



**HAL**  
open science

## **Endocrine and bone disease in appropriately treated adult patients with beta-thalassemia major**

Marina Baldini, Stella Forti, Alessia Marcon, Fabio Massimo Olivieri, Alessandra Orsatti, Benedetta Tampieri, Lorena Airaghi, Laura Zanaboni, Maria Domenica Cappellini

### ► **To cite this version:**

Marina Baldini, Stella Forti, Alessia Marcon, Fabio Massimo Olivieri, Alessandra Orsatti, et al.. Endocrine and bone disease in appropriately treated adult patients with beta-thalassemia major. *Annals of Hematology*, 2010, 89 (12), pp.1207-1213. <10.1007/s00277-010-1007-0>. <hal-00550268>

**HAL Id: hal-00550268**

**<https://hal.science/hal-00550268v1>**

Submitted on 26 Dec 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Editorial Manager(tm) for Annals of Hematology  
Manuscript Draft

Manuscript Number: AOHE-D-10-00052R1

Title: Endocrine and bone disease in appropriately treated adult patients with beta-thalassemia major

Article Type: Original Article

Keywords: Thalassemia, osteoporosis, iron overload, endocrinopathy.

Corresponding Author: Dr marina baldini, MD

Corresponding Author's Institution: Internal Medicine 1 A

First Author: Marina Baldini, MD

Order of Authors: Marina Baldini, MD; Stella Forti; Alessia Marcon, MD; Fabio Massimo Ulivieri, MD; Alessandra Orsatti, MSc; Benedetta Tampieri, MD; Lorena Airaghi, MD; Laura Zanaboni, MD; Maria Domenica Cappellini, PhD

Abstract: The natural history of beta thalassemia major (TM) has significantly changed during the last 2 decades. At present TM patients survive over their thirties and forties, but they have considerable morbidity. Bone demineralization is an important cause of morbidity in older patients; the etiology is multifactorial and partially unknown. We examined cross-sectionally 111 adult TM patients (66 females and 45 males,  $32.6 \pm 6$  years), regularly transfused, properly chelated and replaced for endocrine defects. Bone demineralization was detected in 92.7% of patients, with different severity according to gender and site: osteopenia was the prominent finding at the femur, osteoporosis at lumbar spine ( $p < 0.001$ ), more evident in males. The femoral site was more influenced by biochemical and clinical factors; despite adequate replacement, femoral T-score was lower in the hypogonadic than in the eugonadic group ( $p = 0.047$ ). A significant correlation was found between bone mass and body mass index (BMI), alkaline phosphatase (ALP) and pre-transfusional Hb levels. Multivariate analysis indicated as significant regressors ALP, BMI and hypoparathyroidism (T-score:  $p = 0.005, 0.035, 0.002$ ; Z-score:  $0.002, 0.009, 0.003$ , respectively) at femur, only ALP at lumbar spine ( $p = 0.008$  and  $0.045$  for T and Z scores, respectively). Statistical significance was reached more frequently by T-score, while Z-score demonstrated to have a lower sensitivity.

Despite best care facilities, bone demineralization in thalassemic patients remains a challenge; further exploration of the relationships between bone loss and endocrine, biochemical and hematologic variables is warranted to find effective measures to reduce bone pain and fracture risk.

Response to Reviewers: We are grateful for the useful suggestions of the reviewers. We have revised our paper following their recommendations.

Reviewers' comments:

1) In the Discussion session they comment that "...the correlation between alkaline phosphatase and bone mineralization indicates a prevalent role of reduced deposition over increased reabsorption, as reported by other Authors, at variance with others. We could not confirm the same relationship with osteocalcin, another marker of osteoblastic activity."

First of all the authors have not measured any of the markers of bone resorption. I believe that it is important for the paper and for the the discussion to include at least NTX or CTX measurements. I hope that they have available serum for such measurement. I suggest that CTX is more sensitive than NTX in the serum.

- In the original paper, we did not include any marker of bone resorption because we had only partial data (different markers, incomplete series). It seems now evident that the importance of these indices to our work has been understated. In effect, we had available serum of most patients, so we decided to measure CTX following the useful suggestion of the reviewer; we recognize that this adjunct increases the scientific interest of our results.

As pointed out in the revised paper (page 8, lines 1 to 3) "no significant differences were found in CTX serum levels between thalassemic patients and controls, nor significant correlations with any of the studied parameters. This result may be due to the wide variability of the values in both cohorts; the problem should probably be obviated by a longitudinal study protocol."

2) Second, in order to state that the correlation of bALP with BMD indicates a prevalent role of reduced deposition, someone could expect a strong correlation. On the contrary the correlation was weak ( $r < 0.25$ ). Furthermore, we do not know if the bALP values were reduced (that will indicate reduced formation) or not.

- The correlation was found between total ALP and BMD, not with the bone isoenzyme; this could be due to the low precision of the electrophoretic method utilized for bALP determination. Unfortunately, these are the only variables in our possession for all the patients at the moment. The values of ALP were reduced in thalassemic patients vs normal controls. The discrepancy between osteocalcin and ALP could be explained as stated in the revised paper, page 7, lines 1 to 3 from the bottom: "In effect these parameters, though both related to bone mineralization, differ as alkaline phosphatase assesses osteoblastic activity, osteocalcin expresses bone mineralization. It is well known that mineralization is defective in thalassemic patients (references no 16 and 17 of the revised paper), while data on bone formation are contrasting."

3) Control values (and not the expected values by the manufacturer of the kits) are needed for all studied parameters.

- A matched control group was included and the values for the measured parameters were compared with those obtained in the thalassemic patients.

4) DEXA has to be substituted by DXA throughout the text.

- The term was substituted throughout the text.

**\*Conflict of interest**

[Click here to download Conflict of interest: conflitto interessi-1.jpg](#)

Dear Sir, we are grateful for the useful suggestions of the reviewers. We have revised our paper following their recommendations.

We trust the paper in the present form to be suitable for publication.

Reviewers' comments:

In the Discussion session they comment that "...the correlation between alkaline phosphatase and bone mineralization indicates a prevalent role of reduced deposition over increased reabsorption, as reported by other Authors, at variance with others. We could not confirm the same relationship with osteocalcin, another marker of osteoblastic activity."

First of all the authors have not measured any of the markers of bone resorption. I believe that it is important for the paper and for the the discussion to include at least NTX or CTX measurements. I hope that they have available serum for such measurement. I suggest that CTX is more sensitive than NTX in the serum.

*In the original paper, we did not include any marker of bone resorption because we had only partial data (different markers, incomplete series). It seems now evident that the importance of these indices to our work has been understated. In effect, we had available serum of most patients, so we decided to measure CTX following the useful suggestion of the reviewer; we recognize that this adjunct increases the scientific interest of our results.*

*As pointed out in the revised paper (page 8, lines 1 to 3) "no significant differences were found in CTX serum levels between thalassemic patients and controls, nor significant correlations with any of the studied parameters. This result may be due to the wide variability of the values in both cohorts; the problem should probably be obviated by a longitudinal study protocol."*

Second, in order to state that the correlation of bALP with BMD indicates a prevalent role of reduced deposition, someone could expect a strong correlation. On the contrary the correlation was weak ( $r < -0.25$ ). Furthermore, we do not know if the bALP values were reduced (that will indicate reduced formation) or not.

*The correlation was found between total ALP and BMD, not with the bone isoenzyme; this could be due to the low precision of the electrophoretic method utilized for bALP determination.*

*Unfortunately, these are the only variables in our possession for all the patients at the moment. The values of ALP were reduced in thalassemic patients vs normal controls. The discrepancy between osteocalcin and ALP could be explained as stated in the revised paper, page 7, lines 1 to 3 from the bottom: "In effect these parameters, though both related to bone mineralization, differ as alkaline phosphatase assesses osteoblastic activity, osteocalcin expresses bone mineralization. It is well known that mineralization is defective in thalassemic patients (references no 16 and 17 of the revised paper), while data on bone formation are contrasting."*

Control values (and not the expected values by the manufacturer of the kits) are needed for all studied parameters.

*A matched control group was included and the values for the measured parameters were compared with those obtained in the thalassemic patients.*

DEXA has to be substituted by DXA throughout the text.

*The term was substituted throughout the text.*

1  
2  
3  
4  
5  
6 **Endocrine and bone disease in appropriately treated adult patients with beta-Thalassemia**  
7  
8 **major**  
9

10  
11  
12  
13  
14  
15  
16  
17  
18 Marina Baldini<sup>a</sup>, Stella Forti<sup>b</sup>, Alessia Marcon<sup>a</sup>, Fabio Massimo Ulivieri<sup>d</sup>, Alessandra Orsatti<sup>c</sup>, Benedetta Tampieri<sup>a</sup>,  
19  
20 Lorena Airaghi<sup>e</sup>, Laura Zanaboni<sup>a</sup>, Maria Domenica Cappellini<sup>a</sup>  
21  
22  
23  
24  
25  
26  
27

28  
29 U.O. Internal Medicine 1 A<sup>a</sup> e 1 B<sup>e</sup> Fondazione Ca' Granda, Ospedale Maggiore Policlinico (IRCCS), via F. Sforza 35,  
30  
31 20122 Milan, Italy

32  
33 <sup>b</sup> Audiology Unit, Fondazione Ca' Granda, Ospedale Maggiore Policlinico (IRCCS), via F. Sforza 35, 20122 Milan,  
34  
35 Italy

36  
37 <sup>c</sup> Dipartimento di Medicina Interna, Università degli Studi di Milano

38  
39 <sup>d</sup> U.O. Medicina Nucleare, Centro per la Diagnosi e la Terapia delle Osteoporosi, Fondazione Ca' Granda, Ospedale  
40  
41 Maggiore Policlinico (IRCCS), via F. Sforza 35, 20122 Milan, Italy

42  
43  
44  
45  
46  
47 Corresponding author:

48  
49 Marina Baldini

50  
51 e-mail: [marina.baldini@policlinico.mi.it](mailto:marina.baldini@policlinico.mi.it)

52  
53 tel:+390255033333

54  
55 fax:+390255033665  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42

## Abstract

With optimization of transfusional and chelation regimens, beta Thalassemia has changed from a pediatric disease with poor life expectancy into a chronic disease. Bone demineralization is an important cause of morbidity in older patients; the etiology is multifactorial and partially unknown. We examined cross-sectionally 111 adult patients with beta-Thalassemia Major (66 females and 45 males,  $32.6 \pm 6$  years), regularly transfused, sufficiently chelated and replaced for endocrine defects. Bone demineralization was detected in 92.7% of patients, with different severity according to gender and site: osteopenia was the prominent finding at the femur, osteoporosis at lumbar spine ( $p < 0.001$ ), more evident in the males. The femoral site was more influenced by biochemical and clinical factors; despite adequate replacement, femoral T-score was lower in the hypogonadic than in the eugonadic group ( $p = 0.047$ ). A significant correlation was found between bone mass and body mass index (BMI), alkaline phosphatase (ALP) and pre-transfusional Hb levels. The multivariate analysis indicated as significant regressors ALP, BMI and hypoparathyroidism (T-score:  $p = 0.005, 0.035$  and  $0.002$ ; Z-score:  $0.002, 0.009, 0.003$ , respectively) at the femoral site, only ALP at the lumbar spine ( $p = 0.008$  and  $0.045$  for T and Z scores, respectively). The statistical significance was reached more frequently by T-score, while Z-score seemed to be a less sensitive parameter.

Despite best care facilities, bone demineralization in thalassemic patients remains a challenge; further exploration of the relationships between bone loss and endocrine, biochemical and hematologic parameters is warranted to find effective measures to reduce fracture risk in this disease.

43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Key words:** Thalassemia, osteoporosis, iron overload, endocrinopathy.

## Introduction

1 The life expectancy of patients affected by Thalassemia Major (TM) is significantly increased during the last decade  
2  
3 due to regular transfusion regimen associated to adequate iron chelation. Today most of the patients enter adult life with  
4  
5 a relevant improvement of quality of life[1]. Bone deformities, which could not be forecasted 20 years ago are quite rare  
6  
7 today, nevertheless, low bone mass still remains a significant cause of morbidity in adult patients with TM. An  
8  
9 increased prevalence of osteoporosis, and consequently of fractures due to bone fragility, continues to be reported [2,3]  
10  
11 despite transfusions, effective chelation, calcium and vitamin D supplementation and replaced hormonal deficiencies.  
12  
13 Several concomitant factors may contribute to the pathogenesis of osteoporosis in adult TM patients, making difficult a  
14  
15 proper and preventing therapeutic approach.  
16  
17

18 We designed a cross-sectional evaluation of adult Thalassemia Major patients followed-up at single tertiary outpatient  
19  
20 clinic in Milan, with optimal transfusional and therapeutical regimen, in order to assess the prevalence of bone  
21  
22 demineralization and the relationships between bone status and physiological and pathological factors, including  
23  
24 ferritin, pre-transfusional haemoglobin levels, bone metabolism markers and endocrine complications.  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Patients

1  
2 After the approval of the Ethical Committee of our Institution and after patient's informed consent, 111 adult Caucasian  
3 patients affected by beta-Thalassemia Major (homozygous or double heterozygous for severe beta mutations) were  
4 enrolled in this study (66 females and 45 males, mean age  $32.6 \pm 6$  years (33, range 19-50). All the patients received  
5 regular blood transfusions every 20-25 days, maintaining pre-transfusional haemoglobin around 9-9.5 g/L, according to  
6 the international guidelines [4]. Chelation therapy included deferoxamine (DFO) (39 pts), deferiprone (DFP) (7 pts),  
7 deferasirox (DFX)(46 pts) or combination DFO/DFP (19 pts). The chelation adequacy was estimated based on mean  
8 ferritin levels every 3 months and LIC by MRI when available. Exclusion criteria were medical conditions and  
9 pharmacological treatments interfering with the bone mineral metabolism.

10  
11 The study design was cross-sectional; a periodic endocrinological evaluation was planned and during the same day bone  
12 densitometry, blood and urine biochemical tests and blood hormone evaluations were performed. Data collected  
13 included age, gender, BMI, history of auxologic and sexual development, splenectomy, fractures and liver disease,  
14 drugs used, transfusion regimen and starting age, mean of pre-transfusional haemoglobin and ferritin in the previous  
15 year, liver function, endocrine tests and bone metabolism indices, bone densitometry of femur and lumbar spine. A  
16 control group of healthy subjects (24 men and 35 women, mean age  $33.7 \pm 6.8$  years) was recruited and compared with  
17 thalassemic patients for bone metabolism indices.

18  
19 At the time of the biochemical and densitometric assessment, all hypogonadal patients were on hormonal replacement  
20 therapy, and all hypothyroid ones were on replacement with levothyroxine. Patients with hypoparathyroidism were  
21 treated with conventional vitamin D and calcium supplements, at a dose sufficient to maintain serum calcium within the  
22 normal range or at the lower normal limit.

## Methods

23  
24 Liver and renal function tests, calcium and phosphorus in serum (n. v. 2.1-2.5 and 0.8-1.4 mmol/L, respectively) and in  
25 24-hour urine (n.v. 2.5-7.5 mmol/d and 12.9-42 mmol/d, respectively), intact parathyroid hormone (n.v. 1.5-6.6  
26 pmol/L), osteocalcin (n.v. females 5.3-23.6, mcg/l, males 4.4-26.1), 25-hydroxyvitamin D (n.v.  $> 72$  nmol/L), ferritin  
27 (n.v. 30-400 mcg/L) and gamma-glutamyltranspeptidase (8-62 U/L) were determined by automated routine procedures.  
28 Blood cell count was performed by the Coulter Counter. Alkaline phosphatase was measured by IFCC liquid (n.v. 40-  
29 129 U/L), while the bone isoenzyme was assessed by semiquantitative electrophoretic method with scanning  
30 densitometer (n.v. 20-75%); serum CTX was determined by the Serum Cross Laps OneStep enzyme-linked  
31 immunosorbent assay (ELISA, IDS, Boldon, Tyne & Wear, UK; n.v.: women, post-menopausal 0.142-1.351, pre-  
32 menopausal 0.112-0.73; males 0.115-0.748 ng/mL)

1 Iron overload was properly estimated by Liver Iron Content (LIC) measured by MRI [5], that showed a wide standard  
2 deviation due to the patients' compliance and the different efficacy of therapies.

3  
4 Bone mineral density measurement was performed by means of dual x-ray photon absorptiometry (DXA) using Hologic  
5 bone densitometer (Hologic QDR-Discovery A, Version 12.7.3.1).

6  
7 BMD values were expressed as T-score and Z-score. T-score was calculated as SDs from normal reference population  
8 database, while Z-score as SDs from age and sex matched population.

9  
10 Data were classified according to WHO report (WHO Technical Report, ISCD Official Position Paper 2007); T-score >  
11 -1: normal. -1 > T-score > -2.5: low bone density (osteopenia). T-score < -2.5: osteoporosis.  
12  
13  
14  
15

#### 16 17 18 19 20 Statistical analysis

21  
22 Data are presented as means  $\pm$  SD. Intergroup comparison was performed with the Mann-Whitney test. Associations  
23 between parameters were determined by Pearson's and Spearman's rank correlation coefficients. To find independent  
24 factors affecting bone mineral density in thalassemic patients, a step-wise linear regression analysis was conducted.

25  
26 Two-sided exact tests were used and *p* values of less than 0.05 were considered significant. All statistics were  
27  
28 calculated using the Statistical Package for the Social Sciences 17.0 for Windows software package (SPSS Inc,  
29  
30  
31  
32 Chicago, IL).  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Results

The demographic characteristics of the patients studied are presented in Table 1. All the patients were on iron chelating treatment: 35% with deferoxamine (30+5mg/Kg), 42% with deferasirox (20+15mg/Kg), 6% with deferiprone (75mg/Kg) and the remaining 17% with combined therapy (deferoxamine 30mg/Kg + deferiprone 75mg/Kg).

101 patients (91%) were anti-HCV positive, of whom 50.5% were HCV RNA positive. 68% were splenectomised.

### Evaluation of bone mineral metabolism and endocrine function

The biochemical indices of bone metabolism are reported in Table 2. All the parameters resulted within the normal range apart from 25OH vitamin D serum levels, that were suboptimal in 21 patients even if 13 of them were supplemented with 10.000 IU/week. The levels of alkaline phosphatase and osteocalcin in the control group ( $68.1 \pm 15.5$  U/L and  $10.0 \pm 4.9$  mcg/L respectively) were significant lower (both  $p < 0.001$ ) than in thalassaemic patients ( $85.4 \pm 28.7$  U/L and  $23.4 \pm 30.1$  respectively). Conversely, PTH serum concentrations were significantly higher in the control group ( $3.8 \pm 1.2$ ,  $p = 0.007$  vs  $3.3 \pm 2.2$ ), according to the diagnosis of hypoparathyroidism in 19 out of 111 thalassaemic patients (17.1%). All the hypoparathyroid patients were on treatment with calcium and calcitriol at doses normalizing serum calcium and phosphate concentrations; all of them were also hypogonadic, except one, resulting 16.2% the association of these endocrine diseases. No difference was found for serum levels between thalassaemic and control group ( $0.42 \pm 0.31$  vs  $0.46 \pm 0.28$ ).

Hypogonadism (in all cases hypogonadotrophic) was the most frequent endocrine complication in our cohort of patients, affecting 87 patients (78.4%); all the hypogonadic patients except 4 (95.4%) were on replacement treatment starting from the age of  $18.8 \pm 3$  years. The hypogonadic patients who were not on replacement, being a minority, were excluded from the analysis of densitometric values.

An impairment of glucose metabolism was present in a high proportion of patients, with diabetes mellitus in 20 (18%) and IGT in 24 of them (22%). IGF-1 serum concentrations were evaluated in 78 patients (70%), and resulted lower than normal in 45 of them (41%).

### *Bone mass measurements*

When considering the bone mass measurements in the overall population enrolled, the prevalence of demineralization (including both osteopenia and osteoporosis) was 92.7% (103 out of 111 thalassaemic patients). Indeed, mineralization was normal only in 18 patients (16.2%) at the vertebral site and in 21 patients (18.9%) at the femoral site.

As shown in Table 3, there were no differences in bone mineralization between the hypogonadic and the eugonadic patients at the vertebral site, while at the femoral site the T-score was lower in the hypogonadic group, despite adequate replacement ( $p = 0.047$ ). Further, only at the femoral site, bone mass was significantly different by gender, as females showed lower mean values of T and Z-scores. There were statistically significant differences in T-score at both sites

1 also for the other dicotomic variables examined (hypoparathyroidism, treatment with calcium, treatment with  
2 bisphosphonates), while the statistical significance was not reached in all the analyses using Z-score as dependent  
3 variable.  
4

5 Figure 1 shows the distribution of bone mineralization state (normal / osteopenia / osteoporosis) according to gender  
6 and site. Osteopenia was the prominent finding at the femoral site (63 femoral vs 40 vertebral site,  $p < 0.001$ ), while at  
7 the vertebral site osteoporosis was more prevalent (53 pts vs 27 pts), the difference was statistically significant  
8 ( $p < 0.001$ ). The same distribution was confirmed when the patients were separately considered according to gender,  
9 being more evident in the male group ( $p < 0.001$ ). The patient distributions according to bone mineralization were  
10 significantly different by gender only at the femoral site.  
11

12 Table 4 shows the results of the correlation analysis (correlation indices and statistical significance,  $p < 0.05$ ) between  
13 bone mineralization (expressed by T and Z scores) and clinical/biochemical parametric variables. BMI was positively  
14 correlated with bone mass at femoral site, while advancing age was negatively correlated only with T-score. No  
15 correlation was found between vertebral T-score and the same variables. Among the biochemical indices of bone  
16 metabolism examined, alkaline phosphatase was the only one significantly correlated both with femoral and vertebral  
17 mineralization, being inversely associated with bone mass. Pre-transfusional Hb levels were significantly associated to  
18 vertebral Z-score.  
19

20 The correlations between bone mineralization and the other clinical and biochemical characteristics of the patients (age  
21 at first transfusion, age at beginning of chelation, diabetes, previous splenectomy, LIC, serum concentrations of ferritin,  
22 parathyroid hormone, bone alkaline phosphatase, osteocalcin, 25 OH vitamin D, IGF-1, CTX, and calcium both in  
23 serum and urine) were not significant thus were not reported.  
24

25 The multivariate analysis indicated as possible regressors alkaline phosphatase, BMI and hypoparathyroidism ( $p = 0.005$ ,  
26 0.035 and 0.002 for T-score; 0.002, 0.009, 0.003 for Z-score, respectively) at the femoral site, while alkaline  
27 phosphatase was the only one found regressor for T and Z scores (respectively  $p = 0.008$  and 0.045) at the lumbar spine.  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Discussion

1  
2 Despite the longer life expectancy of thalassaemia major patients due to proper transfusional and chelation regimens  
3  
4 [1], endocrine complications and bone disease still remain important causes of morbidity and disability [2,3,6-9] . In  
5  
6 this study we examined adult patients with Thalassemia Major regularly transfused and sufficiently chelated; the  
7  
8 patients were cared for  $13 \pm 3$  years (mean  $\pm$  SD) at a tertiary single Italian centre where the Thalassemia International  
9  
10 Guidelines [4] for monitoring and treating Thalassemia and its complications are issued and implemented..

11  
12 Despite the availability of the best care facilities, bone demineralization was detected in a high proportion of patients,  
13  
14 involving both sites examined though with different severity: osteopenia prevailed at femoral site, while osteoporosis  
15  
16 was more prominent at lumbar spine (prevalently constituted by trabecular bone). Overall, the femoral site seemed to be  
17  
18 more likely than the vertebral site influenced by biochemical and clinical factors. The only exception was pre-  
19  
20 transfusional Hb, showing a positive correlation with vertebral mineralization; this could be explained by the high  
21  
22 degree of ineffective erythropoiesis in the spine that can occurs in thalassemic syndromes. This finding is consistent  
23  
24 with the interesting hypothesis that the chronic request for blood cell production can play a role in the etiology of  
25  
26 osteoporosis, through overstimulation of the hematopoietic system increasing the number of osteoclasts and osteoblasts  
27  
28 resulting in accelerated bone turnover [10].

29  
30 In our analyses the statistical significance was reached more frequently by T-score, while Z-score seemed to be a less  
31  
32 sensitive parameter. There is a statistical explanation to this behaviour: the relatively small number of cases can render  
33  
34 the statistical power insufficient to detect smaller changes in Z-score, a parameter with high baseline variability.  
35  
36 However, considering the very low prevalence of the disease, this is a sufficiently large cohort, and quite homogeneous  
37  
38 as regards therapeutical approach and clinical management.

39  
40 The statistically significant negative association between T-score and treatment with calcium and with bisphosphonates  
41  
42 was expected, and compatible with the prescription of such drugs to the patients with more severe demineralization; the  
43  
44 therapeutic effect of these medications could not be examined in our study, as the protocol did not include a  
45  
46 longitudinal evaluation. Other parameters correlated to demineralization were gender (females are more involved), age  
47  
48 and BMI, similarly to healthy individuals.

49  
50 Even if we cannot address the issue of the prominent cause of bone disease in Thalassemia [11], the correlation  
51  
52 between alkaline phosphatase and bone mineralization indicates a prevalent role of reduced deposition over increased  
53  
54 reabsorption, as reported by other Authors [12], at variance with others [13, 14] . We could not confirm the same  
55  
56 relationship with osteocalcin, another marker of osteoblastic activity [12, 15]. In effect these parameters, though both  
57  
58 related to bone mineralization, differ as alkaline phosphatase assesses osteoblastic activity, osteocalcin expresses bone  
59  
60

1 mineralization. It is well known that mineralization is defective in thalassemic patients [16, 17], while data on bone  
2 formation are contrasting.

3  
4 As regards bone resorption indices, no significant differences were found in CTX serum levels between thalassemic  
5 patients and controls nor significant correlations with any of the studied parameters. These results may be due to the  
6 wide variability of the values in both cohorts; the problem would probably be obviated by a longitudinal study protocol.  
7

8  
9 As far as the endocrine complications relevant to bone status are concerned, according to previous reports [18] in our  
10 study the hypogonadic patients of both genders were more affected from demineralization than the eugonadic, and,  
11 interestingly, the standard hormone replacement therapy (HRT) does not normalize bone condition. A possible  
12 explanation may be the concurrence of factors interfering with mineralization, such as vitamin D deficiency, impairing  
13 the therapeutic effects of sex steroid hormones and also of bisphosphonates. A recent report [19] stated that an optimal  
14 vitamin D repletion is an essential pre-requisite for maximal efficacy of the anti-resorptive drugs in post-menopausal  
15 osteoporosis; a similar situation could be hypothesized in thalassemic population. In effect, low serum vitamin D levels  
16 have been reported in thalassemic populations; this was ascribed to impaired liver hydroxylation and intestinal  
17 absorption, with a causative role of iron overload suggested by a negative correlation between serum 25-OH-vitamin D  
18 and ferritin [20]. This correlation was not confirmed in our study ( $p = 0.546$ ). Unexpectedly, in our series the serum  
19 vitamin D was often low despite ongoing treatment with metabolites, compatible with a defect in absorption or  
20 metabolism [20] but also with low compliance to the therapy; the latter is quite common using associations with  
21 calcium salts, badly tolerated. Anyway, the contribution of vitamin D to bone status is probably more complex than  
22 presently recognized, and neither vitamin D concentrations nor ongoing treatment did correlate with mineralization  
23 status in our and earlier reports [21]. Conversely, impairment of parathyroid function was inversely related to  
24 mineralization status, indicating a protective effect on bone of this endocrine complication [21, 22]. In our study the  
25 relationship was more evident at the femoral level than at lumbar spine; this was unexpected, as chronic PTH-  
26 deprivation should influence more strongly the sites where trabecular content is maximum. Probably, in this peculiar  
27 population the superimposed pathologic mechanisms concurring to bone disease can produce net effects difficult to  
28 predict in the long-term.  
29

30 Finally, an important consideration emerges from our statistical analysis: the model utilized could account for the  
31 29.4% of T-score variance at the femur and the 32.03% at the spine. This indicates that the variables significant in the  
32 univariate analysis could not explain satisfactorily the mineralization defect. Again, this may be ascribed to insufficient  
33 statistical power, linked to the restricted number of cases in a monocentric study, or alternatively to the influence of  
34 pathogenetically involved factors beyond those included in our analysis. The indices studied reflect bone marrow  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 expansion, iron overload, endocrine function and bone metabolism, but further physiological, pathological, genetic and  
2 iatrogenic factors can work in this multifactorial disease.

3  
4 Furthermore, early preventive intervention and management are needed to influence bone health in adulthood, as  
5 demonstrated by normal Z-scores found in children and young adolescents with Thalassemia Major by Christoforidis et  
6 al [23]. Our patients are probably comparable for the quality of clinical management; however the different results  
7 emerged from our study suggest that transfusion and chelation treatment can prevent bone demineralization only when  
8 applied early from childhood.  
9

10  
11  
12 A deeper learning of the molecular mechanisms causing demineralization, as well as the optimal prevention and  
13 treatment of Thalassemia from infancy, could perhaps allow to avoid complications and further ameliorate the quality of  
14 life in these patients.  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Fig 1** Bone mineralization state (normal / osteopenia / osteoporosis) of the thalassemic patients as distributed on the basis of site and gender, according to the WHO osteoporosis classification. Osteopenia was the prominent finding at the femur, while at lumbar spine osteoporosis was more prevalent ( $p < 0.001$ ). The same distribution was confirmed when the patients were separately considered according to gender, being more evident in the male group

**Table 1** Clinical characteristics of the patients by gender at the time when mineralization was studied

**Table 2** Biochemical and hormonal indices related to bone metabolism in 111 patients affected by Thalassemia Major

**Table 3** Statistic results (mean  $\pm$  standard deviation and  $p$  value) of comparisons between clinical/biochemical dicotomic variables and T or Z- scores at femoral and vertebral sites

**Table 4** Correlation analysis (Pearson's correlation indices and two-tailed  $p$  values) between bone mineralization (expressed by T and Z-scores) and clinical/biochemical parametric variables

## References

1. Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, Ghilardi R, Origa R, Piga A, Romeo MA, Zhao H, Cnaan A (2005) Survival and complications in thalassemia. *Ann N Y Acad Sci* 1054:40-47
2. Vogiatzi MG, Macklin EA, Fung EB, Vichinsky E, Olivieri N, Kwiatkowski J, Cohen A, Neufeld E, Giardina PJ (2006) Prevalence of fractures among the thalassemia syndromes in north america. *Bone* 38(4):571-575
3. Vogiatzi MG, Macklin EA, Fung EB, Cheung AM, Vichinsky E, Olivieri N, Kirby M, Kwiatkowski JL, Cunningham M, Holm IA, Lane J, Schneider R, Fleisher M, Grady RW, Peterson CC, Giardina PJ, Thalassemia Clinical Research Network (2009) Bone disease in thalassemia: A frequent and still unresolved problem. *J Bone Miner Res* 24(3):543-557
4. Cappellini M.D, Cohen A, Eleftheriou A, Piga A, Porter J, Taher A (2008) Guidelines for the clinical management of thalassemia; 2<sup>nd</sup> revised Edition. Thalassemia International Federation. <http://thalassemia.org.cy>
5. Wood JC, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, Coates TD (2005) MRI R2 and R2\* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 106 (4):1460-65
6. Dubey AP, Parakh A, Dubish S (2008) Current trends in the management of beta thalassemia. *Indian J Pediatr* 75(7):739-743
7. Perrotta S, Cappellini MD, Bertoldo F, Servedio V, Iolascon G, D'Agruma L, Gasparini P, Siciliani MC, Iolascon A (2000) Osteoporosis in beta-thalassaemia major patients: Analysis of the genetic background. *Br J Haematol* 111(2):461-466
8. Origa R, Fiumana E, Gamberini MR, Armari S, Mottes M, Sangalli A, Paglietti E, Galanello R, Borgna-Pignatti C (2005) Osteoporosis in beta-thalassemia: Clinical and genetic aspects. *Ann N Y Acad Sci* 1054:451-456
9. Skordis N (2009) The labyrinth of bone disease in thalassaemia: The search for ariadne's thread continues. *Eur J Haematol* 82(1):13-14

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
10. Gurevitch O, Slavin S (2006) The hematological etiology of osteoporosis. *Med Hypotheses* 67(4):729-735
  11. Voskaridou E, Terpos E (2008) Pathogenesis and management of osteoporosis in thalassemia. *Pediatr Endocrinol Rev* 6 Suppl 1:86-93
  12. Salama OS, Al-Tonbary YA, Shahin RA, Eldeen OA (2006) Unbalanced bone turnover in children with beta-thalassemia. *Hematology* 11(3):197-202
  13. Dresner Pollack R, Rachmilewitz E, Blumenfeld A, Idelson M, Goldfarb AW (2000) Bone mineral metabolism in adults with beta-thalassaemia major and intermedia. *Br J Haematol* 111(3):902-907
  14. Voskaridou E, Kyrtsolis MC, Terpos E, Skordili M, Theodoropoulos I, Bergele A, Diamanti E, Kalovidouris A, Loutradi A, Loukopoulos D (2001) Bone resorption is increased in young adults with thalassaemia major. *Br J Haematol* 112(1):36-41
  15. Morabito N, Gaudio A, Lasco A, Atteritano M, Pizzoleo MA, Cincotta M, La Rosa M, Guarino R, Meo A, Frisina N (2004) Osteoprotegerin and RANKL in the pathogenesis of thalassemia-induced osteoporosis: New pieces of the puzzle. *J Bone Miner Res* 19(5):722-727
  16. Hiratsuka H, Katsuta O, Toyota N, Tsuchitani M, Akiba T, Marumo F, Umemura T (1997) Iron deposition at mineralization fronts and osteoid formation following chronic cadmium exposure in ovariectomized rats. *Toxicol Appl Pharmacol* 143:348-356
  17. Mahachoklertwattana P, Sirikulchayanonta V, Chuansumrit A, Karnsombat P, Choubtum L, Sriphrapadang A, Domrongkitchaiporn S, Sirisriro R, Rajatanavin R (2003) Bone histomorphometry in children and adolescents with beta-thalassemia disease: iron-associated focal osteomalacia. *J Clin Endocrinol Metab* 88 ( 8):3966-3972
  18. Carmina E, Di Fede G, Napoli N, Renda G, Vitale G, Lo Pinto C, Bruno D, Malizia R, Rini GB (2004) Hypogonadism and hormone replacement therapy on bone mass of adult women with thalassemia major. *Calcif Tissue Int* 74(1):68-71
  19. Adami S, Giannini S, Bianchi G, Sinigaglia L, Di Munno O, Fiore CE, Minisola S, Rossini M (2009) Vitamin D status and response to treatment in post-menopausal osteoporosis. *Osteoporos Int* 20(2):239-244

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

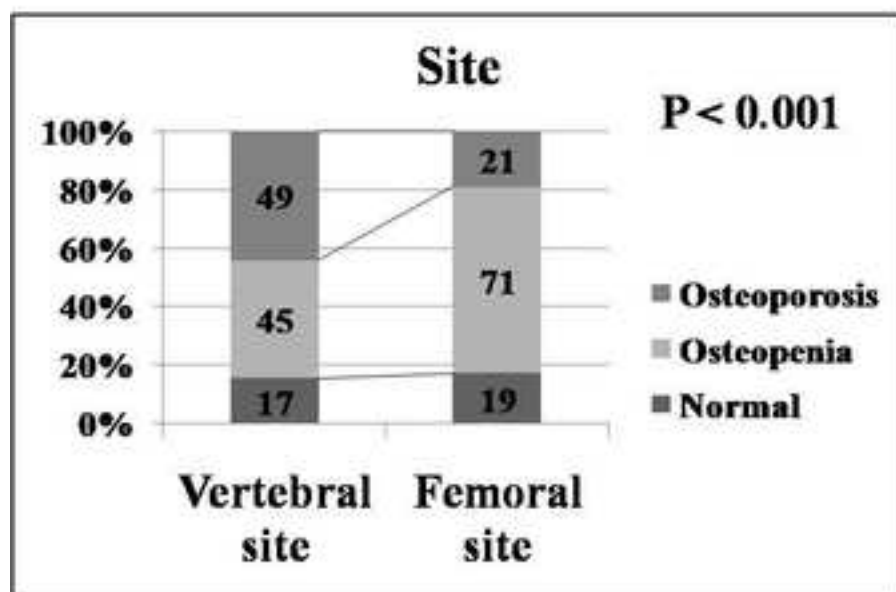
20. Napoli N, Carmina E, Bucchieri S, Sferrazza C, Rini GB, Di Fede G (2006) Low serum levels of 25-hydroxy vitamin D in adults affected by thalassemia major or intermedia. *Bone* 38(6):888-892

21. Angelopoulos NG, Goula A, Rombopoulos G, Kaltzidou V, Katounda E, Kaltsas D, Tolis G (2006) Hypoparathyroidism in transfusion-dependent patients with beta-thalassemia. *J Bone Miner Metab* 24(2):138-145

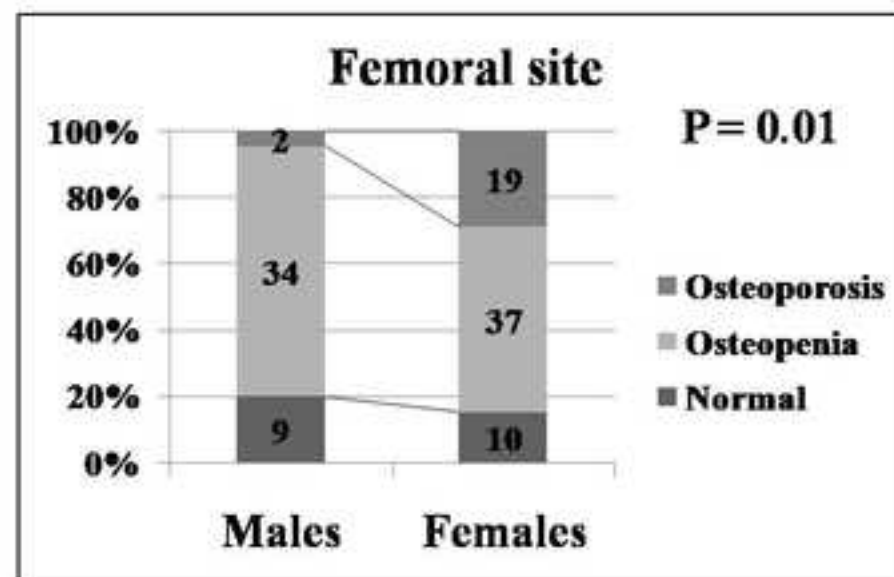
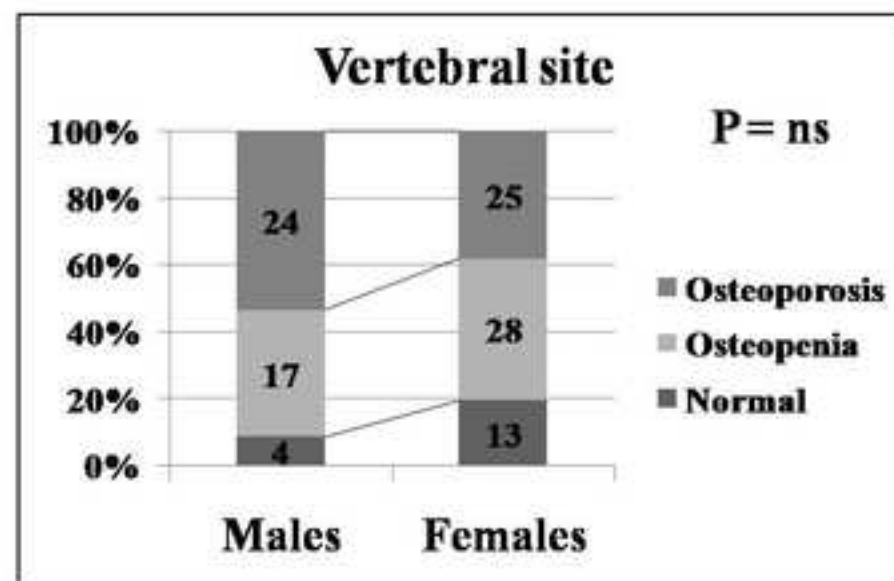
22. Laway BA, Goswami R, Singh N, Gupta N, Seith A (2006) Pattern of bone mineral density in patients with sporadic idiopathic hypoparathyroidism. *Clin Endocrinol (Oxf)* 64(4):405-409

23. Christoforidis A, Kazantzidou E, Tsatra I, Tsantali H, Koliakos G, Hatzipantelis E, Katzos G, Athanassiou-Metaxa M (2007) Normal lumbar bone mineral density in optimally treated children and young adolescents with beta-thalassaemia major. *Hormones (Athens)* 6(4):334-340

## General



## By gender



Table

	males		females	
	Mean $\pm$ SD	range	Mean $\pm$ SD	range
<b>Age (years)</b>	32.5 $\pm$ 4.9	22 - 44	33.9 $\pm$ 6.0	19 - 46
<b>Age at first transfusion (years)</b>	14.3 $\pm$ 11.8	4 - 48	17.3 $\pm$ 16.0	3 - 60
<b>Age at beginning chelation (years)</b>	5.2 $\pm$ 3.7	1 - 15	5.8 $\pm$ 3.5	0 - 15
<b>Body Mass Index (kg/m<sup>2</sup>)</b>	22.9 $\pm$ 3.3	16 - 29	22.3 $\pm$ 3.0	18 - 31
<b>Pre-transfusion hemoglobin (g/dl)</b>	9.4 $\pm$ 0.6	8.4 - 10.4	9.3 $\pm$ 0.6	8 - 10.4
<b>Serum ferritin (mcg/L)</b>	1267 $\pm$ 1168	431 - 6563	1452 $\pm$ 912	354 - 4509
<b>Liver Iron Content (mg Fe/g liver d.w.)</b>	5.1 $\pm$ 3.9	0.6 - 19.3	5.8 $\pm$ 3.7	0.6 - 15.9

	<b>mean ± SD</b>	<b>range</b>	<b>median</b>
<b>Serum calcium (mmol/L)</b>	2.2 ± 0.1	1.6 – 2.6	9
<b>Serum phosphate (mmol/L)</b>	1.3 ± 0.1	0.7 – 2.2	1.2
<b>Urinary calcium (mmol/d)</b>	6.3 ± 3.7	0.4 – 17	5.8
<b>Urinary phosphate (mmol/d)</b>	22.6 ± 9.7	3.2 – 83.9	22.6
<b>Alkaline phosphatase (U/L)</b>	85.4 ± 28.7	33 - 204	85
<b>Bone alkaline phosphatase (%)</b>	62 ± 17	7 - 95	65
<b>GGT (U/L)</b>	21.2 ± 12.9	7 - 72	35
<b>Osteocalcin (mcg/L)</b>	23.4 ± 30.1	0.3 – 209	14.2
<b>Parathyroid hormone (pmol/L)</b>	3.3 ± 2.2	0.2 – 14.9	2.9
<b>25OH Vitamin D (nmol/L)</b>	46.8 ± 29.3	9.6 - 132	38.6
<b>CTX (ng/ml)</b>	0.4 ± 0.3	0.1 – 1.4	0.3

Table

	T-score				Z-score				
	Femoral		Vertebral		Femoral		Vertebral		
	<i>Mean ± SD</i>	<i>p</i>	<i>Mean ± SD</i>	<i>p</i>	<i>Mean ± SD</i>	<i>p</i>	<i>Mean ± SD</i>	<i>p</i>	
<b>Gender</b>	<i>males</i>	-1.58 ± 0.746	0.046	-2.44 ± 1.119	<i>ns</i>	-1.50 ± 0.830	0.027	-2.28 ± 1.196	<i>ns</i>
	<i>females</i>	-1.90 ± 1.018		-2.12 ± 1.267		-1.87 ± 0.967		-2.04 ± 1.219	
<b>Hypogonadism</b>	<i>no</i>	-1.46 ± 0.772	0.047	-2.20 ± 1.095	<i>ns</i>	-1.65 ± 0.744	<i>ns</i>	-2.12 ± 1.098	<i>ns</i>
	<i>yes</i>	-1.85 ± 0.952		-2.26 ± 1.250		-1.73 ± 0.975		-2.15 ± 1.247	
<b>Hypoparathyroidism</b>	<i>no</i>	-1.87 ± 0.855	0.021	-2.36 ± 1.116	0.039	-1.82 ± 0.850	0.017	-2.21 ± 1.126	<i>ns</i>
	<i>yes</i>	-1.31 ± 1.130		-1.71 ± 1.527		-1.20 ± 1.118		-1.79 ± 1.547	
<b>Calcium treatment</b>	<i>no</i>	-1.64 ± 0.819	0.035	-2.05 ± 1.160	0.036	-1.68 ± 0.795	<i>ns</i>	-2.01 ± 1.192	<i>ns</i>
	<i>yes</i>	-1.93 ± 1.030		-2.49 ± 1.247		-1.75 ± 1.081		-2.31 ± 1.224	
<b>Bisphosphonate treatment</b>	<i>no</i>	-1.67 ± 0.905	0.003	-2.09 ± 1.190	<0.001	-1.66 ± 0.928	0.041	-1.99 ± 1.184	0.001
	<i>yes</i>	-2.35 ± 0.674		-3.23 ± 0.634		-2.14 ± 0.823		-3.16 ± 0.614	

	T - score				Z - score			
	Femoral		Vertebral		Femoral		Vertebral	
	<i>Correlation index</i>	<i>p</i>	<i>Correlation index</i>	<i>p</i>	<i>Correlation index</i>	<i>p</i>	<i>Correlation index</i>	<i>p</i>
<b>Age</b>	-0.218	0.022	-0.032	<i>ns</i>	-0.021	<i>ns</i>	0.063	<i>ns</i>
<b>BMI</b>	0.259	0.006	0.064	<i>ns</i>	0.276	0.006	0.049	<i>ns</i>
<b>Pre-transfusional hemoglobin</b>	-0.088	<i>ns</i>	0.179	<i>ns</i>	-0.058	<i>ns</i>	0.198	0.042
<b>Alkaline Phosphatase</b>	-0.223	0.019	-0.233	0.014	-0.024	0.024	0.137	<i>ns</i>
<b>CTX</b>	0.032	<i>ns</i>	-0.028	<i>ns</i>	-0.024	<i>ns</i>	-0.047	<i>ns</i>

1  
2  
3  
4  
5  
6 **Endocrine and bone disease in appropriately treated adult patients with beta-thalassemia**  
7  
8 **major**  
9

10  
11  
12  
13  
14  
15  
16  
17  
18 Marina Baldini<sup>a</sup>, Stella Forti<sup>b</sup>, Alessia Marcon<sup>a</sup>, Fabio Massimo Ulivieri<sup>d</sup>, Alessandra Orsatti<sup>c</sup>, Benedetta Tampieri<sup>a</sup>,  
19  
20 Lorena Airaghi<sup>c</sup>, Laura Zanaboni<sup>a</sup>, Maria Domenica Cappellini<sup>a</sup>  
21  
22  
23  
24  
25  
26  
27

28  
29 U.O. Medicina Interna 1 A<sup>a</sup> e 1 B<sup>c</sup> Fondazione Ca' Granda, Ospedale Maggiore Policlinico (IRCCS), via F. Sforza 35,  
30  
31 20122 Milan, Italy

32  
33 <sup>b</sup> Audiologia, Fondazione Ca' Granda, Ospedale Maggiore Policlinico (IRCCS), via F. Sforza 35, 20122 Milan, Italy

34  
35 <sup>c</sup> Dipartimento di Medicina Interna, Università degli Studi di Milano

36  
37 <sup>d</sup> U.O. Medicina Nucleare, Centro per la Diagnosi e la Terapia delle Osteoporosi, Fondazione Ca' Granda, Ospedale  
38  
39 Maggiore Policlinico (IRCCS), via F. Sforza 35, 20122 Milan, Italy  
40  
41  
42  
43  
44

45 Corresponding author:

46  
47 Marina Baldini

48  
49 e-mail: [marina.baldini@policlinico.mi.it](mailto:marina.baldini@policlinico.mi.it)

50  
51 tel:+390255033333

52  
53 fax:+390255033665  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Abstract**

1 With optimization of transfusional and chelation regimens, beta thalassemia has changed from a pediatric disease with  
2 poor life expectancy into a chronic disease. Bone demineralization is an important cause of morbidity in older patients;  
3 the etiology is multifactorial and partially unknown. We examined cross-sectionally 111 adult patients with beta-  
4 Thalassemia Major (66 females and 45 males,  $32.6 \pm 6$  years), regularly transfused, sufficiently chelated and replaced  
5 for endocrine defects. Bone demineralization was detected in 92.7% of patients, with different severity according to  
6 gender and site: osteopenia was the prominent finding at the femur, osteoporosis at lumbar spine ( $p < 0.001$ ), more  
7 evident in the males. The femoral site was more influenced by biochemical and clinical factors; despite adequate  
8 replacement, femoral T-score was lower in the hypogonadic than in the eugonadic group ( $p = 0.047$ ). A significant  
9 correlation was found between bone mass and body mass index (BMI), alkaline phosphatase (ALP) and pre-  
10 transfusional Hb levels. The multivariate analysis indicated as significant regressors ALP, BMI and hypoparathyroidism  
11 (T-score:  $p = 0.005, 0.035$  and  $0.002$ ; Z-score:  $0.002, 0.009, 0.003$ , respectively) at the femoral site, only ALP at the  
12 lumbar spine ( $p = 0.008$  and  $0.045$  for T and Z scores, respectively). The statistical significance was reached more  
13 frequently by T-score, while Z-score seemed to be a less sensitive parameter.

14 Despite best care facilities, bone demineralization in thalassemic patients remains a challenge; further exploration of the  
15 relationships between bone loss and endocrine, biochemical and hematologic parameters is warranted to find effective  
16 measures to reduce fracture risk in this disease.

17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43 **Key words:** Thalassemia, osteoporosis, iron overload, endocrinopathy.  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Introduction

1 The life expectancy of patients affected by thalassemia major (TM) is significantly increased during the last decade due  
2  
3 to regular transfusion regimen associated to adequate iron chelation . Today most of the patients enter adult life with a  
4  
5 relevant improvement of quality of life[1]. Bone deformities, which could not be forecasted 20 years ago are quite rare  
6  
7 today, nevertheless, low bone mass still remains a significant cause of morbidity in adult patients with TM. An  
8  
9 increased prevalence of osteoporosis, and consequently of fractures due to bone fragility, continues to be reported [2,3]  
10  
11 despite transfusions, effective chelation, calcium and vitamin D supplementation and replaced hormonal deficiencies.  
12  
13 Several concomitant factors may contribute to the pathogenesis of osteoporosis in adult TM patients, making difficult a  
14  
15 proper and preventing therapeutic approach.  
16  
17

18 We designed a cross-sectional evaluation of adult thalassemia major patients followed-up at single tertiary outpatient  
19  
20 clinic in Milan, with optimal transfusional and therapeutical regimen, in order to assess the prevalence of bone  
21  
22 demineralization and the relationships between bone status and physiological and pathological factors, including  
23  
24 ferritin, pre-transfusional haemoglobin levels, bone metabolism markers and endocrine complications.  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Patients

1  
2 After the approval of the Ethical Committee of our Institution and after patient's informed consent, 111 adult Caucasian  
3 patients affected by beta-Thalassemia Major (homozygous or double heterozygous for severe beta mutations) were  
4 enrolled in this study (66 females and 45 males, mean age  $32.6 \pm 6$  years (33, range 19-50). All the patients received  
5 regular blood transfusions every 20-25 days, maintaining pre-transfusional haemoglobin around 9-9.5 g/L, according to  
6 the international guidelines [4]. Chelation therapy included deferoxamine (DFO) (39 pts), deferiprone (DFP) (7 pts),  
7 deferasirox (DFX)(46 pts) or combination DFO/DFP (19 pts). The chelation adequacy was estimated based on mean  
8 levels every 3 months and LIC by MRI when available. Exclusion criteria were medical conditions and pharmacological  
9 treatments interfering with the bone mineral metabolism.

10  
11 The study design was cross-sectional; a periodic endocrinological evaluation was planned and during the same day bone  
12 densitometry, blood and urine biochemical tests and blood hormone evaluations were performed. Data collected  
13 included age, gender, BMI, history of auxologic and sexual development; splenectomy, fractures and liver disease,  
14 drugs used, transfusion regimen and starting age, mean of pre-transfusional haemoglobin and ferritin in the previous  
15 year, liver function and endocrine tests, bone densitometry of femur and lumbar spine.

16  
17 At the time of the biochemical and densitometric assessment, all hypogonadal patients were on hormonal replacement  
18 therapy, and all hypothyroid ones were on replacement with levothyroxine. Patients with hypoparathyroidism were  
19 treated with conventional vitamin D and calcium supplements, at a dose sufficient to maintain serum calcium within the  
20 normal range or at the lower normal limit.

## Methods

21  
22 Liver and renal function tests, calcium and phosphorus in serum (n. v. 2.1-2.5 and 0.8-1.4 mmol/L, respectively) and in  
23 24-hour urine (n.v. 2.5-7.5 mmol/d and 12.9-42 mmol/d, respectively), intact parathyroid hormone (n.v. 1.5-6.6  
24 pmol/L), osteocalcin (n.v.14-42 mcg/L), 25-hydroxyvitamin D (n.v.  $> 72$  nmol/L), ferritin (n.v. 30-400 mcg/L) were  
25 determined by automated routine procedures. Blood cell count was performed by Coulter Counter; alkaline phosphatase  
26 was measured by IFCC liquid (n.v. 40-129 U/L).

27  
28 Iron overload was properly estimated by Liver Iron Content (LIC) measured by MRI [5], that showed a wide standard  
29 deviation due to the patients' compliance and the different efficacy of therapies..

30  
31 Bone mineral density measurement was performed by means of dual x-ray photon absorptiometry (DEXA) using  
32 Hologic bone densitometer (Hologic QDR-Discovery A, Version 12.7.3.1).

33  
34 BMD values were expressed as T-score and Z-score. T-score was calculated as SDs from normal reference population  
35 database, while Z-score as SDs from age and sex matched population.

1 Data were classified according to WHO report (WHO Technical Report, ISCD Official Position Paper 2007); T-score >  
2 -1: normal. -1 > T-score > -2.5: low bone density (osteopenia). T-score < -2.5: osteoporosis.  
3  
4  
5  
6

7  
8 **Statistical analysis**  
9

10 Data are presented as means  $\pm$  SD. Intergroup comparison was performed with the Mann-Whitney test. Associations  
11 between parameters were determined by Pearson's and Spearman's rank correlation coefficients. To find independent  
12 factors affecting bone mineral density in thalassemic patients, a step-wise linear regression analysis was conducted.  
13

14 Two-sided exact tests were used and *p* values of less than 0.5 were considered significant. All statistics were calculated  
15 using the Statistical Package for the Social Sciences 17.0 for Windows software package (SPSS Inc, Chicago, IL).  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Results

The demographic characteristics of the patients studied are presented in Table 1

All the patients were on iron chelating treatment: 35% with deferoxamine (30+5mg/Kg), 42% with deferasirox (20+15mg/Kg), 6% with deferiprone (75mg/Kg) and the remaining 17% with combined therapy (deferoxamine 30mg/Kg + Deferiprone 75mg/Kg).

101 patients (91%) were anti-HCV positive, of whom 50.5% were HCV RNA positive. 68% were splenectomised.

### Evaluation of bone mineral metabolism and endocrine function

The biochemical indices of bone metabolism are reported in Table 2. All the parameters resulted within the normal range apart from 25OH vitamin D serum levels, that were suboptimal in 21 patients even if 13 of them were supplemented with 10.000 IU/week.

Nineteen out of 111 patients (17.1%) were affected by hypoparathyroidism and treated with calcium and calcitriol at doses normalizing serum calcium and phosphate concentrations. All the hypoparathyroid patients were also hypogonadic, except one, resulting 16.2% the association of these endocrine diseases.

Hypogonadism (in all cases hypogonadotropic) was the most frequent endocrine complication in our cohort of patients, affecting 87 patients (78.4%); all the hypogonadic patients except 4 (95.4%) were on replacement treatment starting from the age of  $18.8 \pm 3$  years. The hypogonadic patients who were not on replacement, being a minority, were excluded from the analysis of densitometric values.

An impairment of glucose metabolism was present in a high proportion of patients, with diabetes mellitus in 20 (18%) and IGT in 24 of them (22%). IGF-1 serum concentrations were evaluated in 78 patients (70%), and resulted lower than normal in 45 of them (41%).

### *Bone mass measurements*

When considering the bone mass measurements in the overall population enrolled, the prevalence of demineralization (including both osteopenia and osteoporosis) was 92.7% (103 out of 111 Thalassemic patients). Indeed, mineralization was normal only in 18 patients (16.2%) at the vertebral site and in 21 patients (18.9%) at the femoral site.

As shown in Table 3, there were no differences in bone mineralization between the hypogonadic and the eugonadic patients at the vertebral site, while at the femoral site the T-score was lower in the hypogonadic group, despite adequate replacement ( $p = 0.047$ ). Further, only at the femoral site, bone mass was significantly different by gender, as females showed lower mean values of T and Z-scores. There were statistically significant differences in T-score at both sites also for the other dicotomic variables examined (hypoparathyroidism, treatment with calcium, treatment with bisphosphonates), while the statistical significance was not reached in all the analyses using Z-score as dependent variable.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Figure 1 shows the distribution of bone mineralization state (normal / osteopenia / osteoporosis) according to gender and site. Osteopenia was the prominent finding at the femoral site (63 femoral vs 40 vertebral site,  $p < 0.001$ ), while at the vertebral site osteoporosis was more prevalent (53 pts vs 27 pts), the difference was statistically significant ( $p < 0.001$ ). The same distribution was confirmed when the patients were separately considered according to gender, being more evident in the male group ( $p < 0.001$ ). The patient distributions according to bone mineralization were significantly different by gender only at the femoral site.

Table 4 shows the results of the correlation analysis (correlation indices and statistical significance,  $p < 0.05$ ) between bone mineralization (expressed by T and Z scores) and clinical/biochemical parametric variables. BMI was positively correlated with bone mass at femoral site, while advancing age was negatively correlated only with T-score. No correlation was found between vertebral T-score and the same variables. Among the biochemical indices of bone metabolism examined, alkaline phosphatase was the only one significantly correlated both with femoral and vertebral mineralization, being inversely associated with bone mass. Pre-transfusional Hb levels were significantly associated to vertebral Z-score.

The correlations between bone mineralization and the other clinical and biochemical characteristic of the patients (age at first transfusion, age at beginning of chelation, diabetes, previous splenectomy, LIC, serum concentrations of ferritin, parathyroid hormone, bone alkaline phosphatase, osteocalcin, 25 OH vitamin D, IGF-1, and calcium both in serum and urine) were not significant thus were not reported.

The multivariate analysis indicated as possible regressors alkaline phosphatase, BMI and hypoparathyroidism ( $p = 0.005$ , 0.035 and 0.002 for T-score; 0.002, 0.009, 0.003 for Z-score, respectively) at the femoral site, while alkaline phosphatase was the only one found regressor for T and Z scores (respectively  $p = 0.008$  and 0.045) at the lumbar spine.

## Discussion

1  
2 Despite the longer life expectancy of thalassaemia major patients due to proper transfusional and chelation regimens  
3  
4 ([1], endocrine complications and bone disease still remain important causes of morbidity and disability[2,3,6-9] . In  
5  
6 this study we examined adult patients with thalassemia major regularly transfused and sufficiently chelated; the patients  
7  
8 were cared for  $13 \pm 3$  years (mean  $\pm$  SD) at a tertiary single Italian centre where the Thalassemia International  
9  
10 Guidelines [4] for monitoring and treating Thalassemia and its complications are issued and implemented..

11  
12 Despite the availability of the best care facilities, bone demineralization was detected in a high proportion of patients,  
13  
14 involving both sites examined though with different severity: osteopenia prevailed at femoral site, while osteoporosis  
15  
16 was more prominent at lumbar spine (prevalently constituted by trabecular bone). Overall, the femoral site seemed to  
17  
18 be more likely than the vertebral site influenced by biochemical and clinical factors. The only exception was pre-  
19  
20 transfusional Hb, showing a positive correlation with vertebral mineralization; this could be explained by the high  
21  
22 degree of ineffective erythropoiesis in the spine that can occurs in thalassemia syndromes. This finding is consistent  
23  
24 with the interesting hypothesis that the chronic request for blood cell production can play a role in the etiology of  
25  
26 osteoporosis, through overstimulation of the hematopoietic system increasing the number of osteoclasts and osteoblasts  
27  
28 resulting in accelerated bone turnover [10].

29  
30 In our analyses the statistical significance was reached more frequently by T-score, while Z-score seemed to be a less  
31  
32 sensitive parameter. There is a statistical explanation to this behaviour: the relatively small number of cases can render  
33  
34 the statistical power insufficient to detect smaller changes in Z-score, a parameter with high baseline variability.  
35  
36 However, considering the very low prevalence of the disease, this is a sufficiently large cohort, and quite homogeneous  
37  
38 as regards therapeutical approach and clinical management.

39  
40 The statistically significant negative association between T-score and treatment with calcium and with bisphosphonates  
41  
42 was expected, and compatible with the prescription of such drugs to the patients with more severe demineralization; the  
43  
44 therapeutic effect of these medications could not be examined in our study, as the protocol did not include a  
45  
46 longitudinal evaluation. Other parameters correlated to demineralization were gender (females are more involved), age  
47  
48 and BMI, similarly to healthy individuals.

49  
50 Even if we cannot address the issue of the prominent cause of bone disease in Thalassemia [11], the correlation  
51  
52 between alkaline phosphatase and bone mineralization indicates a prevalent role of reduced deposition over increased  
53  
54 reabsorption, as reported by other Authors [12], at variance with others [13, 14] . We could not confirm the same  
55  
56 relationship with osteocalcin, another marker of osteoblastic activity [12, 15].

57  
58 As far as the endocrine complications relevant to bone status are concerned, according to previous reports [16] in our  
59  
60 study the hypogonadic patients of both genders were more affected from demineralization than the eugonadic, and,  
61  
62

1 interestingly, the standard hormone replacement therapy (HRT) does not normalize bone condition. A possible  
2 explanation may be the concurrence of factors interfering with mineralization, such as vitamin D deficiency, impairing  
3 the therapeutic effects of sex steroid hormones and also of bisphosphonates. A recent report [17] stated that an optimal  
4 vitamin D repletion is an essential pre-requisite for maximal efficacy of the anti-resorptive drugs in post-menopausal  
5 osteoporosis; a similar situation could be hypothesized in thalassemic population. In effect, low serum vitamin D levels  
6 have been reported in thalassemic populations; this was ascribed to impaired liver hydroxylation and intestinal  
7 absorption, with a causative role of iron overload suggested by a negative correlation between serum 25-OH-vitamin D  
8 and ferritin [18]. This correlation was not confirmed in our study ( $p = 0.546$ ). Unexpectedly, in our series the serum  
9 vitamin D was often low despite ongoing treatment with metabolites, compatible with a defect in absorption or  
10 metabolism [18] but also with low compliance to the therapy; the latter is quite common using associations with  
11 calcium salts, badly tolerated. Anyway, the contribution of vitamin D to bone status is probably more complex than  
12 presently recognized, and neither vitamin D concentrations nor ongoing treatment did correlate with mineralization  
13 status in our and earlier reports [19]. Conversely, impairment of parathyroid function was inversely related to  
14 mineralization status, indicating a protective effect on bone of this endocrine complication [19, 20]. In our study the  
15 relationship was more evident at the femoral level than at lumbar spine; this was unexpected, as chronic PTH-  
16 deprivation should influence more strongly the sites where trabecular content is maximum. Probably, in this peculiar  
17 population the superimposed pathologic mechanisms concurring to bone disease can produce net effects difficult to  
18 predict in the long-term.

19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36 Finally, an important consideration emerges from our statistical analysis: the model utilized could account for the  
37 29.4% of T-score variance at the femur and the 32.03% at the spine. This indicates that the variables significant in the  
38 univariate analysis could not explain satisfactorily the mineralization defect. Again, this may be ascribed to insufficient  
39 statistical power, linked to the restricted number of cases in a monocentric study, or alternatively to the influence of  
40 pathogenetically involved factors beyond those included in our analysis. The indices studied reflect bone marrow  
41 expansion, iron overload, endocrine function and bone metabolism, but further physiological, pathological, genetic and  
42 iatrogenic factors can work in this multifactorial disease.

43  
44  
45  
46  
47  
48  
49  
50 Furthermore, early preventive intervention and management are needed to influence bone health in adulthood, as  
51 demonstrated by normal Z-scores found in children and young adolescents with Thalassemia Major by Christoforidis et  
52 al [21]. Our patients are probably comparable for the quality of clinical management; however the different results  
53 emerged from our study suggest that transfusion and chelation treatment can prevent bone demineralization only when  
54 applied early from childhood.

A deeper learning of the molecular mechanisms causing demineralization, as well as the optimal prevention and treatment of thalassemia from infancy, could perhaps allow to avoid complications and further ameliorate the quality of life in these patients.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Fig 1** Bone mineralization state (normal / osteopenia / osteoporosis) of the Thalassemic patients as distributed on the basis of site and gender, according to the WHO osteoporosis classification. Osteopenia was the prominent finding at the femur, while at lumbar spine osteoporosis was more prevalent ( $p < 0.001$ ). The same distribution was confirmed when the patients were separately considered according to gender, being more evident in the male group

**Table 1** Clinical characteristics of the patients by gender at the time when mineralization was studied

**Table 2** Biochemical and hormonal indices related to bone metabolism in 111 patients affected by Thalassemia Major

**Table 3** Statistic results (mean  $\pm$  standard deviation and  $p$  value) of comparisons between clinical/biochemical dicotomic variables and T or Z- scores at femoral and vertebral sites

**Table 4** Correlation analysis (Pearson's correlation indices and two-tailed  $p$  values) between bone mineralization (expressed by T and Z-scores) and clinical/biochemical parametric variables

## References

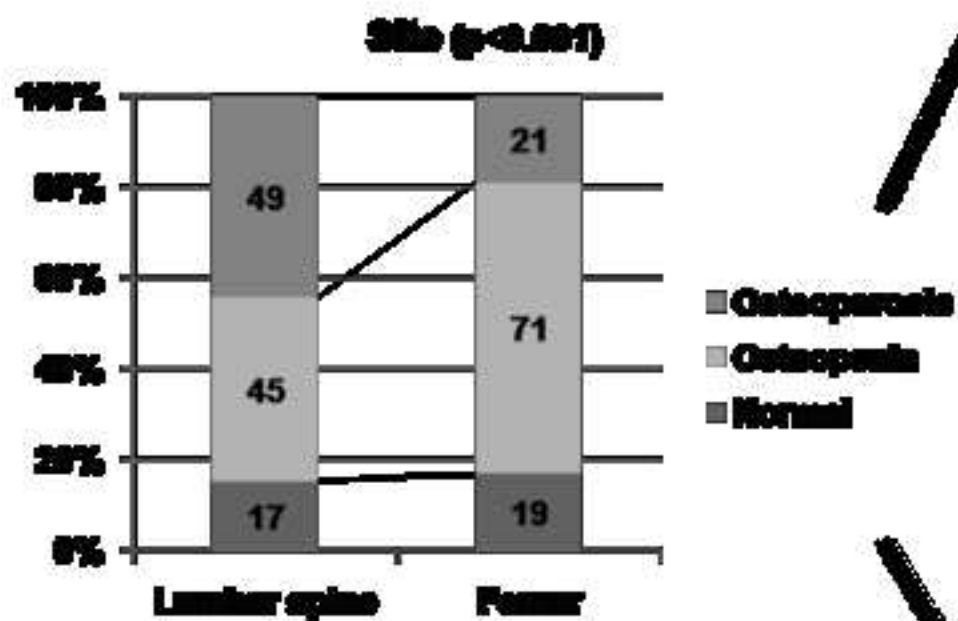
1. Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, Ghilardi R, Origa R, Piga A, Romeo MA, Zhao H, Cnaan A (2005) Survival and complications in thalassemia. *Ann N Y Acad Sci* 1054:40-47
2. Vogiatzi MG, Macklin EA, Fung EB, Vichinsky E, Olivieri N, Kwiatkowski J, Cohen A, Neufeld E, Giardina PJ (2006) Prevalence of fractures among the thalassemia syndromes in north america. *Bone* 38(4):571-575
3. Vogiatzi MG, Macklin EA, Fung EB, Cheung AM, Vichinsky E, Olivieri N, Kirby M, Kwiatkowski JL, Cunningham M, Holm IA, Lane J, Schneider R, Fleisher M, Grady RW, Peterson CC, Giardina PJ, Thalassemia Clinical Research Network (2009) Bone disease in thalassemia: A frequent and still unresolved problem. *J Bone Miner Res* 24(3):543-557
4. Cappellini M.D, Cohen A, Eleftheriou A, Piga A, Porter J, Taher A (2008) Guidelines for the clinical management of thalassemia; 2<sup>nd</sup> revised Edition. Thalassemia International Federation. <http://thalassemia.org.cy>
5. Wood JC, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, Coates TD (2005) MRI R2 and R2\* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 106 (4):1460-65
6. Dubey AP, Parakh A, Dubish S (2008) Current trends in the management of beta thalassemia. *Indian J Pediatr* 75(7):739-743
7. Perrotta S, Cappellini MD, Bertoldo F, Servedio V, Iolascon G, D'Agruma L, Gasparini P, Siciliani MC, Iolascon A (2000) Osteoporosis in beta-thalassaemia major patients: Analysis of the genetic background. *Br J Haematol* 111(2):461-466
8. Origa R, Fiumana E, Gamberini MR, Armari S, Mottes M, Sangalli A, Paglietti E, Galanello R, Borgna-Pignatti C (2005) Osteoporosis in beta-thalassemia: Clinical and genetic aspects. *Ann N Y Acad Sci* 1054:451-456
9. Skordis N (2009) The labyrinth of bone disease in thalassaemia: The search for ariadne's thread continues. *Eur J Haematol* 82(1):13-14

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
10. Gurevitch O, Slavin S (2006) The hematological etiology of osteoporosis. *Med Hypotheses* 67(4):729-735
  11. Voskaridou E, Terpos E (2008) Pathogenesis and management of osteoporosis in thalassemia. *Pediatr Endocrinol Rev* 6 Suppl 1:86-93
  12. Salama OS, Al-Tonbary YA, Shahin RA, Eldeen OA (2006) Unbalanced bone turnover in children with beta-thalassemia. *Hematology* 11(3):197-202
  13. Dresner Pollack R, Rachmilewitz E, Blumenfeld A, Idelson M, Goldfarb AW (2000) Bone mineral metabolism in adults with beta-thalassaemia major and intermedia. *Br J Haematol* 111(3):902-907
  14. Voskaridou E, Kyrtsolis MC, Terpos E, Skordili M, Theodoropoulos I, Bergele A, Diamanti E, Kalovidouris A, Loutradi A, Loukopoulos D (2001) Bone resorption is increased in young adults with thalassaemia major. *Br J Haematol* 112(1):36-41
  15. Morabito N, Gaudio A, Lasco A, Atteritano M, Pizzoleo MA, Cincotta M, La Rosa M, Guarino R, Meo A, Frisina N (2004) Osteoprotegerin and RANKL in the pathogenesis of thalassemia-induced osteoporosis: New pieces of the puzzle. *J Bone Miner Res* 19(5):722-727
  16. Carmina E, Di Fede G, Napoli N, Renda G, Vitale G, Lo Pinto C, Bruno D, Malizia R, Rini GB (2004) Hypogonadism and hormone replacement therapy on bone mass of adult women with thalassemia major. *Calcif Tissue Int* 74(1):68-71
  17. Adami S, Giannini S, Bianchi G, Sinigaglia L, Di Munno O, Fiore CE, Minisola S, Rossini M (2009) Vitamin D status and response to treatment in post-menopausal osteoporosis. *Osteoporos Int* 20(2):239-244
  18. Napoli N, Carmina E, Bucchieri S, Sferrazza C, Rini GB, Di Fede G (2006) Low serum levels of 25-hydroxy vitamin D in adults affected by thalassemia major or intermedia. *Bone* 38(6):888-892
  19. Angelopoulos NG, Goula A, Rombopoulos G, Kaltzidou V, Katounda E, Kaltsas D, Tolis G (2006) Hypoparathyroidism in transfusion-dependent patients with beta-thalassemia. *J Bone Miner Metab* 24(2):138-145
  20. Laway BA, Goswami R, Singh N, Gupta N, Seith A (2006) Pattern of bone mineral density in patients with sporadic idiopathic hypoparathyroidism. *Clin Endocrinol (Oxf)* 64(4):405-409

21. Christoforidis A, Kazantzidou E, Tsatra I, Tsantali H, Koliakos G, Hatzipantelis E, Katzos G, Athanassiou-Metaxa M (2007) Normal lumbar bone mineral density in optimally treated children and young adolescents with beta-thalassaemia major. *Hormones (Athens)* 6(4):334-340

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## General



## By gender

