Sickle cell/β-thalassemia: Comparison of Sβ° and Sβ+ Brazilian patients followed at a single institution

Bruno Deltreggia Benites1, Stephany Oliveira Bastos1, Gabriel Baldanzi1, Allan de Oliveira dos Santos2, Celso Dario Ramos2, Fernando Ferreira Costa1, Simone Cristina Olenscki Gilli1, Sara Teresinha Olalla Saad1

1Hematology and Hemotherapy Center, University of Campinas, Brazil, 2Division of Nuclear Medicine, Department of Radiology, University of Campinas, Brazil

Objectives: In sickle cell/β-thalassemia, mutations in the corresponding β-globin genes are responsible for complex pathological events resulting in diverse clinical complications. The objective of this study was to provide an overview of the clinical and laboratory characteristics of patients with the syndrome, and of the degree of severity of clinical manifestations resulting from the β-thalassemia mutation.

Methods: A retrospective chart review was performed on 46 patients with sickle cell/β-thalassemia (31 Sβ° and 15 Sβ+), evaluating hematological parameters and end organ damage. Statistical analyzes were carried out in order to highlight differences between the two groups according to the nature of the thalassemia mutation.

Results: As expected, patients with the Sβ° phenotype had a higher degree of hematological involvement in comparison to Sβ+ patients; with lower hemoglobin levels, and signs of more intense chronic hemolysis. However, Sβ+ patients were more prone to the occurrence of acute chest syndrome. The impact of the thalassemia mutation upon total body and bone composition was also evident, as Sβ° patients presented lower body mass index (BMI) and bone mineral density. The degree of bone damage correlated to lower BMI and hemoglobin levels, as well as plaquetosis, monocytois and elevated lactate dehydrogenase, possibly reflecting the effects of hemolysis and inflammation upon bone metabolism and body constitution.

Conclusions: This study identified significant differences among sickle cell/β-thalassemia patients according to the beta mutation involvement, pointing to an important predictor of disease severity.

Keywords: Sickle cell anemia, β-thalassemia, Clinical features

Introduction

Sickle cell anemia and the thalassemia syndromes correspond to a group of genetic disorders caused by mutations in the β-globin gene resulting in abnormal hemoglobin variants and their clinical consequences. These diseases greatly affect public health, especially in developing countries, where genetic counseling is still precarious and relatively high frequencies of these genes are present among the population. In the specific case of Brazil, the intense miscegenation involving African and Mediterranean European descents contributed to a singular genetic profile of these diseases in the country, with higher heterozygosity rates for the two conditions.

These hemoglobinopathies lead to a wide range of clinical presentations, even among individuals with similar genotypes. Patients can either exhibit a milder clinical course or present a more intense organic involvement with transfusion dependency. Many factors have been studied to explain these variations, such as genetic polymorphisms in the case of sickle cell anemia, and the nature of the beta thalassemia mutation with the corresponding involvement in the production of beta chains, resulting in decreased (β+) or absent (β0) beta-globin synthesis.

In this aspect, the pathophysiology of both sickle cell disease and thalassemia has been extensively studied, as well as the search for therapies to mitigate the pathological manifestations. However, the specific profile of organic impairment of patients heterozygous for sickle cell/β-thalassemia is not yet well known. The disease is classified as HbSβ° thalassemia, with the absence of HbA and a more severe clinical course,
similar to SS disease, and HbSβ⁺ thalassemia, usually associated with 20–30% of HbA and a milder clinical course. The objective of this study was to give an overview of the clinical and laboratory characteristics of patients with the syndrome, as well as the importance of the degree of impairment of the β-globin gene in the clinical manifestations of the disease.

We were able to demonstrate that Sβ⁰ patients have a higher degree of hematological involvement when compared with Sβ⁺ patients, with significantly lower baseline hemoglobin levels, and higher HbF and HbS levels. These patients also have more pronounced changes in their iron profile, indicating a deeper picture of chronic hemolysis. In addition, the degree of involvement of the thalassemia mutation has proved to be a predictor of changes in the total body composition and bone, demonstrated by lower BMI and bone mineral density (BMD).

**Patients and methods**

**Patients and data collection**

A retrospective chart review was performed on 46 patients with sickle cell/β-thalassemia (67.4% Sβ⁺ and 33.6% Sβ⁰), regularly followed at the Hematology and Hemotherapy Center of the University of Campinas, Brazil, from 1998 to 2014. All patients were referred from primary care units or from other hospitals of the city and neighboring towns. The diagnosis was reached by hemoglobin electrophoresis, and confirmed at our center. For 16 of the patients included, the accurate identification of the mutation was conducted by molecular biology studies. Molecular characterization was performed by polymerase chain reaction and Restriction Fragment Length Polymorphism analysis by digestion with restriction enzymes specific for IVS1-110, IVS1-6, and Cd39 mutations. All patients were informed regarding the purpose and procedures of the study, and provided written consent.

Patients were followed during medical visits every 3 months (or shorter periods in the case of acute complications), with evaluation of end organ damage by laboratory tests and imaging. Five of these patients (2 Sβ⁺ and 3 Sβ⁰) were followed every 3 weeks, as part of the regular transfusion program (due to very low hemoglobin levels with end organ impairment or after the occurrence of complications such as stroke). In the case of complications requiring transfusion or regular transfusions, patients received leukoreduced red cell units, matched at least for Rh (RH: 1, 2, 3, 4, and 5) and Kell (KEL1) antigens. Patients were evaluated at steady state disease and during the data collection for this study, we were careful to ensure that the last transfusion had been performed at least 30 days before analyzes were carried out.

The medical records of these 46 patients were reviewed for laboratory and clinical data comprising: age, gender, weight, height, body mass index (BMI = weight (kg)/height² (m²)), WBC counts, hemoglobin levels, reticulocyte counts, mean cell volume (MCV), mean hemoglobin concentration (MCHC), platelet counts, lactate dehydrogenase (LDH) level, hepatic enzymes (aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase (ALP) and Gamma-Glutamyltransferase, GGT), serum ferritin, serum iron, total iron-binding capacity (TIBC), haptoglobin, direct and indirect bilirubin levels, microalbuminuria and glomerular filtration rates estimated by ⁵¹Cr-EDTA clearance.

BMD was measured in grams per centimeter squared (g/cm²) at the femoral neck, entire femur, and lumbar spine (L2–L4; anteroposterior plane) using a Lunar DPX device (DXA; Lunar DPX, Madison, WI, USA). BMD values were also expressed as T-scores, using the World Health Organization criteria standardized to the normal values for young adults: normal: T-score > -1 standard deviation (SD); osteopenia: T-score between -1 and -2.5 SDs; osteoporosis: T-score < -2.5 SDs.

Hospital admissions were recorded for all sickle related organ involvements, especially acute chest syndrome (ACS) which was recorded according to the current criteria: new infiltrate visible on chest radiograph associated with one or more symptoms, such as fever, cough, tachypnea, breathing difficulties, or new hypoxia onset; retinopathy as the presence of dense capillary bed up to the margin of perfusion, with abrupt termination of small or medium caliber vessels and irregular appearance of the border; avascular bone necrosis defined by a history of osteonecrosis of the femoral head or joint replacement; stroke defined by patient reported cerebrovascular disease, some documented by MRI. Episodes of priapism, leg ulcers, and venous thromboembolism (VTE) were also carefully recorded. Laboratory tests were performed as part of a routine evaluation during steady state condition.

**Statistical analysis**

Exploratory data analyses were used to investigate the distribution of variables. Means and SD or median and range were used to summarize data where applicable. The relationships among variables were tested for significance using Spearman’s rank correlation tests and Wilcoxon/Kruskal–Wallis Rank Sum Tests. Results were considered significant at $P < 0.05$. Statistical analysis was performed using R software version 3.1.3 (2015-03-09) Copyright (C) 2015, The R Foundation for Statistical Computing.
Results

Demographic findings and clinical features
Among the studied population, 25 (54%) were women and 21 (46%) were men. The median age was 36 years, with a range of 15–70 years. 31 patients were diagnosed as Sβ0, and 15 as Sβ+. Patient clinical and laboratory characteristics described detailed in Table 1.

Hematopoiesis and hemolysis
We observed great variability in baseline hemoglobin values in our group of patients (minimum 5.7 and maximum 13.9 g/dl). The median values were relatively high compared to those usually observed in SS patients, and significantly higher in patients with the Sβ+ phenotype rather than Sβ0 (9.9 (7.66–13.7) × 8.7 (5.7–13.9), P = 0.005). Sβ0 patients also showed significantly higher levels of HbS and HbF compared to Sβ+ patients (median HbS 79.95% (24.2–93.7) × 68.2% (25.5–82.4), P = 0.016; median HbF 7.6% (1.3–28.9) × 2.9% (0.3–10.2), P = 0.001).

We observed no differences in relation to hemolysis markers (reticulocyte counts, serum LDH, haptoglobin, and indirect bilirubin levels) between Sβ0 and Sβ+ patients. However, despite the fact that serum ferritin levels and TIBC did not differ between the two groups, serum iron levels and transferrin saturation were significantly higher in Sβ0 patients, possibly reflecting a higher degree of mild, however chronic hemolysis.

Regarding other hematological parameters, total and differential white blood cell counts did not differ between the two groups, however a statistically significant greater number of platelets was observed in Sβ0 patients (418 × 10³/µl (98–818) × 174 (74–644), P = 0.021). This occurrence is probably due to greater and earlier occurrence of auto-splenectomy in these patients, reflecting in diminished survival of platelets.

Regarding the need for blood transfusion, five of the patients included in this study are part of a regular transfusion program (3 Sβ0 and 2 Sβ+, the latter included after being affected by stroke). Thirty-seven patients received transfusions throughout their lives due exclusively to the acute complications of the disease (27 Sβ0 and 10 Sβ+) and four patients never required blood transfusions (3 Sβ+ and 1 Sβ0).

Acute and chronic complications
The occurrence of acute and chronic events related to disease in our population is discriminated in Table 2. We observed that strokes occurred in 6.5%, VTE in 4.4%, splenic sequestration in 2.3%, priapism 14.2% of males, and ACS in 32.6%. The rate of strokes in our cohort is similar to those described for SS patients in other studies, approximately 6% as well as the rates of occurrence of ACS, however the rates are two times higher than those observed for patients with hemoglobinopathy SC treated at our institution (3.67% for stroke and 17.43% for ACS). These data point out to the severity of the HbSβ heterozygous phenotype, with similar rates to those of homozygotes.

The occurrence of priapism in our population was quite lower than that reported in the literature for patients with SS disease (over 40%), however, considerably higher than that found in our group of SC patients (6%). Rates similar to those observed for HbSC were observed uniquely in relation to the occurrence of splenic sequestration (2.7%), and SC patients had higher rates of thromboembolism (6.4%).

Except for acute thoracic syndrome, we found no differences in the occurrence of events between Sβ+ and Sβ0 patients. Interestingly, in our cohort, Sβ+ patients were 4.9 times more prone to the occurrence of ACS than Sβ0 patients (P = 0.035). Previously, the occurrence of ACS in SS patients was correlated to higher steady state Hb levels. We could extrapolate that the highest incidence of ACS in Sβ+ patients in our cohort may correlate to higher hemoglobin levels found in this group, possibly corroborating with higher blood viscosity and therefore facilitating the occurrence of this complication.

Hospital admissions for stroke, VTE, splenic sequestration and ACS represented isolated episodes for each patient. The only exception was a single Sβ+ patient who was hospitalized twice for ACS.

In this cohort, 15.2% of the patients had some degree of retinopathy, 15.2% were diagnosed with hip aseptic necrosis, and 17.3% with biliary calculus disease. However, there was no difference in the occurrence of chronic complications between Sβ+ and Sβ0 patients. There were also no differences between the two groups in relation to the studies of renal and hepatic function.

Twenty-two patients (8 Sβ+ and 14 Sβ0) had an indication for hydroxyurea in the attempt to decrease the incidence of vaso-occlusive crises (pain crisis). One Sβ+ patient started using the drug after repetitive episodes of acute thoracic syndrome, and another patient of this group started using the drug after a stroke.

Body constitution and bone density
We observed lower values of BMI in Sβ0 patients when compared with Sβ+ (19.73 (17.75–24.42) × 25.15 (19.53–37.52), P < 0.001). In fact, two of the Sβ0 patients can be classified as malnourished (BMI less than 18.5). Interestingly, 40% of Sβ+ patients were in the overweight or obesity ranges. The degree of β-chain involvement may probably affect energy balance and metabolism of these patients, for various reasons such as basal inflammation secondary to chronic hemolysis and hormonal changes as those known to be characteristic to thalassemia patients (as hypopituitarism and hypogonadism).
Regarding bone constitution, 32% of the HbSβ patients exhibited osteoporosis/osteopenia according to WHO criteria. This overall prevalence is similar to that observed in a previous study conducted in a Greek cohort and much lower than the prevalence observed in a previous study from our institution involving only patients with the SS genotype (approximately 80%). When comparing our group of HbSβ patients in relation to the thalassemia mutation, the values for BMD, expressed in g/cm² and as T-score, were significantly lower in Sβ⁰ patients compared to Sβ⁺; at all three examined sites (lumbar spine, femoral neck, and total femur), as shown in Table 3. When evaluating all patients together, we observed a significant statistical correlation between low BMI values and worse indices of BMD (g/cm² and T-score) in the three examined sites (as shown in Table 4). These findings, taken together, may reflect the same systemic pathophysiological mechanisms leading to impaired energy balance and bone metabolism in these patients.

In addition, we observed that low BMD in the three examined sites was associated with lower hemoglobin levels, as well as higher LDH levels, platelets and monocytes (Table 4). Therefore, we speculated that in patients with Sβ-thalassemia, regardless of the status of the thalassemia mutation, the degree of bone damage may be related to the levels of hemolysis and chronic inflammation, and their possible direct

### Table 1 Patient clinical and laboratory data. Values are expressed in mean ± SD

| Parameters          | Sβ⁺ (n = 15) | Sβ⁰ (n = 31) | P-value |
|---------------------|-------------|-------------|---------|
| Age, y              | 37 (23–70)  | 35 (15–53)  | 0.131   |
| Weight, kg          | 69.4 (50–124.3) | 51 (35.3–64.9) | 0.002   |
| Stature, m          | 1.63 (1.52–1.82) | 1.62 (1.41–1.69) | 0.504   |
| Body mass index (BMI) kg/m² | 25.1 (19.5–37.5) | 19.7 (17.7–24.4) | <0.001   |
| Serum creatinin     | 0.69 (0.48–1.58) | 0.57 (0.32–2.6) | 0.090   |
| Chromium EDTA       | 106.65 (54.6–157) | 105 (81–151) | 0.842   |
| MicroalbuminRest    | 7.77 (2.62–8.16) | 4.96 (1.99–203) | 0.923   |
| Hemoglobin, g/l     | 9.9 (7.6–13.7) | 8.7 (5.7–13.9) | 0.005   |
| Reticulocyte counts, x10⁹/l | 187.5 (66.78–561.1) | 231.3 (67.5–540) | 0.644   |
| MCV II              | 72.1 (65.9–94.1) | 77.4 (59.2–101.9) | 0.234   |
| MCHC pg             | 31.5 (30.5–36.7) | 33.2 (29.9–35.4) | 0.414   |
| Leukocytes count x10⁹/l | 7.4 (3.01–16.01) | 8.48 (3.99–15.07) | 0.826   |
| Neutrophils         | 3.73 (0.84–9.89) | 4.0 (2.03–9.36) | 0.738   |
| Lymphocyte          | 2.0 (0.99–4.73) | 2.86 (0.96–5.97) | 0.347   |
| Monocyte            | 0.32 (0.06–0.84) | 0.45 (0.1–1.51) | 0.431   |
| Eosinophil          | 0.24 (0.01–1.52) | 0.24 (0–2) | 0.700   |
| Platelets count x10⁹/l | 174 (74–644) | 418 (98–818) | 0.021   |
| MPV                 | 7.2 (6.1–13.5) | 7.75 (6–11.6) | 0.045   |
| HbF (%)             | 2.9 (0.3–10.2) | 7.6 (1.3–28.9) | 0.001   |
| Serum ferritin ng/l | 316.1 (22.8–1955) | 415.75 (64.5–6382) | 0.929   |
| Serum iron µg/dl    | 70.5 (39–201) | 114.5 (44–363) | 0.014   |
| TIBC pg/dl          | 273 (214–524) | 230 (172–538) | 0.115   |
| Transferrin saturation (%) | 27.03 (13.13–67) | 47.52 (16.54–95.34) | 0.004   |
| Lactate dehydrogenase, U/l | 469.5 (260–1974) | 612 (328–1159) | 0.063   |
| AST IU/l            | 27 (14–102) | 33.5 (16–104) | (0.475) |
| ALT IU/l            | 23 (12–135) | 22 (8–161) | 0.065   |
| ALP IU/l            | 86 (42–607) | 83 (41–694) | 0.525   |
| GGT IU/l            | 42 (19–257) | 27 (9–316) | 0.074   |
| Conjugated bilirubin mg/dl | 0.66 (0.38–4.4) | 0.60 (0.25–2.9) | 0.826   |
| Unconjugated bilirubin mg/dl | 0.9 (0.73–8.1) | 1.2 (0.7–8) | 0.764   |
| Microalbuminuria (mg/24 hours) | 4.96 (1.99–203) | 7.77 (2.62–816) | 0.923   |
| GFR (ml/kg/1.73 m²) | 106.6 (54.6–157) | 105 (81–151) | 0.842   |
| Genotype (N)        | I9V-110 (4) | Cd39 (9) | n/a     |
|                     | IV51-6 (2) |               |         |
|                     | Cd39 (1)  |               |         |

Hydroxyurea treatment (N) 8 14 n/a

Notes: The bold values indicates P<0.05. MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MPV, mean platelet volume; TIBC, total iron-binding capacity; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; GFR, glomerular filtration rate (estimated by 51Cr-EDTA clearance).

### Table 2 Frequency of acute and chronic complications affecting HbSβ⁺ and HbSβ⁰ patients

| Condition               | HbSβ⁺ patients (%) | HbSβ⁰ patients (%) |
|-------------------------|---------------------|--------------------|
| Retinopathy             | 4 (26.6%)           | 3 (9.6%)           |
| Acute chest syndrome    | 8 (53.3%)           | 6 (19.3%)          |
| Stroke                  | 1 (6.6%)            | 2 (6.4%)           |
| VTE                     | 1 (6.6%)            | 1 (3.2%)           |
| Splenic sequestration   | 0                   | 1 (3.2%)           |
| Priapism                | 0                   | 3 (27.2% of the men) |
| Cholelithiasis          | 7 (46.6%)           | 18 (58%)           |
| Hip aseptic necrosis    | 4 (26.6%)           | 3 (9.6%)           |
Table 3 Comparison of bone mass density (absolute values and T-score) and body mass index between Sβ+ and Sβ0 patients, expressed in mean ± SD

|                      | Sβ+     | Sβ0     | P value |
|----------------------|---------|---------|---------|
| Femoral neck (g/cm²) | 0.98 ± 0.27 | 0.87 ± 0.18 | 0.021  |
| (T-score)            | 0.70 ± 2.07 | −0.1 ± 2.01 | 0.002  |
| Lumbar spine (g/cm²) | 1.19 ± 0.22 | 1.00 ± 0.13 | 0.041  |
| (T-score)            | 0.95 ± 2.26 | −0.8 ± 1.14 | 0.032  |
| Entire femur (g/cm²) | 1.18 ± 0.24 | 0.99 ± 0.18 | 0.017  |
| (T-score)            | 1.20 ± 1.79 | −0.2 ± 1.05 | 0.015  |
| Body mass index (BMI)| 25.1 (19.5–37.5) | 19.7 (17.7–24.4) | <0.001 |

effects on osteoblastic/osteoclastic function. In the lumbar spine and entire femur, low BMD was also associated to higher levels of HbS. This information testifies to the importance of sickle cell mutation in bone involvement, as is well observed in the case of homozygous SS patients and their higher rates of osteoporosis.

Discussion

Silvestrconi and Bianco were the first to describe the compound heterozygosity (S/βthal) for the sickle gene and the β thalassemia gene, and since then, HbS-β thalassemia has been reported in different ethnic groups. Clinical and hematologic features in HbS-β thalassemias are quite variable and clinical severity depends largely upon the nature of the β thalassemia mutations. HbS-β thalassemias are classified as HbS-β0 thalassemia, with the absence of HbA and a severe clinical course similar to SS disease and HbS-β+ thalassemia is usually associated with 20–30% of HbA and a milder clinical course.

In 1973, Serjeant et al. first reported hematological and clinical features of 64 patients diagnosed with sickle cell/β-thalassemia. The study primarily evaluated broader clinical conditions with scarce description of events and complications strictly linked to the pathophysiology of hemoglobinopathies. Despite this fact, the authors were able to highlight the importance of the thalassemia mutation on the manifestations of the disease (at the time, the two groups were called ‘HbA type’ and ‘non HbA type,’ according to the electrophoretic pattern, and exhibited different degrees of severity). Since then, little data has emerged in the medical literature describing the profile of clinical complications of HbSβ patients, and there is even less information comparing the level of clinical impairment of these patients regarding the impact of the thalassemia mutation. This particular group of patients show an intense endothelial activation profile even under steady state, with high concentrations of inflammatory markers and a severe profile of organic impairment, as demonstrated by high rates of pulmonary hypertension and renal dysfunction. However, in general, what is known regarding the biology and clinical manifestations of sickle cell/β-thalassemia up to date comes largely from case reports or studies with limited sample numbers.

A larger study conducted in 2011 correlated clinical and hematological characteristics of 261 HbSβ patients with well identified β-thalassemia mutations, and correlated these mutations to the degree of disease severity. In other studies, specific mutations were shown to result in more unfavorable phenotypes, which could not alternatively be explained by other factors, such as HbF levels. Furthermore, mutations described as associated to a milder profile of complications did not necessarily lead to favorable clinical presentation when in compound heterozygosity with HbS.

This study aimed to draw a profile of hematological parameters and end organ damage in patients with sickle cell/β-thalassemia followed in a single Brazilian institution. We were able to demonstrate that Sβ0 patients have a higher degree of hematological involvement when compared with Sβ+ patients, with significantly lower baseline hemoglobin levels, and higher levels of HbF and HbS. These patients have more pronounced changes in their iron profile, indicating a deeper picture of chronic hemolysis, and significant plaquetosis, probably reflecting earlier and more intense auto-splenectomy. Interestingly, the Sβ+ patients in this cohort were more prone to the occurrence of ACS than Sβ0 patients (P = 0.035). We speculated whether this higher incidence of ACS could be related to higher steady state hemoglobin levels,

Table 4 Relationship between BMD values (T-scores) at the three examined sites and clinical and laboratory parameters (significant at P < 0.05).

|                      | Lumbar spine | Femoral neck | Entire femur |
|----------------------|--------------|--------------|--------------|
| Hemoglobin           | P = 0.002    | P = 0.011    | P = 0.214    |
| Platelets            | P = 0.006    | P = 0.045    | P = 0.310    |
| Monocytes            | P = 0.001    | P = 0.153    | P = 0.326    |
| LDH                  | P = 0.070    | P = 0.021    | P = 0.362    |
| HbS                  | P = 0.001    | P = 0.067    | P = 0.029    |
| BMI                  | P = 0.013    | P = 0.041    | P = 0.016    |

Notes: The bold values indicates P<0.05. LDH, Lactate dehydrogenase; BMI, body mass index.
possibly reflected by higher blood viscosity facilitating the occurrence of this complication. We might also consider the fact that the average BMI was higher in $\beta^+$ patients (40% of patients in this group were in the overweight or obesity range), which could be a risk factor for ACS.

In fact, the degree of involvement of the thalassemia mutation proved to be a predictor of changes in total body and bone composition, demonstrated by lower BMI and BMD in $\beta^+$ patients. Osteopenia and osteoporosis are well-described complications of both sickle cell anemia and thalassemia major. However, the information regarding bone involvement and its pathophysiology in individuals bearing the heterozygote phenotype ($HbS/\beta$-thalassemia) remains scarce. Voskaridou et al. demonstrated that one third of $HbS/\beta$-thalassemia patients had osteopenia/osteoporosis with features of enhanced bone reabsorption. This cohort also presented increased erythropoietic activity and higher levels of osteoprotegerin, the decoy receptor of nuclear factor-Kb ligand (RANKL), a potent enhancer of osteoclastic activity. These skeletal events generate high morbidity and may be worsened by the coexistence of malnutrition in these patients. Individuals with hemoglobinopathies have higher rates of malnutrition and delayed growth for several reasons, which include protein hypermetabolism, increased red cell turnover and cardiac demands.

In fact, the pathogenesis of bone disease in thalassemia is multifactorial, as growth hormone, insulin growth factors and sex steroids which affect bone formation, and these hormones have impaired secretion in thalassemic patients due to pituitary damage secondary to chronic anemia and iron deposition. Therefore, it is reasonable to extrapolate that the differences between the two groups analyzed in this study may reflect the level of involvement generated by $\beta$-thalassemia phenotype, probably differentially compromising the hormonal function in these patients, and thus directly affecting bone metabolism. This same mechanism could also be involved in the differences in body constitution, evidenced by BMI, as these hormones also influence the balance of anabolic functions and tissue metabolism.

A major concern in the analysis of our data was the use of $T$-score comparison (which was primarily validated for postmenopausal women) in such a heterogeneous population, comprising male individuals and young patients. A working group convened by the International Osteoporosis Foundation (IOF) has recently reviewed the diagnosis of osteoporosis in the young population and has proposed to maintain the $T$-score-based definition of the disease for young adults, unless the patient appears to still be growing. Thus, according to this consensus, in the young adults who suffer from a chronic disorder which is known to affect bone metabolism, a $T$-score below $-2.5$ at spine or hip should be considered as diagnostic for osteoporosis. We concluded that this definition was appropriate for our cohort, which consisted of patients older than 15 years and diagnosed with a condition known to affect bone density.

Another point to be considered when we analyze the data from this study is the relatively small number of patients, limiting the possibility of more assertive conclusions regarding the results. However, we must take into consideration that heterozygous states of sickle cell disease are rare, rendering the surveys with large cohorts extremely difficult. In any event, the results reported in this manuscript demonstrate significant differences between patients, and must be taken into consideration when discussing this group of diseases.

Conclusions
The results from this study provide more detailed information regarding the clinical and laboratory characteristics of patients diagnosed with sickle cell/ $\beta$-thalassemia, with considerable differences in clinical outcomes and laboratory findings according to the impact of the thalassemia mutation. Interestingly, in this cohort, $\beta^+$ patients were more prone to the occurrence of ACS. We also demonstrated the impact of the thalassemia mutation upon total body and bone composition, with $\beta^+$ patients presenting with lower BMI and BMD. The degree of bone damage correlated to lower BMI and hematological parameters, possibly reflecting the effects of hemolysis and inflammation upon bone metabolism and body constitution. These differences encourage the reflection regarding the need for future research aiming a more individualized approach on the management of sickle cell disease patients, considering the existing large clinical variability in the field of hemoglobinopathies and the rarity of heterozygous phenotypes.

Disclaimer statement
Contributors Raquel Foglio contributed to the revision of the text in English language and Roberto Zulli contributed to the statistical analysis.

Funding None.

Conflict of interest The authors declare no conflicts of interest.

Ethics approval Ethical approval was not required.

References
1 Old JM. Screening and genetic diagnosis of haemoglobin disorders. Blood Rev. 2003;17(1):43–53.
2 Finotti A, Breda L, Lederer CW, Bianchi N, Zuccato C, Kleanthous M, et al. Recent trends in the gene therapy of $\beta$-thalassemia. J Blood Med. 2015;6:69–85.
3 Colah R, Gorakshakar A, Nadkarni A. Global burden, distribution and prevention of β-thalassemias and hemoglobin E disorders. Expert Rev Hematol. 2010;3(1):103–17.

4 Gage MA, Costa FF, Freitas TC, Bottura C. Clinical, hematological and genetic features of sickle cell-β anemia and sickle cell-beta thalassemia in a Brazilian population. Clin Genet. 1980;18(1):58–64.

5 Thein SL. Genetic association studies in beta-hemoglobinopathies. Hematology Am Soc Hematol Educ Program. 2013;2013:554–61.

6 Lettce G. The search for genetic modifiers of disease severity in the beta-hemoglobinopathies. Cold Spring Harb Perspect Med. 2012;2(10):a015032–a015032.

7 Fertado LS. Genetic polymorphisms in sickle cell disease: implications for clinical diversity and treatment. Expert Rev Hematol. 2010;3(4):443–58.

8 Steinberg MH. Genetic etiologies for phenotypic diversity in sickle cell anemia. Sci World J. 2009;9:46–67.

9 Daniel Y, Hill K, Inusa B, Thein SL, Howard J. Sickle cell β+ thalassemia associated with the 1393 bp deletion can be associated with a severe phenotype. Hemoglobin. 2011;35(4):406–10.

10 Thein SL. Genetic modifiers of beta-thalassemia. Haematologica. 2005;90(5):649–60.

11 Weatherall DJ. Current trends in the diagnosis and management of haemoglobinopathies. Scand J Clin Lab Invest. 2007;67(1):1–2.

12 Powars D, Wilson B, Imbus C, Pegelow C, Allen J. The natural history of stroke in sickle cell disease. Am J Med. 1979;65(3):461–71.

13 Njannshi AK, Mbong EN, Wonkam A, Tzianetra E, Kalotychou V, et al. Adhesion molecules and high-sensitivity C-reactive protein levels in patients with sickle cell beta-thalassemia. Eur J Clin Invest. 2012;42(1):27–33.

14 Voskaridou E, Tsetsos G, Tsoutsias A, Spyropoulo E, Christoulas D, Terpos E. Pulmonary hypertension in patients with sickle cell-β thalassemia: incidence and correlation with serum N-terminal pro-brain natriuretic peptide concentrations. Haematologica. 2007;92(6):738–43.

15 Voskaridou E, Terpos E, Michail S, Hantzi E, Anagnostopoulos A, Margeli A, et al. Early markers of renal dysfunction in patients with sickle cell-β thalassemia. Kidney Int. 2006;69(1):2037–42.

16 Tedla FM, Friedman EA. Multiorgan failure during a sickle cell crisis in sickle cell-β-thalassemia. Am J Kidney Dis. 2003;42(2):e41–e43.

17 Kosturi PR, Kovarik P. Acute splenic sequestration crisis in an adult with sickle-β thalassemia. Ann Hematol. 2006;85(9):633–5.

18 Aslam AF, Aslam AK, Dipillo F. Fatal splenic sequestration crisis with multiorgan failure in an adult woman with sickle cell-β thalassemia. Am J Med Sci. 2005;329(3):141–3.

19 Serjeant GR, Serjeant BE, Fraser RA, Hambleton IR, Higgs DR, Kulozik AE, et al. Hb S-beta-thalassemia: molecular, hematological and clinical comparisons. Hemoglobin. 2011;35(1):1–12.

20 Schnugue M, Wayse JS, Basran RK, Zurbriggen K, Frischknecht H. The Hb S-beta-thalassemia phenotype demonstrates that the IVS-I (–2) (A>C) mutation is a mild β-thalassemia allele. Hemoglobin. 2008;32(3):303–7.

21 Osunkwo I. An update on the recent literature on sickle cell bone disease. Curr Opin Endocrinol Diabetes Obes. 2013;20(6):539–46.

22 Kolozik AE, Bail S, Kar BC, Serjeant BE, Serjeant GE. Sickle cell-beta+ thalassemia in Orissa State, India. Br J Haematol. 1991;77(2):215–20.

23 Mukherjee MB, Nadkarni AH, Gorakshakar AC, Ghosh K, Mohanty D. Clinical, hematologic and molecular variability of sickle cell-beta thalassemia in western India. Indian J Hum Genet. 2010;16(3):154–8.

24 Perseu L, Ristaldi MS, Ditommedietto SP, Testa R, Schillirò G, Pirastu M, et al. The effect of the beta thalassemia mutation on the clinical severity of the sickle beta thalassemia syndrome. Haematologica. 1989;74(4):341–5.

25 Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001;79(8):704–12.

26 Serjeant GR, Ennis JT, Middlemiss H. Sickle cell beta thalassemia in Jamaica. Br J Radiol. 1973;46(551):8.

27 Kanavaki I, Makrythanasis P, Lazaropoulou C, Kattamis A, Tzanetra E, Kalotychou V, et al. Adhesion molecules and high-sensitivity C-reactive protein levels in patients with sickle cell beta-thalassemia. Eur J Clin Invest. 2012;42(1):27–33.

28 Voskaridou E, Tsetsos G, Tsoutsias A, Spyropoulou E, Christoulas D, Terpos E. Pulmonary hypertension in patients with sickle cell-β thalassemia: incidence and correlation with serum N-terminal pro-brain natriuretic peptide concentrations. Haematologica. 2007;92(6):738–43.

29 Voskaridou E, Terpos E, Michail S, Hantzi E, Anagnostopoulos A, Margeli A, et al. Early markers of renal dysfunction in patients with sickle cell-β thalassemia. Kidney Int. 2006;69(1):2037–42.

30 Tedla FM, Friedman EA. Multiorgan failure during a sickle cell crisis in sickle cell-β-thalassemia. Am J Kidney Dis. 2003;42(2):e41–e43.

31 Koudri PR, Kovarik P. Acute splenic sequestration crisis in an adult with sickle-β thalassemia. Ann Hematol. 2006;85(9):633–5.

32 Aslam AF, Aslam AK, Dipillo F. Fatal splenic sequestration crisis with multiorgan failure in an adult woman with sickle cell-β thalassemia. Am J Med Sci. 2005;329(3):141–3.

33 Serjeant GR, Serjeant BE, Fraser RA, Hambleton IR, Higgs DR, Kulozik AE, et al. Hb S-beta-thalassemia: molecular, hematological and clinical comparisons. Hemoglobin. 2011;35(1):1–12.

34 Schnugue M, Wayse JS, Basran RK, Zurbriggen K, Frischknecht H. The Hb S-beta-thalassemia phenotype demonstrates that the IVS-I (–2) (A>C) mutation is a mild β-thalassemia allele. Hemoglobin. 2008;32(3):303–7.

35 Osunkwo I. An update on the recent literature on sickle cell bone disease. Curr Opin Endocrinol Diabetes Obes. 2013;20(6):539–46.

36 De Sanctis V, Soloni AK, Hadziyannis H, Yassin M, Canadan D, Kiliç Y, et al. Osteoporosis in thalassemia major: an update and the I-CEST 2013 recommendations for surveillance and treatment. Pediatr Endocrinol Rev. 2013;11(2):167–80.

37 Hyacinth HI, Adekeye OA, Yilgwan CS. Malnutrition in sickle cell anemia and sickle cell-beta thalassemia. J Hum Nutr Diet. 2006;19(2):125–32.

38 Soliman AT, Yassin M, Sanad A. Sickle cell disease: implications for infection, growth, and maturation. J Soc Behav Health Sci. 2013;7(1). doi:10.5590/JBSHS.2013.07.02.

39 Sharma R, Setha A, Chandrab J, Gohainb S, Kapoorc S, Singhc P, et al. Osteoporosis in young adults: pathophysiology, diagnosis, and management. Osteoporos Int. 2012;23(12):2735–48.