Dual-Energy X-ray Absorptiometry Predictors of Vertebral Deformities in Beta-Thalassemia Major

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DXA Predictors of Vertebral Deformities in Beta-Thalassemia Major

Running Title: DXA and Vertebral Fractures in β-Thalassemia

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Abstract

Purpose. Vertebral fractures in beta-thalassemia major are increasingly found because of the longer life expectancy of patients, with a major negative impact on their quality of life. We performed a retrospective cross-sectional study to investigate the prevalence of vertebral deformities in thalassemic patients and identify their best DXA predictor among Trabecular Bone Score, Bone Mineral Density and Z-score.

Methods. Eighty-two outpatients with beta-thalassemia major on regular conventional treatment were studied at a single academic center. All patients underwent plain thoracic-lumbar spine X-rays and lumbar DXA to assess number and severity of vertebral deformities (Genant's method), Spinal Deformity Index, lumbar spine DXA parameters (Bone Mineral Density, Trabecular Bone Score, Z-score) and presence of platyspondyly.

Results. Twenty-nine patients (35%) had vertebral deformities and showed significantly lower Trabecular Bone Score than the remainders (1.141 ± 0.083 *vs* 1.254 ± 0.072, p <0.0001). The analysis of variance (ANOVA) of the Trabecular Bone Score between the group of patients without vertebral deformities (SDI=0) and the remaining groups showed a significant difference (p <0.001). Trabecular Bone Score had better sensitivity (86.2%), specificity (75.5%) and diagnostic accuracy (79.3%) than Bone Mineral Density and Z-score in discriminating patients with and without vertebral deformities. Combining Trabecular Bone Score with Bone Mineral Density or Z-score, the diagnostic accuracy of the first in discriminating patients with and without vertebral deformities improved from 79.3% to 85.4% and 87.8%, respectively. Presence of platyspondyly and low body mass index were significant predictors of vertebral deformities in the multivariate model.

Conclusions. Vertebral deformities in well-treated patients with beta-thalassemia major are common and often unrecognized. In our hands, Trabecular Bone Score was better than Bone Mineral Density and Z-score in predicting vertebral deformities. Plain X-rays of the spine

should be performed also in asymptomatic patients, especially when Trabecular Bone Score is low.

Key words: DXA; TBS; beta-thalassemia; vertebral deformities; platyspondyly.

Introduction

Beta-thalassemia is an inherited autosomal recessive disorder caused by mutations of the beta-globin gene, located on the chromosome 11. In beta-thalassemia major (i.e., homozygous beta-thalassemia), both beta-globin genes are mutated with consequent critical deficiency in the production of Hemoglobin (HbA), ineffective erythropoiesis, and severe transfusion-dependent anemia (1). The conventional transfusion protocols and the iron chelation therapy have significantly prolonged life expectancy of affected patients (2). As a consequence, long-term complications of the disease have become more frequent. Among these, fragility fractures (i.e. any fracture due to non-efficient trauma, like a fall from a standing height or less) deserve special attention for their negative impact on the quality of life.

The pathogenesis of bone involvement in beta-thalassemia major is multifactorial. Both genetic causes (e.g., primary disease itself, polymorphism of the collagen type 1 [COLIA 1] and of the vitamin D-receptor [VDR] genes) (3, 4) and acquired factors (e.g., ineffective hematopoiesis with marrow expansion, delayed puberty, hypogonadism, increased frequency of diabetes and hypothyroidism, insulin growth factor 1 deficiency, parathyroid gland dysfunction, hypercalciuria, calcium and vitamin D deficiencies, inadequate physical activity, toxic effects of iron and iron chelators) have been implicated)(5-10). A low bone mass is commonly reported in thalassemic patients, along with fractures of extremities, but the prevalence of vertebral fractures is probably underestimated (11-14). This is attributable to mild vertebral deformities that usually present with no apparent hyperkyphosis in patients with few or no symptoms. In the radiologic reports, moreover, mild vertebral deformities may be misdiagnosed as a consequence of bone marrow expansion.

Dual-energy X-ray absorptiometry (DXA), the gold standard technique for the evaluation of bone mineral density (BMD) in the general population and in thalassemic osteopathy as well,

provides no information about bone quality (15). Trabecular bone score (TBS) is a gray-level textural metric related to bone texture that can be extracted from the two-dimensional lumbar spine DXA image (16). It is based on experimental variograms of the projected DXA image. The results of TBS seem to be well correlated with bone fragility, regardless of BMD, and recently the WHO FRAX calculator allows the use of TBS as an independent clinical risk factor for computing the absolute ten years risk of fractures (17). TBS is associated with fracture risk in various clinical conditions related to bone fragility, other than postmenopausal osteoporosis, such as diabetes, primary hyperparathyroidism, subclinical hypercortisolism, rheumatoid arthritis and growth hormone deficiency (17,18). In a recent study comparing BMD and TBS at lumbar spine, and BMD at total femur and femoral neck in a cohort of 124 adult patients with beta-thalassemia major and in a control group of 65 non-thalassemic patients (untreated older females), vertebral deformities were not discussed (19).

The aim of our study was to compare the performance of DXA and TBS for predicting vertebral deformities in a cohort of 82 patients with beta-thalassemia major in conventional transfusion and iron chelation protocols.

Patients and Methods

We retrospectively evaluated a series of 82 young adults (15-45 years, median 33 years) affected by beta-thalassemia major and cared at the reference Centre for Hemoglobinopathies of our Hospital. In the last 5 years the Centre cared for about 103 young adults in the studied age range (15-45 years); thus, the sample of 82 selected subjects (80%) is well representative of the whole series. The inclusion criteria were the availability of at least one lumbar spine DXA evaluation and one x-ray of the spine performed within an interval time equal or less than 6 months each from the other. The imaging evaluation was not driven by a clinical

suspect of vertebral fracture; rather, both tests (DXA and spine x-ray) are part of a routinely performed screening for the assessment of bone health at the Centre. The analysis was limited to the last 5 years to ensure the use of the same device for all the DXA measurements. Medical records of each subject were extracted from the database of the Centre and reviewed. All DXA scans and x-rays of the spine were carefully re-analyzed. All patients were treated by monthly blood transfusions, to maintain a mean pre-transfusion level of Hb ≥9,5 g/dL, and by daily iron chelation according to predefined protocols. Clinical evaluation and lab tests for thyroid, parathyroid, pancreatic and gonadic functions were performed routinely, and hormonal replacement therapies were administered when indicated according to current guidelines. None of the patients included in the study was affected by hypoparathyroidism. Vitamin D supplements were administered in subjects with low calcium dietary intake and low serum levels of 25-hydroxyvitamin D (< 20 ng/mL). Plain thoracic-lumbar spine X-rays were obtained using standardized technique. Vertebral deformities were defined using the semi-quantitative method proposed by Genant et al. (20). Two trained radiologists (AM.P and SM.P., with 10 years of experience in conventional radiology) examined the X-ray films in a blinded and independent manner, and graded vertebrae as normal (SQ0), or bearing mild (SQ1), moderate (SQ2), or severe (SQ3) deformities. Deformities were defined as 20 to 25% (mild), 25 to 40% (moderate), or greater than 40% (severe) decreases in anterior, central or posterior vertebral height (from T4 to L4) (20). Spinal Deformity Index (SDI), a semi-quantitative index that integrates both the number and the severity of vertebral deformities, was calculated for every patient by summing the SQ grade for each of the 13 vertebrae from T4 to L4 (21). Lumbar spine (L1-L4) bone mineral density (BMD) was measured by Hologic Discovery A (version 12.7.3.2, Waltham, MA, USA) and BMD values were expressed as grams/cm^2 and as Z-scores (i.e., Standard Deviations [SD] from age and sex matched population). Because of the young age of most patients, we did not consider the T-scores in

our analysis. Lumbar spine TBS was automatically derived from each lumbar DXA using the TBS iNsight® software (version 2.1.0, Medimaps Group, Geneva, Switzerland, distributed by TechnoLogic, Turin, Italy).

We performed the study in accordance with the World Medical Association Declaration of Helsinki and the guidance on Good Clinical Practice (GCP). All patients gave their informed consent for the anonymous use of personal health information in a scientific paper and the study was approved by the Ethical Committee of our Hospital.

Statistical Analysis

The Kolmogorov-Smirnov test was performed and showed that TBS, BMD and Z-score were parametric with normal distribution. Thus, according to the defined groups, data concerning TBS, BMD and Z-score levels were expressed as mean values ± standard deviation (SD), with 95% confidence intervals, and were also represented using box and whiskers plots. The correlation between TBS with BMD and Z-score was evaluated by using the Pearson's correlation coefficients. Furthermore, for TBS and Z-score, the Chi-squared test with Yates' correction for continuity was used to test the correlation of each classification system with vertebral deformity. To test between group differences in TBS, BMD and Z-score, the *t* test for equal or unequal variance was performed after the equality of variance was tested with the *F* test. Logistic regression models were estimated in order to evaluate the ability of TBS, BMD and Z-score levels to discriminate between groups with computation of odds ratios (OR). The discrimination abilities were evaluated by the Area Under the Receiver Operating Characteristic curve (AUROC) that was used to find the optimal TBS, BMD and Z-score cut-points. The optimal cut-points were determined according to the Youden Index with computation of sensitivity, specificity and diagnostic accuracy for each quantitative parameter. Moreover, AUROC of TBS, BMD and Z-score were compared to each other using

the method proposed by DeLong et al. (22). Additional analyses aimed to control for potential confounding factors (gender, age, platyspondyly, and BMI) were performed by including statistically significant factors at univariate analysis in each multivariate logistic regression model obtained separately for TBS, BMD and Z-score. Considering TBS, cases that had an erroneous group assignment with the use of the optimal cut-off point, were reassigned to one of the two groups based on optimal cut-off point determined for BMD or Z-score in order to evaluate the improvement of accuracy and the relative AUROC by associating TBS and BMD or TBS and Z-score. Furthermore, the Chi-squared test with Yates' correction for continuity was used to test the correlation of platyspondyly with vertebral deformity, TBS, BMD and Z-score. Finally, the inter-rater agreement of SDI between the two readers was assessed using the Cohen's kappa (*k*, range: from 0 [no agreement] to 1 [perfect agreement]). To test the correlation between SDI and TBS, the Spearman's rank correlation and the one-way analysis of variance were performed according to the Newman-Keuls multiple comparisons test after confirming the equality of variance with the Levene test. A *p* value of less than 0.05 was considered indicative of a statistically significant difference. All statistical analyses were performed with MedCalc (release 15.6.1; MedCalc Software bvba, Ostend, Belgium).

Results

We studied 82 patients, aged 15-45 years, median 33 years, (43 males, 39 females; mean age 32.6 ± 6.9 years). Mean height was 159.8 ± 8.2 (range 142-178) cm, mean weight was 57.4 ± 12.3 (range 35-95) Kg, and mean BMI was 22.4 ± 4.2 (range 14.6-41.7) kg/m^2. We found vertebral deformities in 29/82 patients (35.4%). Using the Genant semi-quantitative method, a total of 61 vertebral deformities (or 62, in the opinion of one radiologist) were detected and classified as "mild", 6 vertebral deformities were classified as "moderate" and 2 as "severe".

Ten patients showed the presence of only 1 vertebral deformity, 4 (or 5, depending on the reader) were affected by 2 deformities, 8 (or 9) patients by 3 deformities, 5 by 4 and 1 by 5 deformities. Platyspondyly (vertebrae becoming cranio-caudally flattened and elongated anteriorly), a typical skeletal change conceivably related to iron chelation therapy (23), was present in 33/82 patients (40%). Fifty-five out of 82 patients (67%) showed a DXA Z-score < -2.0.

SDI: relationship with TBS

For SDI, the inter-reader agreement between the two radiologists was excellent with a Cohen's Kappa of 0.975 (95%CI: 0.946-1). In only one out of 82 cases (1.2%) a discordance, with a SDI of 2 for one reader and 3 for the other, was recorded.

Overall, SDI ranged from 0 to 5 with SDI equal to 0 in 53/82 patients (64.6%), 1 in 7/82 (8.5%), 2 in 4/82 (4.9%), 2.5 in 1/82 (1.2%), 3 in 7/82 (8.5%), 4 in 8/82 (9.8%), and 5 in 2/82 (2.4%). By comparing SDI and TBS, the mean TBS of patients with SDI=0 (1.254) was significantly higher than the mean TBS of patients with SDI from 1 to 5 (1.141) ($p<0.001$), whereas no significant differences in mean TBS was found between patients with SDI from 1 to 5 ($p=0.397$) (**Fig. 1**). Nevertheless, when SDI was tested for its correlation with TBS by using the Spearman's rank correlation coefficient, a significant correlation was found because TBS values progressively decreased with the increase of SDI (rho = -0.609; 95%CI: -0.729/-0.452; $p<0.0001$).

Relationship between TBS, BMD and Z-score

TBS showed a significant positive correlation with BMD ($r = 0.544$, $p<0.001$) and Z-score ($r = 0.480$, $p<0.001$) (**Fig. 2**).

Relationship between TBS and vertebral deformities

The mean of TBS ± SD and 95%CI for group A (vertebral deformity) and group B (normal vertebral bodies) is reported in **Table 1**. The TBS differed significantly between groups

(p<0.0001), being higher in patients without vertebral deformities (**Fig. 3a**). The AUROC of TBS in discriminating between groups was 0.856 (95%CI: 0.761-0.924) with an optimal cut-point of 1.214 (Youden Index J=0.617) (**Fig. 4a**). By using this cut-point for group discrimination (diagnosis of vertebral deformities), sensitivity was 86.2% (25/29, 95%CI: 73.4-99.0), specificity was 75.5% (40/53, 95%CI: 63.7-87.3), and diagnostic accuracy was 79.3% (65/82, 95%CI: 70.4-88.2). Logistic regression models for evaluating the ability of TBS in differentiation of groups are reported in **Table 2**. At univariate analysis, the probability of finding vertebral deformities decreased with the increase of TBS (OR *per* 0.001 increase, 0.979; 95%CI: 0.969-0.989; p<0.0001). TBS maintained its significance as an independent predictor of vertebral deformities after including potential confounding factors in the multivariate model (OR *per* 0.001 increase, 0.975; 95%CI: 0.963-0.988; p=0.0002).

Relationship between BMD and vertebral deformities

As reported in **Table 1**, patients in group A had a significantly lower BMD compared to patients in group B (p<0.001) (**Fig. 3b**). The AUROC of BMD in discriminating between groups was 0.719 (95%CI: 0.609-0.813) with an optimal cut-point of 0.796 (Youden Index J=0.356) (**Fig. 4b**). Applying this cut-point, sensitivity was 82.8% (24/29, 95%CI: 68.8-96.8), specificity was 52.8% (28/53, 95%CI: 39.1-66.5), and diagnostic accuracy was 63.4% (52/82, 95%CI: 52.8-74.0). The probability of finding vertebral deformities decreased with the increase of BMD (OR *per* 0.001 increase, 0.992; 95%CI: 0.987-0.997; p=0.002). BMD was also a significant predictor parameter of vertebral deformities after including potential confounding factors in the multivariate model (OR *per* 0.001 increase, 0.990; 95%CI: 0.982-0.997; p=0.004) (**Table 2**).

Relationship between Z-score and vertebral deformities

Similarly to TBS and BMD, the mean Z-score of group A and B was significantly different (p<0.005) (**Table 1, Fig. 3c**). The AUROC of Z-score in differentiating between groups was

0.711 (95%CI: 0.601-0.806) with an optimal cut-point of -2.4 (Youden Index J=0.375) (**Fig. 4c**). Applying this cut-point, sensitivity was 82.8% (24/29, 95%CI: 68.8-96.8), specificity 54.7% (29/53, 95%CI: 41.0-68.4), and diagnostic accuracy 64.6% (53/82, 95%CI: 54.0-72.2). The probability of finding vertebral deformities decreased with the increase of Z-score (OR *per* -0.1 increase, 0.919; 95%CI: 0.869-0.972; p=0.003). However, Z-score did not yield statistical significance as a predictor of vertebral deformities after including potential confounding factors in the multivariate model (OR *per* -0.01 increase, 0.900; 95%CI: 0.735-1.071; p=0.091) (**Table 2**).

Ability of TBS, BMD and Z-score in predicting vertebral deformities: comparison of ROC curves

The difference between the AUROC of TBS and BMD or Z-score was equal to 0.137 and 0.145, respectively, with a statistically significant better performance of TBS in predicting vertebral deformities (p value of 0.024 and 0.026, respectively) (**Fig. 4d**). Moreover, compared to BMD, TBS had a significantly greater specificity (p=0.004) and diagnostic accuracy (p=0.038). Conversely, although sensitivity and accuracy of TBS was higher than that of BMD and Z-score, these differences were not significant (p>0.05).

Improvement of accuracy in predicting vertebral deformities by associating Z-score or BMD to TBS

Seven out of the seventeen patients who had an incorrect group assignment with the use of TBS, were reassigned to the correct group by using the optimal cut-point of Z-score. The diagnostic accuracy improved from 79.3% to 87.8% (72/82), even though this difference was not significant (p=0.179). The AUROC improved from 0.856 to 0.920 (95%CI: 0.839-0.968; p=0.042). Similarly, by using the optimal cut-point of BMD, five out of the seventeen patients had a correct diagnosis. The diagnostic accuracy increased from 79.3% to 85.4% (70/82; p=0.426). The AUROC improved from 0.856 to 0.912 (95%CI: 0.829-0.963; p=0.069).

Relationship between other factors and vertebral deformities

Gender, age, presence of platyspondyly and BMI were included in the logistic model to ascertain their ability in differentiating between groups. In the univariate model, significant factors were age (OR *per* 1-year increase, 1.092; 95%CI: 1.015-1.174; p=0.018), platyspondyly (OR, 6.838; 95%CI: 2.502-18.684; p=0.0001) and BMI (OR *per* 1-Kg/m^2 increase, 1.149; 95%CI: 1.017-1.230; p=0.026), while gender was not significant (OR, 1.045; 95%CI: 0.422-2.587; p=0.924). At multivariate analysis, platyspondyly and BMI maintained statistical significance as predictors of vertebral deformity in all models obtained for TBS (p=0.005 and 0.047, respectively), BMD (p=0.018 and 0.006, respectively) and Z-score (p=0.019 and 0.006, respectively).

Platyspondyly: relationship with TBS, BMD and Z-score

In the study group, 33/82 (40.2%) patients had platyspondyly. The presence of platyspondyly was significantly associated with vertebral deformity. Indeed, platyspondyly was found in 20/29 (68.9%) patients of group A and in 13/53 (24.5%) patients of group B (p=0.0002). Furthermore, TBS, BMD and Z-score were all significantly different between patients with normal vertebral bodies and platyspondyly (**Table 3, Fig. 5**).

Discussion

BMD, easily and accurately measured by DXA, is one of the many determinants of skeletal strength. Other factors, included in the definition of "bone quality", are equally relevant in determining the ability of bone to oppose fractures, but by far harder to be assessed. The microarchitecture of the trabecular bone is an important contributor (24). DXA is the gold standard technique for the evaluation of BMD in the general population and in thalassemic osteopathy (15). However, BMD values considerably overlap in thalassemic patients with and

without fragility fractures (19). Pertinently, measurement of areal bone density does not provide information about bone microarchitecture. Moreover, spinal abnormalities (e.g., platyspondyly) interfere with DXA analysis based on reference scores obtained in subjects with vertebrae of normal shape. Quantitative computed tomography (QCT), by providing a volumetric measure with distinction of cortical and trabecular BMD, is considered a reliable tool for bone strength examination. However, its limited availability, the higher radiation exposure, and the interference in the X-ray attenuation values (due to local iron deposition in not adequately chelated patients) are major limits (19). Bone biopsy and histomorphometric analysis provide an accurate description of the microarchitecture but cannot be employed in daily clinical practice (25).

TBS, a parameter that evaluates pixel gray-level variations in DXA images of the lumbar spine, was developed to meet the need for a simple, easily performed and non-invasive method of assessing bone texture. For spatial resolution and technical reasons (as an example, the pixel size of the available DXA machines is about four times larger than the mean trabecular size), TBS correlates with microarchitectural parameters, although it is not a direct measure of bone microarchitecture and exact skeletal properties assessed by TBS remain poorly understood (26). However, results in large populations showed that TBS, similarly to BMD, could predict fragility fractures with a consequent improvement in diagnostic accuracy by combining both methods (26). To date, TBS has been evaluated in postmenopausal osteoporosis and in various other clinical conditions related to bone fragility such as diabetes, primary hyperparathyroidism, adrenal incidentalomas with subclinical hypercortisolism, rheumatoid arthritis and growth hormone deficiency (17). In 2014, Baldini et al. compared TBS values measured in 124 thalassemic patients with those measured in 65 non-thalassemic osteoporotic controls (19). In that study, TBS was found as a valuable non-invasive tool to assess bone quality, possibly related to fragility fracture risk in thalassemic osteopathy. There

was a significant correlation between TBS and BMD, and between TBS and age. Fragility fractures were found only in a few patients.

In the present study, we have chosen a different approach looking for asymptomatic vertebral deformities by using a lateral X-ray of the spine and a lumbar spine DXA performed within 6 months each from the other. An important result of the study is a higher than expected prevalence of at least one vertebral deformity (35.4%). A recent study reported a 13% prevalence of asymptomatic vertebral fractures in 150 thalassemic patients (27). Their patients, however, were younger than our ones (median age of 15.7 years *vs* mean age of 32.6 years). Moreover, they were affected by different forms of hemoglobinopathies (with only 9% of beta-thalassemia major) and were by far undertreated as regards anemia and iron overload. In a retrospective study (questionnaire interview), 14% of 201 Thai thalassemic patients (68% beta-thalassemia and 32% alpha-thalassemia) reported a spine/back/pelvis fracture (all but one beta-thalassemic) (28). The mean age of patients (35 years) was similar to our series but spinal imaging was performed only when vertebral fractures were suspected (pain, trauma). Since only about one-fourth to one-third of vertebral deformities are symptomatic (29), a lower figure in that study is expected.

In our study, a platyspondyly appearance (harmonic flattening of more vertebral bodies, that has been related to prolonged desferrioxamine chelation in hypertransfused patients) (23, 30) was observed in a high proportion of subjects (40%). It was significantly associated with the presence of vertebral deformities (defined according to Genant's method) and with low levels of TBS, BMD and Z-score. Fragility fractures may share pathogenetic mechanisms that lead also to platyspondyly. Accordingly, it is suggested that this skeletal abnormality is a marker of more severe and/or over-treated disease, with enhanced risk of osteoporosis and bone fragility (31).

Strengths of the present study are the number of patients evaluated, considering that beta-thalassemia is a rare disease, and that all patients were followed-up at a single centre applying uniform transfusion and iron chelation protocols according to guidelines, with a special attention to correct vitamin D and other hormonal deficiencies.

A limit of the present study could be considered its cross-sectional design, which allows for detecting associations and not causality. We paid attention to X-rays and DXA evaluations and did not consider a number of data (e.g., indices of liver function, endocrine and metabolic variables, vitamin D status, ferritin levels, drugs used for iron chelation) that could play a role in the pathogenesis of osteoporosis, microarchitectural impairment, and fragility fractures. The high prevalence of vertebral deformities could be partially due to a selection bias (enrollment of subjects affected by more severe bone damage). However, the indication to perform spine X-rays was not driven by the presence of symptoms, and DXA scans were routinely scheduled as periodic controls. The diagnosis of mild (grade 1) vertebral fractures remains an open question because the Genant semiquantitative (SQ) method could lead to overdiagnosis of this kind of vertebral deformities. The Genant SQ method has not been specifically validated in thalassemia, as in many other diseases known to affect bone metabolism, but is the more frequently employed method for evaluating the prevalence of vertebral fractures. Also Engkakul et al. (27) reported data on the prevalence of vertebral fractures in thalassemic patients using Genant SQ method and our study is the only other paper dealing with the prevalence of asymptomatic vertebral deformities in thalassemia. The blinded and independent evaluation performed by two expert radiologists and their excellent inter-rater agreement provides robust evidence of the real existence of even a mild vertebral "deformity". Moreover, it should be pointed out that the mean TBS of patients with SDI of 0 was significantly higher than that of patients with SDI from 1 to 5. This finding suggests that subgroups of patients with SDI of 0 and 1 are different as to TBS. Furthermore, when SDI was

tested for its correlation with TBS by using the Spearman's rank correlation, a significant correlation was found because TBS values progressively decreased with the increase of SDI. Even if an overestimation of the prevalence of vertebral deformities cannot be fully ruled out, the main purpose of the study was to compare the performance of DXA and TBS, alone or in combination, for predicting their occurrence. In our study, TBS was the best predictor of vertebral deformities; thus, the present data prompt its application in clinical practice because it is widely available, not invasive, cheap and may be obtained simultaneously with lumbar spine DXA evaluation.

In conclusion, vertebral deformities in well-treated patients with beta-thalassemia are still common and often unrecognized. TBS shows the best diagnostic accuracy in predicting vertebral deformities in comparison to BMD and Z-score, although our results need to be validated in independent samples of patients before recommending its use in clinical practice.

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Figure Legends

Fig. 1 Box-and-whiskers plots show TBS values of patients grouped on the basis of SDI score. The graphs report data from the 25th to the 75th percentile (boxes), the median or 50th percentile (horizontal line inside the boxes), and the range from the largest to smallest observed data point within 1.5xIQR of the higher and lower quartile, respectively (vertical lines with whiskers). The values outside this range (SDI=0) are displayed as individual points.

Fig. 2 Graphs show the relationship between TBS and BMD (a), as well as TBS and Z score (b) expressed by the Pearson correlation coefficient.

Fig. 3 Box-and-whiskers plots represent differences in values of TBS (a), BMD (b) and Z score (c) for patients with vertebral deformities compared to patients without vertebral deformities.

Fig. 4 Receiver operating characteristic (ROC) curve analysis for predicting vertebral deformities by using TBS (a), BMD (b), and Z score (c). The areas under the ROC curve are compared for each parameter (d).

Fig. 5 Box-and-whiskers plots demonstrate differences in values of TBS (a), BMD (b) and Z score (c) for patients with platyspondyly and without platyspondyly.

Table 1. Basic demographics and differences in mean values of TBS, BMD and Z score for patients with or without vertebral deformities (group A and B)

Parameter	**Group A (n = 29)**	**Group B (n = 53)**	***p****
Sex (M=male; F=female)	14 M; 15 F	25 M; 28 F	NS^
Age (years)	35.3 ± 5.2 (33.4/37.3)	31.2 ± 7.4 (29.2/33.3)	0.009
Height (cm)	158.9 ± 8.9 (155.5/162.2)	160.3 ± 7.8 (158.1/162.4)	NS
Weight (Kg)	60.5 ± 13.9 (55.2/65.8)	55.7 ± 11.1 (52.6/58.7)	NS
BMI (Kg/m^2)	23.9 ± 5.0 (21.9/25.8)	21.6 ± 3.5 (20.6/22.5)	0.0326**
TBS, L1-L4 (mm^{-1})	1.141 ± 0.083 (1.109/1.173)	1.254 ± 0.072 (1.234/1.274)	< 0.0001
BMD, L1-L4 (g/cm^2)	0.719 ± 0.092 (0.684/0.754)	0.802 ± 0.106 (0.773/0.831)	0.0007
Z-score	-3.010 ± 0.838 (-3.329/-2.692)	-2.306 ± 0.962 (-2.571/-2.041)	0.0014

n = number of subjects.
Data are means ± standard deviations with 95% confidence intervals in parentheses.
* independent samples t-test
^ Chi-squared test
** t-test corrected for unequal variances (Welch test; F-test, p=0.024)

Table 2. Logistic regression models for evaluating abilities of different parameters in differentiating patients with or without vertebral deformities

Parameter	**Univariate model**	**Multivariate model§**
	Odds Ratio	**Odds Ratio**
TBS *per* 0.001 increase	0.979 (0.969/0.989)	0.975 (0.963/0.988)
BMD *per* 0.001 increase	0.992 (0.987/0.997)	0.990 (0.982/0.997)
Z-score *per* -0.1 increase	0.919 (0.869/0.972)	0.900 (0.735/1.071)

Numbers in parentheses are 95% confidence intervals.
§ Multivariate model was obtained separately for TBS, BMD and Z-score by including in each model age, platyspondyly and BMI. Gender was not included in the multivariate model because of its not significance in the univariate model.

Table 3. Differences in mean values of TBS, BMD and Z score for patients with or without platyspondyly			
Parameter	**Platyspondyly**		***p****
	Yes (n = 33)	**No (n = 49)**	
TBS, L1-L4 (mm^{-1})	1.176 ± 0.077 (1.148/1.203)	1.239 ± 0.095 (1.212/1.267)	0.002
BMD, L1-L4 (g/cm^2)	0.723 ± 0.119 (0.687/0.771)	0.802 ± 0.090 (0.776/0.828)	0.002
Z-score	-2.936 ± 1.022 (-3.299/-2.574)	-2.299 ± 0.862 (-2.545/-2.050)	0.003
n = number of subjects. Data are means ± standard deviations with 95% confidence intervals in parentheses. * Chi-square test			

FIGURA 1.

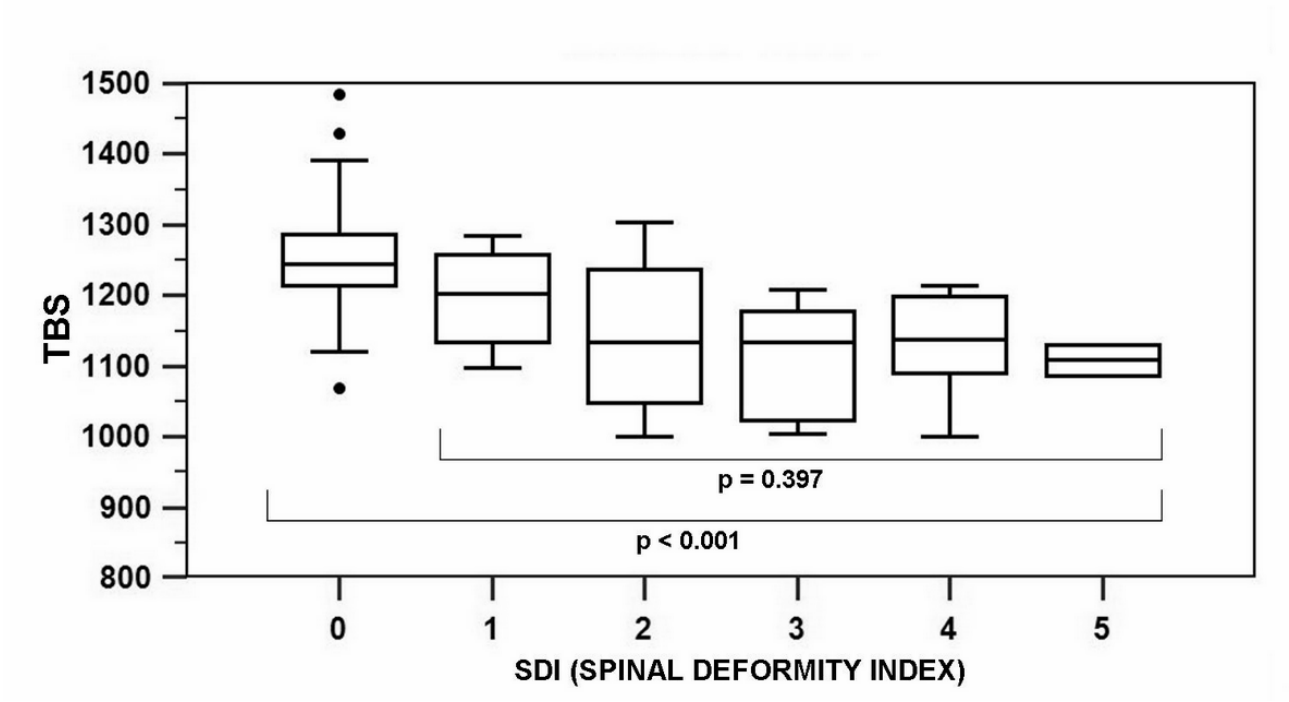

FIGURA 2.

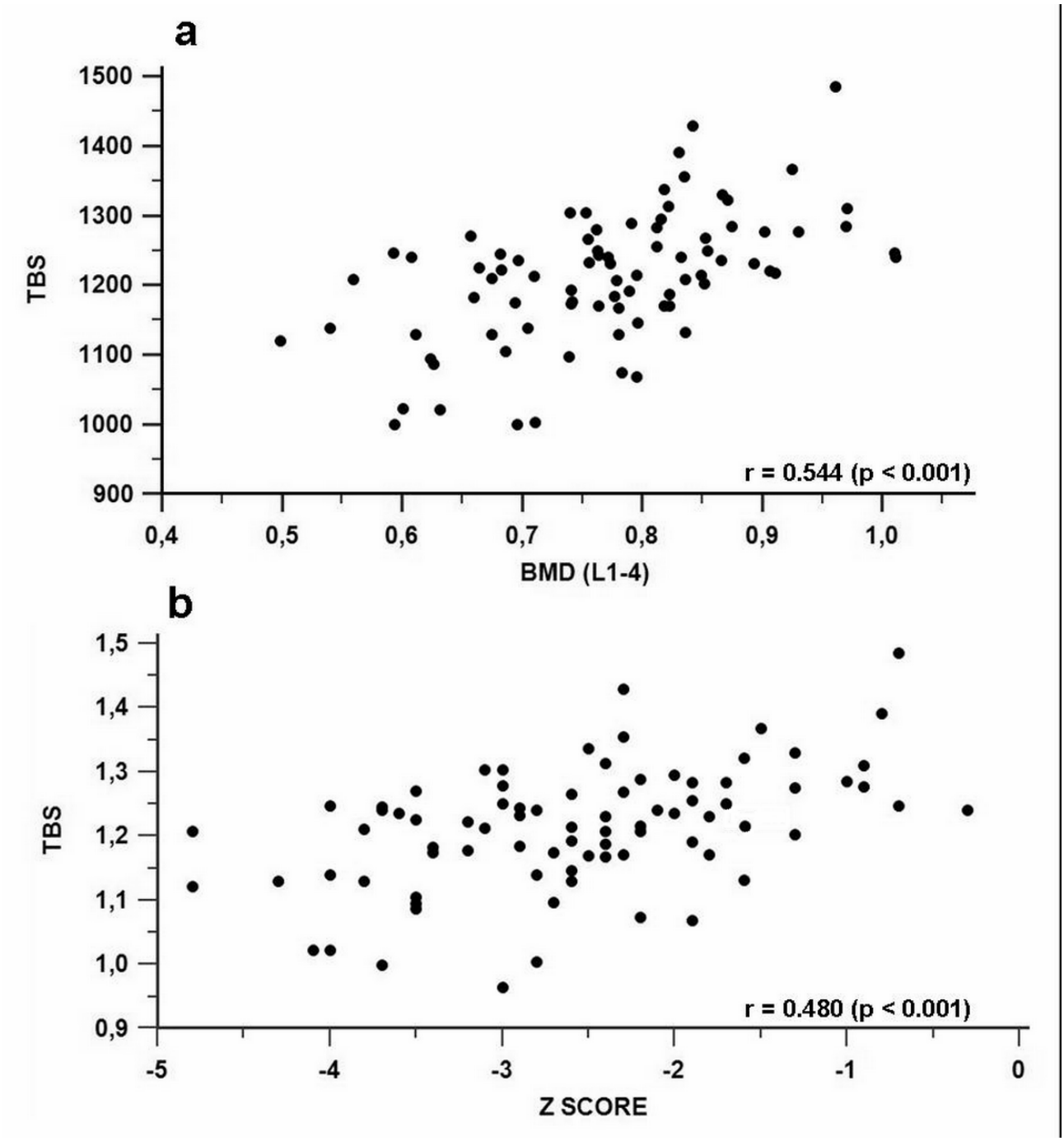

FIGURA 3.

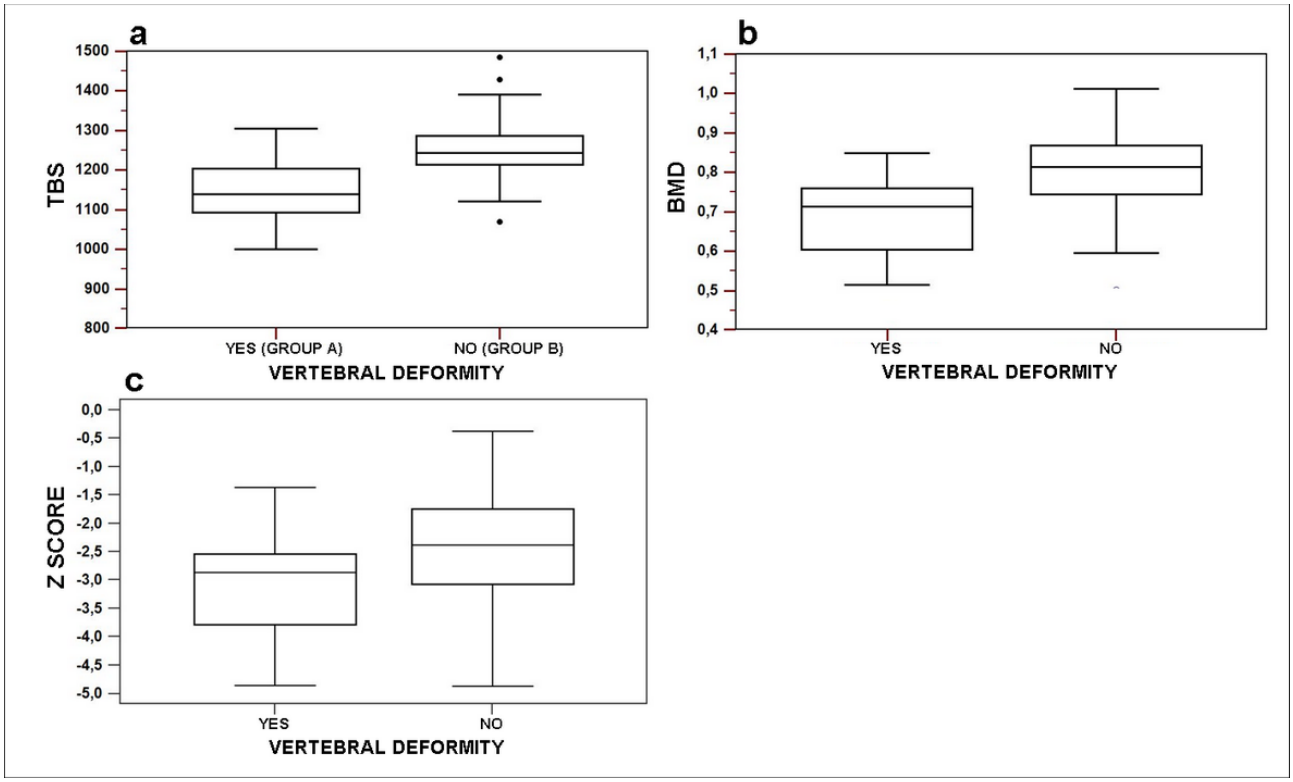

FIGURA 4.

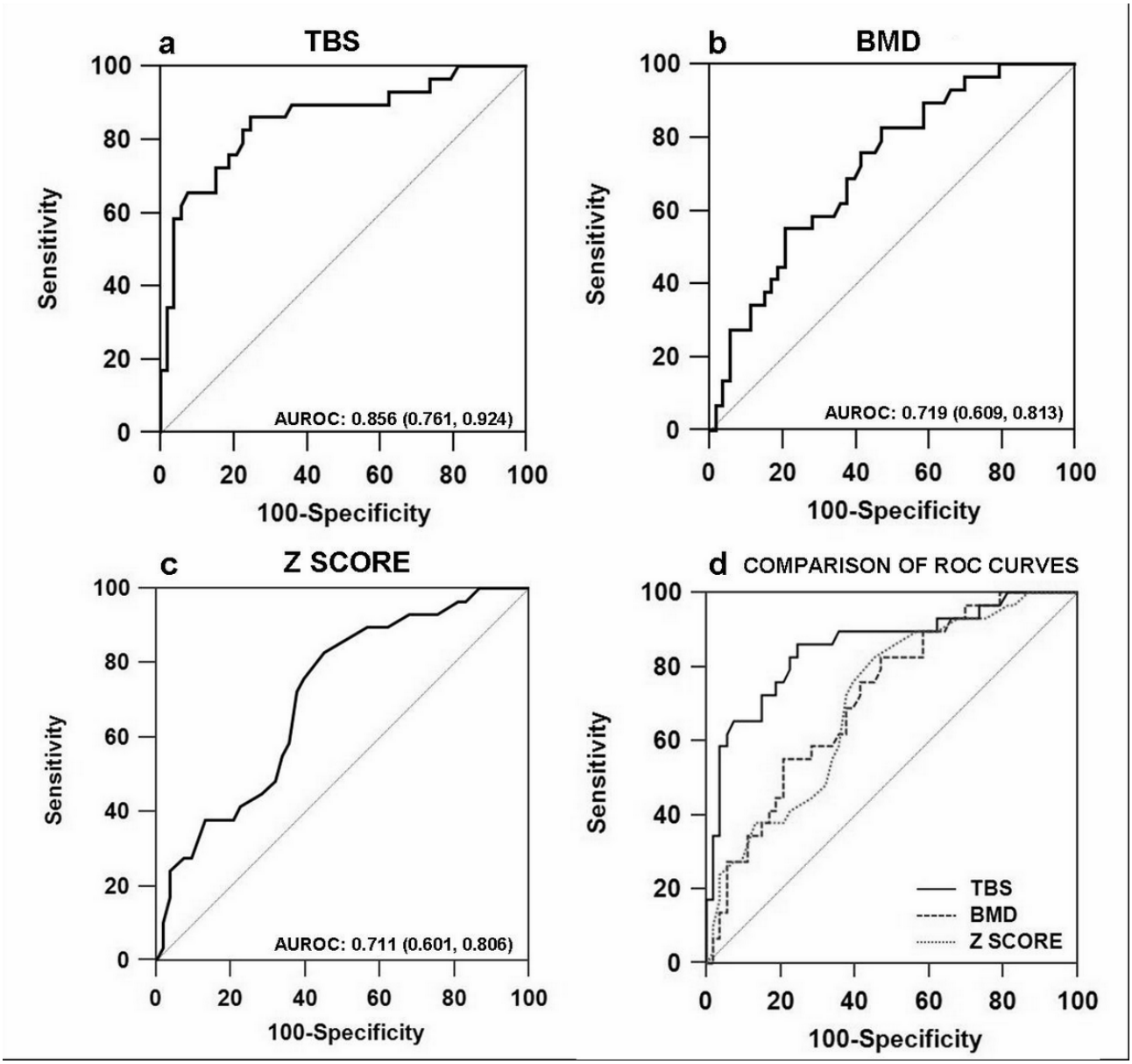

FIGURA 5.