studied site or in bone pain scores; on the contrary, they had an aggravation in bone resorption. Therefore, this study confirmed that ZA is an effective treatment for increasing BMD and reducing bone resorption in thalassemia-induced osteoporosis with no serious side effects.<sup>28</sup> As the duration of ZA therapy had not been evaluated in any trial, we followed-up our patients for 24 months after discontinuation of ZA for

groups A and B and for 12 months for group C (patients of group C received ZA, 4 mg every 3 months, i.v., for 12 months after their placebo 12-month administration). We found, interestingly, that at the 36th month, patients of group B continued to show an increase in the BMD of all studied sites despite the discontinuation of ZA. Furthermore, patients of groups A and C showed a dramatic increase in BMD of all studied sites compared with baseline values ( $P < 0.01$ ) The increase of BMD observed in groups A and C was accompanied by a comparable reduction in bone resorption marker CTX at the 36th month, which had not reported at the 12th month; on the contrary in group C there was an increase in CTX at the 12th month. These observations suggest that ZA continues to act after its discontinuation.<sup>39</sup>

In another recent study, we confirmed that the increase of erythropoietic activity in TI, which continues irrespectively of the improvement of BMD produced by ZA, seems to be a major cause of bone loss in this hemoglobinopathy. Soluble transferrin receptor (sTfR) and erythropoietin (Epo) serum levels are increased in TI but we showed for the first time in the literature that this elevation was further increased by time, although BMD was improved by ZA.<sup>40</sup>

All described studies confirm the effectiveness of bisphosphonates in the treatment of thalassemia-induced osteoporosis. Alendronate, pamidronate, and ZA seem to have the greater efficacy. However, more trials must be conducted 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 thalassemia-induced osteoporosis.

## Conclusion and future perspectives

Thalassemia-associated osteoporosis is multifactorial and, therefore, very difficult in its management. Osteoporosis is a progressive disease; thus, prevention and early diagnosis are very important. Adequate hormonal replacement, effective iron chelation, improvement of hemoglobin levels, calcium and vitamin D administration, physical activity, and smoking cessation are the main to-date 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. Therefore, the role of bisphosphonates arises as major in the management of osteoporosis in these patients. However, many aspects have to be clarified before the broad use of bisphosphonates in TM-induced osteoporosis: which one? how long? and at what dose? The combination of bisphosphonates with other effective agents has also to be evaluated in randomized trials. Other novel agents that stimulate bone formation such as teriparatide, a recombinant peptide fragment of parathyroid hormone, strontium ranelate, a second anabolic agent, that seem to prevent osteoporotic fractures in postmenopausal women, are being studied but their effects in TM-induced osteoporosis remains to be proven. Finally, antibodies against RANKL, such as denosumab, which has just been licensed by FDA for the treatment of postmenopausal osteoporosis, and antibodies against Dkk-1 or against sclerostin may be future agents for the effective management of this difficult complication of thalassemia.

### Conflicts of interest

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### References

- Voskaridou, E. & E. Terpos. 2004. New insights into the pathophysiology and management of osteoporosis in patients with beta thalassaemia. *Br. J. Haematol.* **127**: 127–139.
- Garofalo, F., A. Piga, R. Lala, *et al.* 1998. Bone metabolism in thalassemia. *Ann. N.Y. Acad. Sci.* **850**: 475–478.
- Voskaridou, E. & E. Terpos. 2008. Pathogenesis and management of osteoporosis in thalassemia. *Pediatr. Endocrinol. Rev.* **6**(Suppl. 1): 86–93.
- Hatori, M., J. Sparkman, C.C. Teixeira, *et al.* 1995. Effects of deferoximine on chondrocyte alkaline phosphatase activity: prooxidant role of deferoximine in thalassemia. *Calcif. Tissue Int.* **57**: 229–236.
- Olivieri, N.F. 1999. The beta-thalassemias. *N. Engl. J. Med.* **341**: 99–109.
- Lasco, A., N. Morabito, A. Gaudio, *et al.* 2001. Effects of hormonal replacement therapy on bone metabolism in young adults with beta-thalassemia major. *Osteoporos. Int.* **12**: 570–575.
- Carmina, E., G. Di Fede, N. Napoli, *et al.* 2004. Hypogonadism and hormone replacement therapy on bone mass of adult women with thalassemia major. *Calcif. Tissue Int.* **74**: 68–71.
- World Health Organization. 1994. Assessment of fracture risks and its application to screening for post-menopausal osteoporosis. Report of a WHO Study Group. *World Health Organ. Tech. Rep. Ser.* **843**: 1–129.
- Binkley, N.C., P. Schmeier, R.D. Wasnich & L. Lenchik. 2002. What are the criteria by which a densitometric diagnosis of osteoporosis can be made in males and non-Caucasians? *J. Clin. Densitom.* **5**(Suppl.): 19–27.
- Wonke, B., C. Jensen, J.J. Hanslip, *et al.* 1998. Genetic and acquired predisposing factors and treatment of osteoporosis in thalassaemia major. *J. Pediatr. Endocrinol. Metab.* **11**(Suppl. 3): 795–801.
- Uitterlinden, A.G., A.E. Weel, H. Burger, *et al.* 2001. Interaction between the vitamin D receptor gene and collagen type I alpha 1 gene in susceptibility for fracture. *J. Bone Miner. Res.* **16**: 379–385.
- Uitterlinden, A.G., H. Burger, C.M. van Duijn, *et al.* 2000. Adjacent genes, for COL2A1 and the vitamin D receptor, are associated with separate features of radiographic osteoarthritis of the knee. *Arthritis Rheum.* **43**: 1456–1464.
- Perrotta, S., M.D. Cappellini, F. Bertoldo, *et al.* 2000. Osteoporosis in beta-thalassaemia major patients: analysis of the genetic background. *Br. J. Haematol.* **111**: 461–466.
- Berkovitch, M., T. Bistrizter, S.D. Milone, *et al.* 2000. Iron deposition in the anterior pituitary in homozygous beta-thalassemia: MRI evaluation and correlation with gonadal function. *J. Pediatr. Endocrinol. Metab.* **13**: 179–184.
- Riggs, B.L., S. Khosla & L.J. Melton III. 1998. A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. *J. Bone Miner. Res.* **13**: 763–773.
- Geusens, P.P. & S. Boonen. 2002. Osteoporosis and the growth hormone-insulin-like growth factor axis. *Horm. Res.* **58**(Suppl. 3): 49–55.
- Rucker, D., S. Ezzat, A. Diamandi, *et al.* 2004. IGF-I and testosterone levels as predictors of bone mineral density in healthy, community-dwelling men. *Clin. Endocrinol.* **60**: 491–499.
- Soliman, A.T., N. El Banna, M. Abdel Fattah, *et al.* 1998. Bone mineral density in prepubertal children with beta-thalassemia: correlation with growth and hormonal data. *Metabolism* **47**: 541–548.
- Morabito, N., A. Gaudio, A. Lasco, *et al.* 2004. Osteoprotegerin and RANKL in the pathogenesis of thalassemia-induced osteoporosis: new pieces of the puzzle. *J. Bone Miner. Res.* **19**: 722–727.
- Mahachoklertwattana, P., V. Srikulchayanonta, A. Chuan-sumrit, *et al.* 2003. Bone histomorphometry in children and adolescents with beta-thalassemia disease: iron-associated focal osteomalacia. *J. Clin. Endocrinol. Metab.* **88**: 3966–3972.
- De Sanctis, V., A. Pinamonti, A. Di Palma, *et al.* 1996. Growth and development in thalassaemia major patients with severe bone lesions due to desferrioxamine. *Eur. J. Pediatr.* **155**: 368–372.
- Ma, E.S., K.K. Lam, A.Y. Chan, *et al.* 2003. Transferrin receptor-2 polymorphisms and iron overload in transfusion independent  $\beta$ -thalassaemia intermedia. *Haematologica* **88**: 345–346 [letter].
- Michelson, J. & A. Cohen. 1988. Incidence and treatment of fractures in thalassemia. *J. Orthop. Trauma* **2**: 29–32.
- Voskaridou, E., M.C. Kyrtonis, E. Terpos, *et al.* 2001. Bone resorption is increased in young adults with thalassaemia major. *Br. J. Haematol.* **112**: 36–41.

25. Voskaridou, E., E. Terpos, G. Spina, *et al.* 2003. Pamidronate is an effective treatment for osteoporosis in patients with beta-thalassaemia. *Br. J. Haematol.* **123**: 730–737.
26. Dresner Pollack, R., E. Rachmilewitz, A. Blumenfeld, *et al.* 2000. Bone mineral metabolism in adults with beta-thalassaemia major and intermedia. *Br. J. Haematol.* **111**: 902–907.
27. Voskaridou, E. & E. Terpos. 2005. Osteoprotegerin to soluble receptor activator of nuclear factor kappa-B ligand ratio is reduced in patients with thalassaemia-related osteoporosis who receive vitamin D3. *Eur. J. Haematol.* **74**: 359–361.
28. Voskaridou, E., A. Anagnostopoulos, K. Konstantopoulos, *et al.* 2006. Zoledronic acid for the treatment of osteoporosis in patients with beta-thalassemia: results from a single-center, randomized, placebo-controlled trial. *Haematologica* **91**: 1193–1202.
29. Morabito, N., G.T. Russo, A. Gaudio, *et al.* 2007. The “lively” cytokines network in beta-Thalassemia Major-related osteoporosis. *Bone* **40**: 1588–1594.
30. Domrongkitchaiporn, S., V. Sirikulchayanonta, P. Anchaisuksiri, *et al.* 2003. Abnormalities in bone mineral density and bone histology in thalassemia. *J. Bone Miner. Res.* **18**: 1682–1688.
31. Voskaridou, E., D. Christoulas, C. Xirakia, *et al.* 2009. Serum Dickkopf-1 is increased and correlates with reduced bone mineral density in patients with thalassemia-induced osteoporosis. Reduction post-zoledronic acid administration. *Haematologica* **94**: 725–728.
32. Lindsay, R. 1993. Prevention and treatment of osteoporosis. *Lancet* **341**: 801–805.
33. Anapliotou, M.L., I.T. Kastanias, P. Psara, *et al.* 1995. The contribution of hypogonadism to the development of osteoporosis in thalassaemia major: new therapeutic approaches. *Clin. Endocrinol.* **42**: 279–287.
34. Canatan, D., N. Akar & A. Arcasoy. 1995. Effects of calcitonin therapy on osteoporosis in patients with thalassemia. *Acta Haematol.* **93**: 20–24.
35. Angastiniotis, M., N. Pavlides, K. Aristidou, *et al.* 1998. Bone pain in thalassaemia: assessment of DEXA and MRI findings. *J. Pediatr. Endocrinol. Metab.* **11**(Suppl. 3): 779–784.
36. Kosaryan, M., M.F. Zadeh & V.K. Shahi. 2004. The bone density of thalassaemic patients of Boe Ali Sina hospital, Sari, Iran in 2002; does hydroxyurea help? *Pediatr. Endocrinol. Rev.* **2**(Suppl. 2): 303–306.
37. Morabito, N., A. Lasco, A. Gaudio, *et al.* 2002. Bisphosphonates in the treatment of thalassemia-induced osteoporosis. *Osteoporos. Int.* **13**: 644–649.
38. Pennisi, P., G. Pizzarelli, M. Spina, *et al.* 2003. Quantitative ultrasound of bone and clodronate effects in thalassemia-induced osteoporosis. *J. Bone Miner. Metab.* **21**: 402–408.
39. Voskaridou, E., D. Christoulas, M. Konstantinidou, *et al.* 2008. Continuous improvement of bone mineral density two years post zoledronic acid discontinuation in patients with thalassemia-induced osteoporosis: long term follow up a randomized, placebo-controlled trial. *Haematologica* **93**: 1588–1590.
40. Voskaridou, E., D. Christoulas, E. Antoniadou & E. Terpos. 2008. Continuous increase in erythropoietic activity despite the improvement in bone mineral density by zoledronic acid in patients with thalassemia induced osteoporosis. *Acta Haematol.* **119**: 40–44.