Continuous improvement of bone mineral density two years post zoledronic acid discontinuation in patients with thalassemia-induced osteoporosis: long-term follow-up of a randomized, placebo-controlled trial

β-thalassemia major (TM) is often accompanied by osteopenia or osteoporosis.[1,2] Bisphosphonates have been used in the management of TM-induced bone loss.[3-7] We report here the results of the long-term follow-up of our TM patients with osteoporosis who participated in a randomized, placebo-controlled trial with zoledronic acid (ZOL), the results of which at 12 months had been previously reported.[5] According to that study, 66 patients with TM-induced osteoporosis (21M/45F; median age 35.5 years) were studied. Patients were blindly randomized to receive 4 mg ZOL, iv, in 15 min. infusion, every six months (group A, n=23) or every three months (group B, n=21), or to receive placebo every three months (group C, n=22), for a period of one year.

Patients of groups A and B then discontinued ZOL treatment, while patients of group C were given 4 mg ZOL, every three months, for 12 months, and subsequently stopped ZOL therapy.[5] Informed consent was obtained from all patients prior to inclusion in the study. The study was conducted with the approval of the Greek National Drug Organization Committee (Ref. No Aα-KΔ-79/01/03) in keeping with the guidelines of Helsinki. Bone Mineral Density of the lumbar spine (L1-L4), femoral neck (FN) and wrist was evaluated in all patients prior to starting therapy and then after 12 and 36 months, using DEXA.[5] Serum markers of bone remodeling [C-telopeptide of collagen type-I (CTX) and bone-specific alkaline phosphatase (bALP)] were measured in patients and in a control group of 40, age and gender-matched, healthy blood donors, as previously described.[5] Patients were asked to quantify their degree of bone pain on Huskisson's visual analog scale (VAS) and the McGill–Melzack scoring system before entering the trial, and then every six months for 36 months. Statistical analysis was described in our initial report.[5]

Patients of groups A and C showed no differences in BMD of all evaluated sites at the 12th month, while patients of group B achieved a significant increase only in their L1-L4 BMD[5] (Figure 1A-1C). ZOL also reduced bone pain in groups A+B, while there was no pain relief in the placebo group after 12 months of therapy (Table 1). Similar results have been described in two subsequent trials where ZOL was given in TM patients with osteoporosis for 12 months[6,7] confirming the beneficial effect of ZOL in this setting. However, the treatment duration has not been clarified in any trial.

Our patients were followed for 24 months after discontinuation of ZOL for groups A and B, and for 12 months for group C. Interestingly, at the 36th month, patients of both groups A and B showed a significant increase in BMD of all studied sites compared with baseline values ($p<0.01$) (Figure 1A-1B). Patients of group C, who had received ZOL for 12 months after the placebo period, also increased their BMD of all studied sites at the 36th month ($p<0.01$; Figure 1C), while they also reduced bone pain scores (Table 1). Furthermore, there was no more difference in BMD T-values of L1-L4 and forearm between patients of groups A, B, and C, while FN BMD continued to be higher in group B (either as a T-score absolute value or as a T-score percentage change;

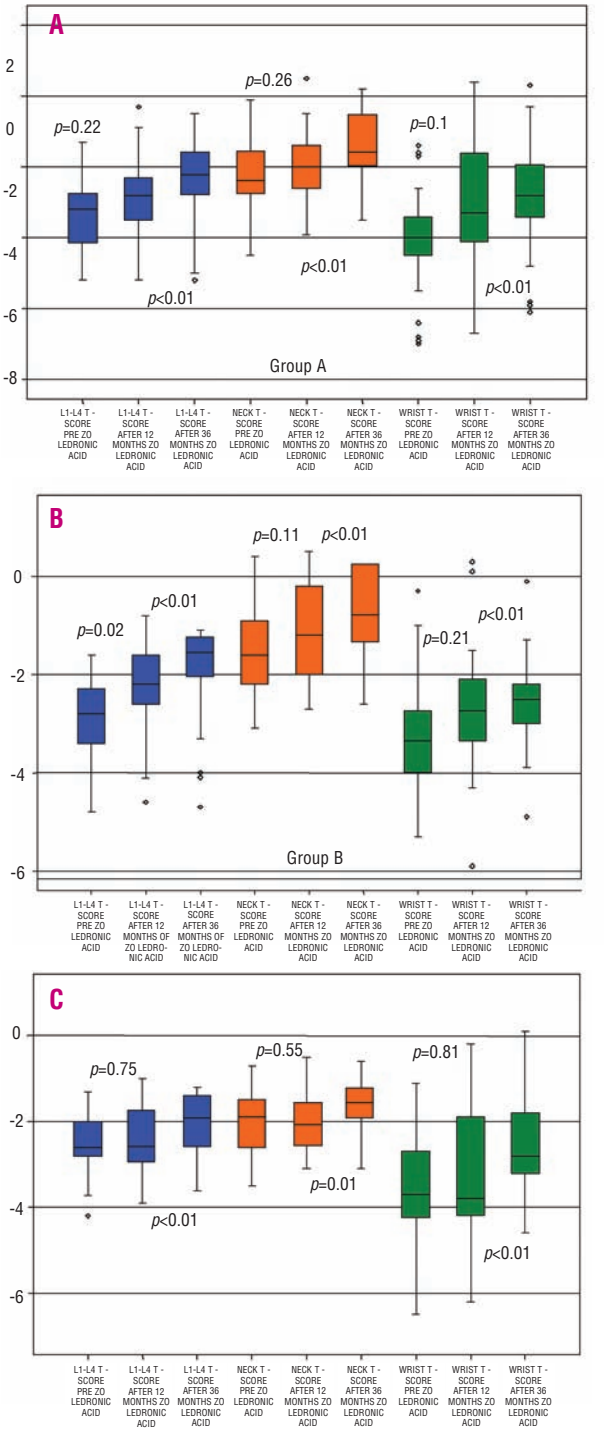

Figure 1. (A) Patients of group A showed a slight, but not significant, increase of T-score in BMD of all studied sites at 12 months, which dramatically increased at month 36 (mean ± SD): L1-L4: from -3.3 (±0.9) at baseline, to -2.8 (±1.19) at 12th month and -2.2 (±1.0) at 36th month; Femoral neck: from -1.9 (±1.5) at baseline, to -1.8 (±0.8) at 12th month and -1.6 (±0.7) at 36th month Wrist: from -4.0 (±1.7) at baseline, to -3.7 (±2.0) at 12th month and -2.8 (±1.1) at 36th month. (B) Patients of group B showed a statistically significant increase of L1-L4 BMD at 12 months which was continued at 36th month: from -2.9 (±0.9) at baseline, to -2.2 (±1.0) at 12th month and -1.7 (±0.7) at 36th month. They also showed a borderline increase in FN BMD at 12 months [from -1.7 (±0.8) to -1.4 (±0.6)], which became significant at 36th month (-0.9±0.9). The respective values for T-score of the wrist were: -3.1 (±1.1) at baseline, -2.7 (±1.0) at 12th month and -2.3 (±0.9) at 36th month. (C) Finally, patients of group C showed no significant alterations of BMD in all studied sites at 12th month that improved at 36th month after zoledronic acid administration: L1-L4: from -2.5 (±0.7) at baseline, to -2.5 (±0.9) at 12th month and to -1.9 (±0.9) at 36th month; Femoral neck: from -2.0 (±0.7) at baseline, to -2.1 (±0.8) at 12th month and -1.6 (±0.6) at 36th month Wrist: from -3.8 (±1.5) at baseline, to -3.9 (±1.8) at 12th month and -2.5 (±1.1) at 36th month.

Table 1. Pain scores and markers of bone remodeling during study period.

Parameter	*Group A median (range)*	*Group B median (range)*	*Group C median (range)*
Pain Scoring System			
VAS			
baseline	4.6 (0-7.5)	4.8 (0-10)	4.5 (0-7)
12th month	0.8 (0-2)[a]	0.3 (0-1)[a]	4.8 (0-7)
36th month	0.5 (0-1.5)[a]	0.2 (0-1)[a]	1.0 (0-4)[a]
McGill-Melrack			
baseline	2 (0-4)	2.5 (0-5)	2.0 (0-3)
12th month	0 (0-1)[a]	0 (0-1)[a]	2.0 (0-3)
36th month	0 (0-1)[a]	0 (0-1)[a]	0.5 (0-2)[a]
CTX (ng/mL)			
baseline	0.60 (0.35-2.40)	0.70 (0.32-2.44)	0.62 (0.33-3.15)
12th month	0.63 (0.12-1.53)	0.23 (0.09-0.78)[a]	0.95 (0.56-4.18)[b]
36th month	0.25 (0.11-0.76)[a]	0.20 (0.02-0.49)[a]	0.31 (0.10-0.84)[a]
bALP (U/L)			
baseline	33.0 (13.6-57.0)	30.3 (14.0-67.4)	32.7 (11.4-117.3)
12th month	23.5 (8.85-49.6)[b]	17.4 (7.68-34.2)[a]	29.3 (12.1-78.3)
36th month	20.8 (9.23-31.8)[b]	18.0 (8.04-30.1)[a]	20.5 (10.9-32.9)[b]

[a]$p<0.001$ compared to baseline; [b]$p<0.01$ compared to baseline.

p=0.01). The BMD elevation observed in groups A and C was accompanied by a comparable reduction in CTX at the 36th month, which had not been reported at the 12th month (Table 1). These observations suggest that ZOL continues to act after its discontinuation. ZOL may need more time before its efficacy becomes evident in patients who receive it every six months. Pharmacokinetic studies showed that bisphosphonates remain in bone matrix for many years, normalize bone turnover rates within weeks and no further suppression is seen during long-term use.[8] This indicates that long-term treatment may not be very different from short-term treatment.[8]

Thus some of the skeletal effects of bisphosphonates may last for years after treatment stops. Bisphosphonates bind strongly to hydroxyapatite. When bone containing bisphosphonate is resorbed, some of the bisphosphonate released recirculates locally and systemically, and binds again to bone surfaces. Therefore, when long-term treatment with bisphosphonates stops, the residual and recirculating bisphosphonate continues to inhibit bone resorption, although to a lesser extent than continued treatment.[9,10]

The retention of ZOL in bone may justify the continuous effect after its discontinuation observed in our study. In addition, recent pre-clinical reports established that the bone protection of zoledronic acid was dose-dependent and lasted for up to 32 weeks,[11] thus supporting the rationale for an annual intravenous dosing regimen of ZOL for treatment of postmenopausal osteoporosis and confirming our results for the prolonged effect of zoledronic acid in our patients. In a very interesting study on the effect of alendronate on BMD and markers of bone remodeling, authors compared patients who continuously received alendronate with those who received alendronate and then stopped for five years.[12] After five years, the cumulative risk of non-vertebral fractures was not significantly different between those continuing and discontinuing alendronate. Moreover, BMD continued to be elevated and CTX to be reduced compared to baseline after five years of alendronate discontinuation.[12] Comparable results in terms of vertebral fractures have been reported with pamidronate in postmenopausal osteoporosis.[10] In our study, we observed no new fractures during the study period although ZOL acid had been stopped. Furthermore, the changes in bone markers after the discontinuation of ZOL in groups A and B also suggest some residual effect of the drug for 24 months after its discontinuation.

No significant side-effects, including osteonecrosis of the jaw, were observed during this study. In conclusion, our study shows for the first time in the literature that ZOL continues to improve BMD, bone metabolism and pain scores 24 months after its discontinuation in TM patients with osteoporosis. We also confirm the safety of ZOL for up to 24 months after treatment. However, well-controlled studies with longer follow-up periods are needed to determine the best dose and treatment schedule of ZOL in the management of TM-induced osteoporosis.

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Large deletion in *UGT1A1* gene encompassing the promoter and the exon 1 responsible for Crigler-Najjar type I syndrome

Crigler-Najjar type I syndrome (CN-I, MIM #218800) is due to a complete and non-inductile deficiency of bilirubin-UDP-glucuronosyltransferase activity (EC 2.4.1.17, gene *UGT1A1* located on 2q37.1).[1] Currently, over 90 genetic alterations such as mutations, small insertions or small deletions have been described in the five exons of the *UGT1A1* gene responsible for bilirubin conjugation activity deficiency. Large deletions (>20 bp) are rare genetic alterations in human genetics comprising only 5.8% of all genetic lesions referenced (Human Gene Mutation Database; *http://www.hgmd.cf.ad.uk*). Such genomic rearrangements could be due either to homologous or non-homologous recombination.[2,3] The only large deletion described in the *UGT1A1* gene has been reported by Seppen *et al.* in 1994.[4] The patient was homozygous for a deletion of the exon 2 responsible for CN-I but exact breakpoints have not been characterized.

We report the case of a male CN-I child in whom molecular studies allowed us to identify a large deletion encompassing the promoter and the exon 1 of *UGT1A1* gene. The infant was born at full term after an uneventful pregnancy. During the neonatal period, he presented an elevation of the serum bilirubin to 250-300 µmol/L, entirely unconjugated, with peaks at 600 µmol/L, contrasting with an absence of neurological manifestations. Intensive phototherapy and enzymatic induction by phenobarbital were inefficient in reducing the serum bilirubin concentration. At two weeks, the diagnosis of Crigler-Najjar was suspected and blood was sampled for molecular studies. Blood from parents, who are first cousins, were also sampled. Genomic DNA was extracted from peripheral leucocytes of the child and his parents. The promoter and the five exons with the flanking intron-exon junctions were PCR-amplified as previously described.[5,6] On two different blood samples, no amplification of the promoter and the exon 1 for the child was available. On the other hand, the parents' promoter and exon 1 were correctly amplified and no genetic sequence alteration was observed after sequencing. Since they are consanguineous, an identical large deletion, including at least the promoter and the exon 1, was suspected at the heterozygote state in the parents explaining their *normal* electrophoretic profile. This deletion – transmitted to the

Table 1. Genetic markers analyzed to determine the breakpoints of the deletion including the promoter and the exon 1.

Marker	*Primer OLF*	*Primer OLR*	*Size (bp)*	*Position in relation to ATG (bp)*	*Presence in the child*	*Presence in the parents*
D04	5' tacactagtaaaggtcactc 3'	5' ccctctagccattctggatc 3'	290	-3496	+	+
D03	5' tacactagtaaaggtcactc 3'	5' tttgcatatctgtccttgct 3'	816	-3496	+	+
D02	5' tacactagtaaaggtcactc 3'	5' gttagaaatggtccttgcct 3'	1266	-3496	–	+
D01	5' ctggccagtgatgtgtatgg 3'	5' gcaagtattgtgcagccag 3'	125	-1545	–	+
D11	5' gccaatgggtctgcatgtat 3'	5' ggttggcacctttcttctca 3'	111	1896	–	+
D12	5' tgtaggagaggcaccgaact 3'	5' ccaaacaaggcaacaacaaa 3'	112	2797	+	+
D13	5' agccatttaccaacgctcag 3'	5' agggtctgcaccacatctct 3'	108	4361	+	+
D14	5' gaagggtttcccctggagt 3'	5' cactgaccagcagaacaacg 3'	118	5939	+	+

Primers were designed with the Primer3 web site (frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Genetic positions were determined with Ensembl Project in relation to the adenine of the first codon of UGT1A1.

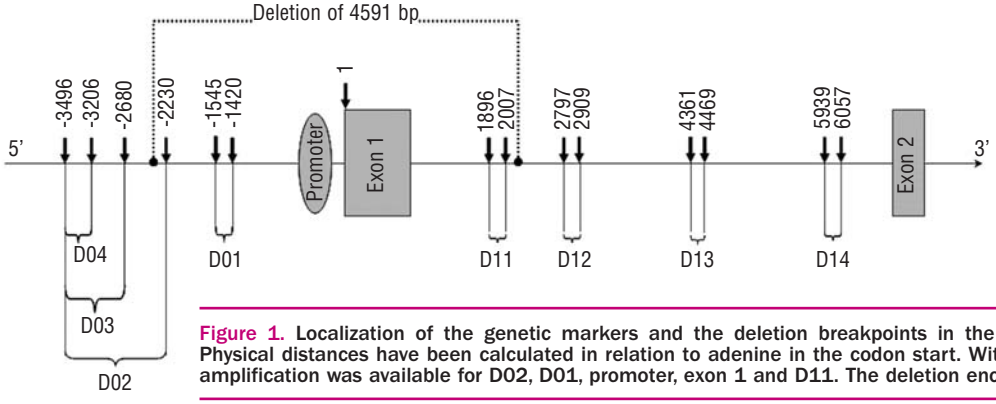

Figure 1. Localization of the genetic markers and the deletion breakpoints in the *UGT1A1* genetic region. Physical distances have been calculated in relation to adenine in the codon start. With child genomic DNA, no amplification was available for D02, D01, promoter, exon 1 and D11. The deletion encompasses 4591 bp.