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ORIGINAL ARTICLE

Skeletal status assessed by quantitative ultrasound and dual-energy X-ray absorptiometry in children with inflammatory bowel disease: A 2-year prospective study

Katarzyna Bąk-Drabik[a,*], **Piotr Adamczyk**[b], **Agata Chobot**[c], **Wojciech Pluskiewicz**[d]

[a] *Department of Peadiatrics, Faculty of Medical Sciences in Zabrze, Medical University of Silesia Katowice, Poland*
[b] *Department of Peadiatrics, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*
[c] *Department of Peadiatrics, Institute of Medical Sciences, University of Opole, Poland*
[d] *Chair of Internal Medicine, Diabetology and Nephrology, Metabolic Bone Diseases Unit, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, Katowice, Poland*

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KEYWORDS
Inflammatory bowel disease;
Quantitative ultrasound;
Dual-energy X-ray absorptiometry;
Children

Summary
Purpose: To assess the bone status in children with inflammatory bowel diseases (IBD) using quantitative ultrasound (QUS) measurement and dual-energy X-ray absorptiometry (DXA) at baseline and after two years of adequate treatment of the IBD and bone protection medication.
Methods: Sixteen children (six boys) with IBD, aged 13.4 ± 2.4 years, were examined at baseline and two years later. DXA was used to asses bone mineral density (BMD) and reference data were provided by the device's manufacturer (Hologic Explorer). QUS measurements were performed in patients and controls — 48 healthy children.
Results: Mean Z-scores for TB- and s-BMD were significantly below zero for both, baseline and follow-up (−2.61 ± 0.99 and −2.48 ± 0.88 for TB, and −1.83 ± 1.33 and −1.61 ± 1.19 for s-BMD, respectively), and did not differ significantly, as well as mean Ad-SoS Z-score. The changes in time of TB Z-score and body weight Z-score correlated positively ($r = 0.63$; $P < 0.01$). The QUS results did not differ between patients and controls. There was a negative correlation between the baseline nutritional status and the activity of the disease, as well as of the number of flares before the enrolment and Ad-SoS Z-score.

* Corresponding author.
E-mail address: bak-drabik@wp.pl (K. Bąk-Drabik).

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Conclusions: BMD was found to be lowered both at baseline and follow-up. No further deterioration was observed during 2-year follow-up. Proper treatment, defined as treatment following ECCO Guidelines, may allow to keep a similar trend in the development of bone tissue as in healthy children. The bone properties assessed by QUS method did not differ between patients and controls. QUS at hand phalanges appears not to be proper diagnostic tool in IBD children.

Introduction

The incidence of inflammatory bowel disease (IBD) is increasing, also in the paediatric population [1]. Both, ulcerative colitis (UC) and Crohn's disease (CD), may be accompanied by extraintestinal complications, such as malnutrition and derangements in bone mineralization [2]. In various studies the prevalence of low bone mineral density (BMD) in paediatric IBD patients ranged from 5 to 70% [3—6]. It has been also shown that patients with CD are more likely to have lower BMD than subjects with UC [7]. BMD measurements can be the basis for estimating the bone mass.

The World Health Organization (WHO) defines osteoporosis as a remarkable reduction of BMD along with microarchitectural changes that enhance bone fragility [8]. However, in developmental period, it is recommended to use the terms ''low bone mass'' or ''low bone mineral density'' instead of ''osteoporosis'' or ''osteopenia'', to describe dual-energy X-ray absorptiometry (DXA) results [9]. DXA measurements at lumbar spine (s-BMD) can provide reproducible measures for infants and young children (0—5 years) whereas whole (total) body assessments (TB-BMD) are suitable for examining only children aged 3 years or older [9]. DXA measures the bone in two dimensions providing only estimation of bone density. Thus in growing children BMD is closely related to the large biologic variation in BMD measurements, mainly because of the age related changes in bone geometry [10]. According to the recommendations of the International Society for Clinical Densitometry (ISCD) [9], the assessment of bone status should identify children and adolescents, who may benefit from interventions, and the diagnosis of osteoporosis 5- to 19-years-olds should not be based only on densitometric criteria alone. In case of absence of vertebral compression fractures, it can be diagnosed when low BMD Z-score (≤ 2.0) and significant fracture history (two or more long bone fractures by the age of 10 years or three or more long bone fractures at any age up to 19 years) coexist.

Quantitative ultrasound (QUS) is based on the attenuation of the ultrasound wave, and measures its speed when it passes through the investigated region of interest. This diagnostic tool is especially valuable in the paediatric population because of the lack of the ionizing radiation. It has gained much popularity in recent years for the diagnosis of skeletal changes, but only peripheral skeletal sites (calcaneus, tibia, radius or phalanges) can be examined. The QUS result depends on various bone mechanical properties and the trabecular structural orientation. The velocity of transmission and the amplitude of the ultrasound signal are influenced by the bone tissue, reflecting its architecture, elasticity and density, but the mentioned components are not assessed separately. There is no simple correlation between Ad-SoS and DXA results, because the latter measures only bone density (bone mass). Nevertheless a lot of children with low bone density have lower Ad-SoS results. Even though bone size may affect QUS measurement results, only 6% of the Ad-SoS value assessed at the proximal phalanges of the hand may be related to finger width [10]. By now the usefulness of QUS in detecting skeletal changes in children was confirmed in pediatric patients with type 1 diabetes, end stage renal disease, and celiac disease [11—13]. There are also some studies that demonstrated reduced values of QUS variables in patients with IBD [14,15]. On the other hand there is observation that QUS was not sufficiently sensitive to predict lower BMD in IBD patients [16—18].

The presented study aimed to compare QUS and DXA results in children with IBD at baseline and after two years. Additionally, we aimed to identify factors potentially influencing the results of both bone status measurements.

Material and methods

Subjects

A longitudinal observation was started in the group of 51 patients with IBD, treated in the Public Clinical Hospital in Zabrze, as presented in our previous study [18]. The follow-up after two years was completed by 16 (10 girls and 6 boys) children. The reasons for this significant drop-out were: change of paediatric centre, achieving adulthood or lack of consent for repeated skeletal examination. Patients aged between 6 and 18 years with an established diagnosis of IBD based on the Porto Criteria were eligible for this study [19]. Within the study group there were eight patients with UC and eight with CD. All children were treated according to the ECCO (European Crohn's and Colitis Organization) guidelines. The control group for the QUS examination included 48 children and adolescents matched by age, sex and body size. Thy were selected from previously examined healthy subjects [20]. The characteristics of the study and control group is presented in Tables 1 and 2.

Patients and controls had no history of fractures. If the baseline QUS and DXA measurements revealed any bone derangements, each patient received calcium (800—1200 mg/day). Vitamin D (cholecalciferol), which was used in each patient before the first skeletal assessment, was continued in a dose determined individually depending on the concentration of 25(OH)D.

The study protocol was approved by Ethics Committee of the Medical University of Silesia. The parents or caregivers of study participants gave informed written consent prior to the enrolment into the study.

Table 1 The basic characteristics of the study group and controls.

Value	Patients ($n = 16$) baseline measurement	Controls ($n = 48$) baseline measurement	Patients ($n = 16$) second measurement	Controls ($n = 48$) second measurement
Age (years) ± SD	13.41 ± 2.42	13.41 ± 2.35	15.49 ± 2.42	15.49 ± 2.30
Weight (kg)	40.06 ± 14.75	42.08 ± 11.67	46.71 ± 11.43	47.88 ± 11.9
Weight Z-score	−0.89149.43 ± 15.94	−0.69	−0.83	−0.69
Height (m)	−1,2	152.51 ± 14.8	157.5 ± 13.11	158.3 ± 10.8
Height Z-score		−0.66	−1.02	−0.77

No significant differences between subjects studied and controls were noted for presented parameters.

Table 2 The characteristics of disease activity and treatment in patients studied at baseline and at follow up.

Value	Baseline measurement ($n = 16$)	Follow-up after 2 years ($n = 16$)
Cole's index[a]	92.87 ± 12.97	93.38 ± 14.38
Disease activity		
Mild form of the disease	12 (75%)	13 (81.5%)
Moderate form of the disease	2 (12.5%)	2 (12.5%)
Severe form of the disease	2 (12.5%)	1 (6%)
Number of flares		
1—2 flares	6 (37.5%)	2 (12.5%)
> 3flares	10 (62.5%)	14 (87.5%)
Type of treatment		
Aminosalicylates	6 (37.5%)	2 (12.5%)
Aminosalicylates + Steroids	4 (25%)	2 (12.5%)
Aminosalicylates + Steroids+ Azathioprine	6 (37.5%)	12 (75%)

DXA: dual-energy X-ray absorptiometry; QUS: quantitative ultrasound; s-BMD: lumbar spine bone mineral density; TB-BMD: total body mineral density; Ad-SOS: amplitude-dependent speed of sound; LSC: the least significant change.
[a] No significant difference between baseline and follow-up examination

Methods

Clinical parameters

In each patient following clinical parameters were collected: anthropometric data (weight and height), activity of IBD, methods of treatment, nutritional status and sexual maturity. IBD activity was determined using the respective scales: PUCAI (Pediatric Ulcerative Colitis Activity Index) and PCDAI (Pediatric Crohn's Disease Activity Index) in modification of Ryżko and Woynarowski [21]. Nutritional status was assessed using Cole's index (CI). Patients were divided into four subgroups according to CI:

- > 120% (obesity);
- 110—120% (overweight);
- 90—110% (normal nutritional status);
- < 90% (malnutrition) — classification given by MacLaren [22].

Sexual maturity was assessed using Tanner stages.

Bone densitometry

DXA assessments were performed using the Hologic Explorer (Hologic Inc., Waltham, MA, USA; software version: 13.0:3) device. BMD (g/cm^2) of the lumbar spine (s-BMD) and total body (TB-BMD) was measured. The scans of total body were analyzed by means of the ''auto whole body'' option, that is incorporated in the software of the densitometer. All analyzes were performed by one experienced technician. Results were expressed as Z-scores which were calculated using paediatric gender-specific and age-matched reference data provided by the Hologic Explorer manufacturer. The precision (root-mean-square coefficient of variation (CV%)), for spine measurements was established on the basis of 50 examinations (two scans in 25 subjects), was 1.6%. Due to the ethical reasons the CV% for TB-BMD during this study was not established, but according to the manufacturer the TB-BMD CV% equals 1.8%.

QUS measurement

QUS measurements were performed with the use of the DBM Sonic 1200 device (IGEA, Carpi, Italy). The measurement determines the amplitude-dependent speed of sound (Ad-SoS, m/s) at proximal phalanges of fingers II—V of the dominant hand. The mean of the results obtained for four fingers was taken into account. Speed of sound in bone tissue was calculated considering the first signal with amplitude of 2 mV at the receiving probe. Thus, the measured speed of sound is called amplitude-dependent. All QUS measurements were carried out manually by the same experienced operator. To normalize Ad-SoS for age and gender, a Z-score was calculated. As normative values data from a previously performed, population-based study were used [20]. The CV%

Table 3 Comparison between dual-energy X-ray absorptiometry (DXA) and quantitative ultrasound (QUS) measurements at baseline and at follow up (mean ± SD; range).

Value	Baseline measurement	Follow-up after 2 years
TB-BMD (g/cm^2)	0.77 ± 0.12; (0.56 − 1.03)	0.85 ± 0.12; (0.63 − 1.06)[a]
TB-BMD Z-score	−2.61 ± 0.99; (−4.2 − +0.7)	−2.48 ± 0.88; (−3.7 − −0.7)[c]
s-BMD (g/cm^2)	0.65 ± 0.20; (0.35 − 1.05)	0.75 ± 0.17; (0.5 to 1.05)[a]
s-BMD Z-score	−1.83 ± 1.33; (−4.2 − +0.6)	−1.61 ± 1.19; (−3.9 to +0.5)[c]
Ad-SoS (m/s)	2001.125 ± 66.54 (1906 − 2145)	2037.94 ± 59.17 (2039 to 1913)[b]
Ad-SoS Z-score	−0.08 ± 1.26 (−2.6 − +1.96)	0.24 ± 0.83 (−1.5 − +2.1)[c]

DXA: dual-energy X-ray absorptiometry; QUS: quantitative ultrasound; s-BMD: lumbar spine bone mineral density; TB-BMD: total body mineral density; Ad-SOS: amplitude-dependent speed of sound; LSC: the least significant change.
[a] Significantly higher than at baseline examination; $P < 0.0001$.
[b] Significantly higher than at baseline examination; $P < 0.05$.
[c] No significant difference.

for QUS results equalled 0.64% and was previously established by 5 serial measurements [20].

Statistics

Statistical analysis was performed, using the Statistica software (StatSoft, Tulsa, OK, USA). Descriptive statistics for continuous variables were presented as mean values and standard deviations. The normality of data distribution was checked by the Shapiro-Wilk test. For comparative analysis (comparisons between study groups) the Student's *t*-test for independent samples or the Mann-Whitney U-test (in the case of data that had or did not have a normal distribution, respectively) were employed. The differences between measurements obtained at baseline and follow-up (longitudinal comparisons) were assessed by the t-test for dependent samples or the Wilcoxon signed rank test, whichever was appropriate according to the data distribution. Correlation analysis was done by Pearson's or Spearman's correlation tests, whichever was appropriate according to data character (continuous or categorized) and normality of distribution. Analysis of covariance (ANCOVA), with age as a covariate was also applied. To effectively follow individual changes in skeletal measurements (BMD and Ad-SoS), the least significant change (LSC) for each of applied diagnostic method was calculated. The LSC, or critical difference, denotes the minimum difference between two consecutive results in an individual that can be considered to reflect a real change. The LSC was calculated according to the following formula: root-mean-square-coefficient of variation × 2.77, which represents a statistically significant difference at the 95% confidence level. Significance for results of all statistical analyses was assumed at $P < 0.05$.

Results

DXA measurements.

Mean values of s-BMD and TB-BMD expressed as Z-scores were significantly below zero, both at baseline and follow-up (Table 3), which indirectly indicates that they were lower than expected in healthy subjects. Taking into consideration TB-BMD Z-scores, decreased BMD (Z-score < −1.0) was revealed in 87% of patients at baseline as well as at follow-up. TB-BMD Z-scores of ≤ -2.0 were found respectively in 75% and 81% patients. Low s-BMD (Z-score < −1.0) was present in 68% of cases at both time points, and values ≤ -2.0 were revealed in 56% of patients at baseline and 43% at follow-up. We noted no significant differences between children with UC and CD (data not shown).

Mean absolute s-BMD and TB-BMD values at the follow-up examination were significantly higher in comparison to the baseline results. However, when respective Z-scores were calculated based on these absolute numbers, there was no significant difference between the baseline and follow-up measurements, neither for s-BMD nor for TB-BMD. Details are given in Table 3.

QUS measurements.

Mean Ad-SoS values and Ad-SoS Z-scores did not differ between IBD subjects and healthy controls, neither at baseline (2001 ± 66 vs. 2004 ± 67 and −0.08 ± 1.26 vs. −0.01 ± 0.93, respectively) nor at follow-up (2038 ± 59 vs. 2059 ± 82 and 0.24 ± 0.83 vs. 0.19 ± 0.85, respectively). There was also no difference in Ad-SoS and Ad-SoS Z-score between children with UC and CD (data not shown). Longitudinal comparisons revealed a significant increase of the Ad-SoS value at follow-up, whereas the mean Ad-SoS Z-score did not change significantly (Table 3).

Regarding anthropometric parameters, Ad-SoS results correlated significantly at baseline with weight ($r = 0.54$, $P < 0.05$) and height ($r = 0.5$, $P < 0.05$). The results measured at follow-up correlated significantly only with weight ($r = 0.5$, $P < 0.05$).

Individual changes in DXA and QUS measurements

To follow individual changes of the assessed DXA and QUS parameters, the differences between the results from the two time points were calculated separately for each study participant. Those individual changes were related to the LSC values, that were calculated according to the formula provided in the 'Statistics' section, and based on the CV% values given in the 'Methods' section. The average LSC values obtained in our study cohort for s-BMD, TB-BMD

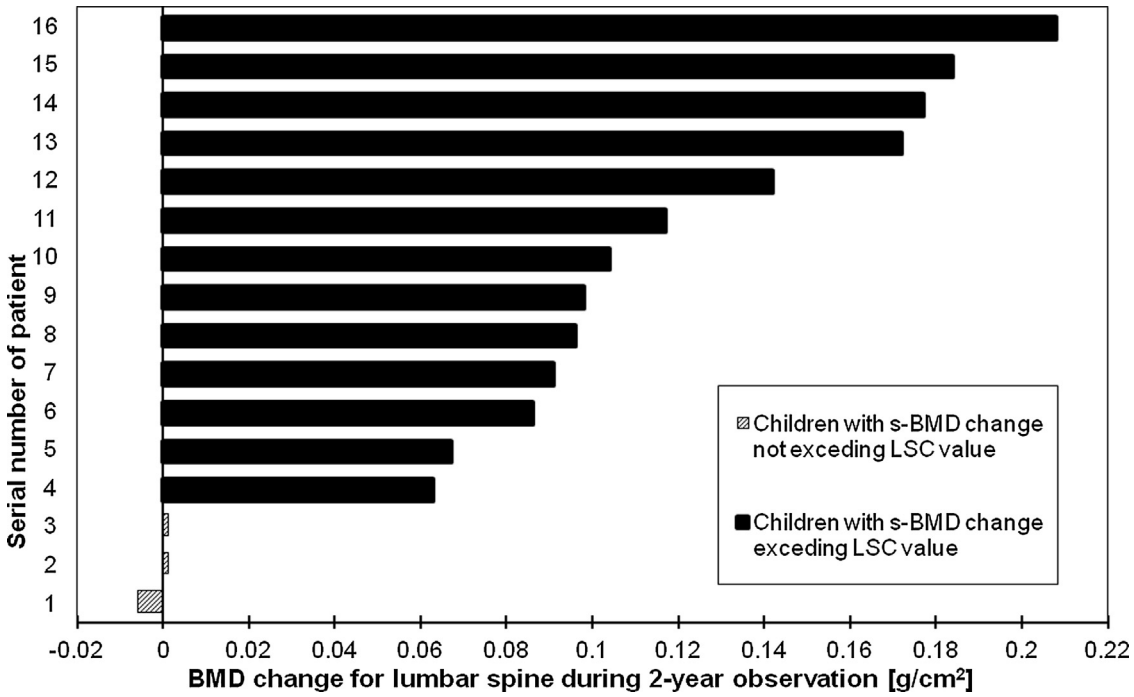

Figure 1 Individual changes in s-BMD value over a period of observation. DXA: dual-energy X-ray absorptiometry; QUS: quantitative ultrasound; s-BMD: lumbar spine bone mineral density; TB-BMD: total body mineral density; Ad-SOS: amplitude-dependent speed of sound; LSC: the least significant change.

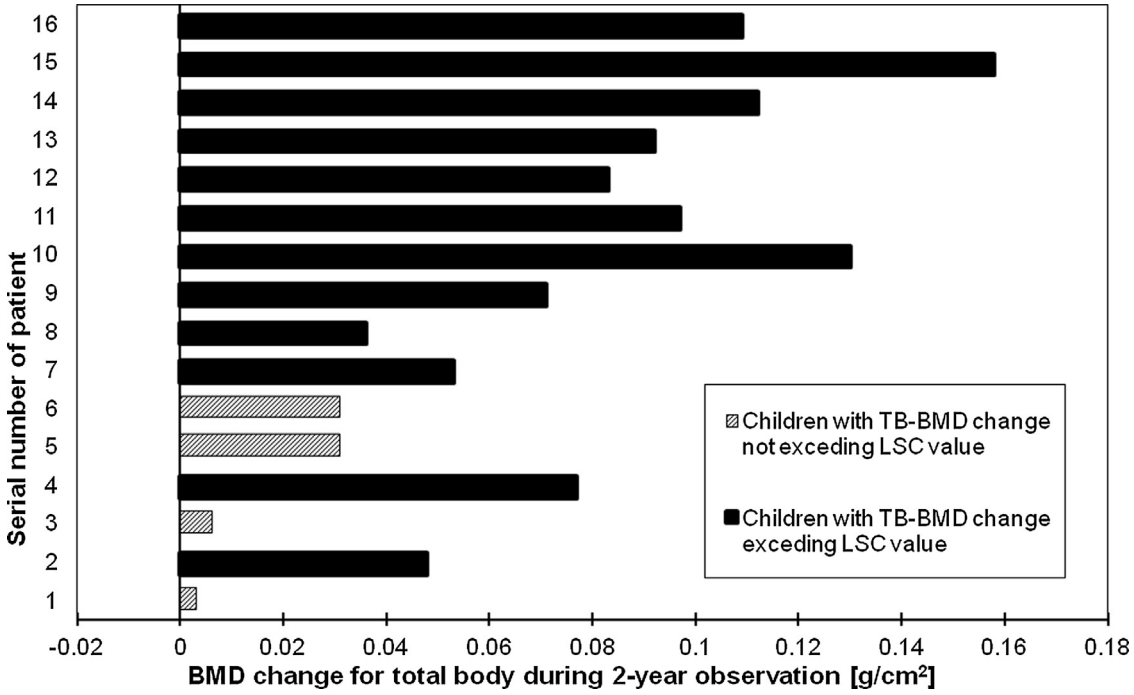

Figure 2 Individual changes in TB-BMD value over a period of observation. DXA: dual-energy X-ray absorptiometry; QUS: quantitative ultrasound; s-BMD: lumbar spine bone mineral density; TB-BMD: total body mineral density; Ad-SOS: amplitude-dependent speed of sound; LSC: the least significant change.

and Ad-SoS were 0.029 g/cm^2, 0.038 g/cm^2 and 35.4 m/s, respectively. A difference between two successive measurements lower than the LSC value may be interpreted as a lack of a real change in the analyzed parameter (regarding the precision of applied methodology of measurement). Taking into consideration the LSC threshold, in 13 of 16 patients the increase in s-BMD exceeded the LSC value. In the remaining 3 subjects the change was lower than the LSC, that may

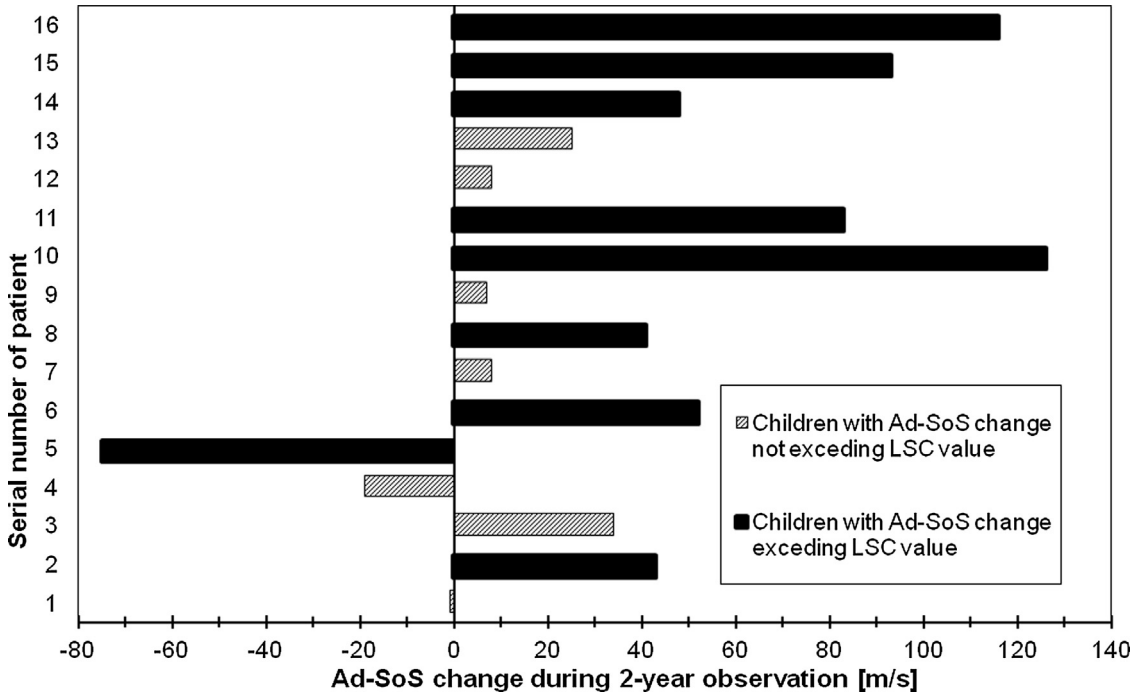

Figure 3 Individual changes in Ad-SoS value over a period of observation. DXA: dual-energy X-ray absorptiometry; QUS: quantitative ultrasound; s-BMD: lumbar spine bone mineral density; TB-BMD: total body mineral density; Ad-SOS: amplitude-dependent speed of sound; LSC: the least significant change.

suggest that s-BMD neither increased nor decreased. The data are presented in Fig. 1. In case of TB-BMD, in 12 children the increase exceeded the LSC value, and in 4 subjects the change was below the LSC threshold. These results are presented in Fig. 2. Individual changes of Ad-SoS in the studied group referred to the LSC are shown in Fig. 3. In only 8 patients the increase in Ad-SoS values was greater than the LSC. In 7 children with IBD the Ad-SoS change was lower than the LSC value (suggesting no real change), whereas one patient presented with the decrease exceeding the LSC. In Figs. 1—3, each study participant is marked with an individual 'serial number of the patient', which allows additionally to compare the trends for the applied diagnostic methods not only within the cohort, but also in each patient separately. This manner of presentation of this longitudinal observation allowed a qualitative assessment: in most of patients with IBD there was a real increase of BMD, whereas the fluctuations of Ad-SoS results were less coherent and rather not concordant with BMD changes.

Correlation between DXA and QUS measurements

There was a strong, significant correlation between results obtained in both DXA modalities, noticeable not only for values directly measured (BMD), but also for the calculated Z-scores. The coefficient of correlation for BMD (s-BMD vs. TB-BMD) at baseline and follow-up were 0.93 ($P < 0.0001$) and 0.90 ($P < 0.0001$), respectively. For the Z-scores (s-BMD Z-score vs. TB-BMD Z-score) the coefficients were 0.89 ($P < 0.0001$) and 0.77 ($P < 0.0001$) at respective time points. Ad-SoS values correlated significantly with BMD results measured in both DXA variations and at both times of assessment. However the analysis of correlation between results presented as Z-scores revealed no interdependence between QUS and DXA methods. Detailed results are presented in Table 4 (baseline and follow-up examination). This suggests a lack of satisfactory conformity between QUS and DXA diagnostic tools in the studied patients.

Other analyses

We found no correlation between the CI and the DXA or QUS measurement results at both times of assessment. The number of flares of the IBD was also not relevant in respect to the nutritional status. Noteworthy the nutritional status differed significantly depending on the activity of the disease, but only at the baseline measurement ($r = -0.5$; $P < 0.05$).

The number of flares was negatively related to the Ad-SoS Z-score ($r = -0.7$ $P < 0.001$) at baseline. There was no association between the number of flares and DXA or QUS results at follow-up.

Spearman rank correlation revealed a significant relationship between Tanner stages and most of the applied skeletal measurements: s-BMD ($r = 0.61$; $P < 0.05$) and TB-BMD ($r = 0.65$; $P < 0.01$) at baseline, as well as Ad-SoS ($r = 0.56$; $P < 0.05$), s-BMD ($r = 0.61$; $P < 0.05$) and TB-BMD ($r = 0.63$; $P < 0.01$) at the second measurement. However ANCOVA with age as a covariate showed no associations between DXA or QUS results and Tanner stages (data not shown).

Table 4 Correlation between quantitative ultrasound (QUS) and dual-energy X-ray absorptiometry (DXA) results at baseline measurement and after 2 years observation.

All patients ($n = 16$)	s-BMD (g/cm^2) baseline	s-BMD Z-score follow-up	TB-BMD (g/cm^2) baseline	TB-BMD Z-score baseline	s-BMD (g/cm^2) follow-up	s-BMD Z-score follow-up	TB-BMD (g/cm^2) follow-up	TB-BMD Z-score follow-up
Ad-SoS (m/s)	$r = 0.71$ $P < 0.01$		$r = 0.73$ $P < 0.01$		$r = 0.71$ $P < 0.01$		$r = 0.82$ $P < 0.001$	
Ad-SoS Z-score		$r = 0.29$		$r = 0.38$		$r = 0.30$		$r = 0.16$

DXA: dual-energy X-ray absorptiometry; QUS: quantitative ultrasound; s-BMD: lumbar spine bone mineral density; TB-BMD: total body mineral density; Ad-SOS: amplitude-dependent speed of sound; LSC: the least significant change.

Discussion

Mean Ad-SoS in the patients with IBD differed significantly between the baseline and follow-up assessments and correlated significantly at baseline with weight and height. Ad-SoS results measured after 2 years significantly correlated only with weight and Tanner stage, but the last association became insignificant after age-adjustment. The study by Baroncelli at al. [23] in healthy children revealed that age, weight, BMI, and pubertal stage were independent predictors of Ad-SoS in males, age and pubertal stage were independent predictors of Ad-SoS in females. Other authors also reported positive correlations between Ad-SoS and anthropometric variables, such as weight and height, in healthy children [24,25].

In regard to the DXA results we have similar observations as presented in other studies. s-BMD and TB-BMD differed significantly at baseline and at follow up. TB-BMD correlated with weight, height and Tanner stage at both assessments. s-BMD correlated with weight and Tanner stage at baseline study and with weight, height and Tanner stage two years later. Yilmaz et al. [26] evaluated 174 healthy pubertal children stated that weight was significantly associated with BMD in both genders. The BMD values increased also significantly until Tanner stage IV in girls. As well in boys, the BMD values increased during puberty, but it was significantly higher in stage IV compared with that in earlier pubertal stages. A study carried out among Korean children aged 2—18 years showed that height, body weight, fat content, body mass index and Tanner stage had a significant impact on BMC (bone mineral content) and BMD measured at different sites [27].

Our observation of the group of IBD patients showed a similar trend as in the group of healthy controls. This suggests that in children with IBD who received optimal treatment, the development of the skeleton proceeded with no progressive deterioration. In addition the longitudinal observation of individual subjects (Figs. 1, 2 and 3) showed also an improvement in the skeletal status over the observation period. Childhood and adolescence are important phases for the development of peak bone mass, as it is the time when there is a gradual increase in bone tissue. Several studies showed that BMD progressively increases between maturational stages in both genders in the different sites analysed, such as the lumbar spine, femur, and whole body [28—31].

The Z-scores of the DXA and QUS results did not correlate with each other, although in our previous study Ad-SoS Z-score was significantly related to TB-BMD Z-score [18]. This discrepancy is not surprising, because Ad-SoS does not always correlate with BMD. These parameters describe different components of the bone structure. BMD is a very specific measure of the consolidated mineral matrix of the bone, and requires several months to show a detectable change. On the other hand Ad-SOS assessed at the same anatomical region varies according to the behavior of the nonmineralized matrix, which in children predominantly reflects the organic matrix bone accretion. The timing of both processes is asynchronous and asymmetric.

There were few studies carried out that compared QUS and DXA in healthy children. The results of both methods were similar when comparing the maturational stages between individuals of the same gender. BMD progressively increased between maturational stages in both genders in the different sites analysed, such as the lumbar spine, femur, and whole body [28—30] corroborating the results of the QUS. Pubertal development is one of the factors that influence bone mass, and is positively associated with age, weight, height, and BMI, what confirms that the development and increase of bone mass are directly associated with maturation and growth aspects. In a systematic literature review [30], QUS of phalanges was described as a good method to evaluate the progressive acquisition of bone mass during growth and maturation of children by monitoring alterations that occur with increasing age and pubertal stage. Contrary to healthy children, studies assessing the usefulness of QUS in IBD patients provided different findings. In children ''at risk'' of low bone mass QUS measurements were not able to recognize patients with low bone mass. Reproducibility of the QUS results was also moderate as well a correlation between QUS and DXA [17]. In another study DXA revealed low bone mass in 50% of children with CD whereas QUS detected only 19.2% of those patients, what suggests that QUS measurement may not be sensitive enough to pick up low BMD in paediatric patients with CD. Also other authors, including our previous study, confirmed this observation [18,31,32]. Nevertheless some investigations demonstrated reduced values of QUS variables in patients with IBD [14,15].

More data concerning the application of QUS is available for diseases other than IBD. A recent study in adult patients with osteoporotic fractures revealed that QUS

measurements of calcaneus were valuable for evaluating the bone metabolism activity and the process of bone turnover – bone matrix deposition and degradation [33]. QUS provided also reliable information in patients with chronic kidney disease (CKD) [11,34,35]. A possible explanation of the fact that we did not observe changes in QUS of hand phalanges in IBD patients may be the different pathomechanism of skeletal disturbances in these two clinical conditions. In the course of CKD there levels of the parathyroid hormone (PTH) are increased, and cortical bone, which is mostly represented in the bone of phalanges, is very sensitive to hyperparathyroidism. Contrary, in IBD there are no features of PTH-related disorders, but the bone tissue derangements result rather from chronic inflammation, malnutrition or steroid treatment. The negative influence of steroids is expected to be more pronounced at trabecular bone, whereas QUS measured at hand phalanges provides the assessment mainly of cortical bone. Such an explanation is also concordant with the results of our previous study, which revealed a significant correlation between Z-score TB-BMD (also dependent mostly on cortical bone status) and Z-score Ad-SoS [18]. However in the current investigation such an association could not be confirmed. Z-score s-BMD, reflecting mainly mineralization of steroid-sensitive, trabecular bone, did not correlate with QUS results. Observations of patients with celiac disease revealed changes in QUS measurements, contrary to DXA, in patients with gluten containing diet. The authors pointed out that QUS methods do not measure identical properties of bone tissue as DXA, and that QUS might be sufficient to recognize additional bone abnormalities (not only related to the bone mineralization but also to some qualitative features of bone microarchitecture) in comparison to DXA [13]. A similar observation was published regarding patients with type 1 diabetes [12].

The findings of this study suggest that QUS seems not to be an appropriate method for the assessment of bone changes in paediatric patients with IBD, because it was insufficient to detect skeletal impairment revealed by the DXA examination. Probably QUS should be considered as a complementary diagnostic tool in clinical practice.

We described decreased DXA results in patients with IBD. s-BMD and TB-BMD were lower than in the healthy subjects, which is expressed by strongly negative average values of Z-scores. We found a very high percentage of subjects with bone mineral derangements 75% and 81% with Z-score ≤ -2.0 for TB-BMD, respectively at baseline and second measurement, and only slightly lower indices for s-BMD results (56% and 43% with Z-score ≤ -2.0 at baseline and second measurement, respectively). Other studies confirmed this observation, but found lower prevalence of bone derangements. In a polish study of 42 young patients with IBD bone mineral alterations occurred in 57% of cases, and they were classified as osteoporosis and osteopenia with equal frequency of 28.6%. No difference in bone health was found between UC and CD [13]. Moreover a longitudinal study carried out in a large paediatric group with IBD showed that s-BMD Z-score was significantly lower in comparison to healthy counterparts [5]. s-BMD Z-score <-1.0 was found in almost 50% and ≤ -2.0 was detected in 25% patients. There were also no differences between IBD forms. Krzesiek et al. revealed significantly lower bone density in children with IBD, with higher frequency of that complication in CD than in UC (46.2% vs. 25.9%) [4]. Laakso et al. also confirmed that IBD patients had lower s-BMD and areal TB-BMD Z- scores, even after adjustment of the DXA results for bone age. No difference between CD and UC patients were showed [36]. Contrary Nobile at al. proved that patients with CD had a higher prevalence of low spine BMD compared with UC patients (15.9 vs. 3.4%), The prevalence of low BMD was 12.5% (spine BMD) and 27% (total-body BMD) [37].

In our study patients and controls had no history of fractures. Studies examining whether the risk of fractures is increased in pediatric patients with IBD have shown contradictory results. Persad et al. found no statistically significant difference in the prevalence of fracture in 132 children with IBD [38]. In another study involving 733 children with CD, 488 with UC, and 3287 controls, IBD was not associated with a higher risk of fracture at any or multiple sites in children older than 12 years. Also steroid exposure was not associated with the occurrence of fractures [39]. Nevertheless Nobile et al. revealed that patients with a positive history of fractures had lower Z-scores for spine BMD (-1.20 vs. -0.69, $P = 0.020$) and total-body BMD (-1.30 vs. -0.75, $P = 0.014$) compared with those without a no fractures in the past. The prevalence of lifetime fractures was 11.8% [37].

Nutritional deficiencies are very frequent in IBD patients and nutritional therapy is often necessary [2]. We found no correlation between nutritional status and bone status measurements, contrary to our previous observation [18]. Krzesiek et al. revealed that only 16.3% normally nourished subjects had bone mineral disturbances, while all severely malnourished children had low bone mineral density [4]. Unexpectedly in our study at baseline and follow-up measurements there was a high percentage (50%) of normally nourished or overweight patients. During our current follow-up study most of the patients were in remission or had mild activity of the disease. Nutritional status correlated with disease activity at baseline and second study. Malnutrition was present mostly in children with moderate and severe IBD. The results are concordant with findings of other authors [4].

One of the limitations of this study is the small sample size, and, in consequence, small subgroups. Furthermore we did not estimate the Tanner stage in the control group. s-BMD and TB-BMD were not adjusted for height. The DXA measurements for total body were analyzed with the ''auto whole body'' option, provided in the software of the used densitometer, whereas the ''total body less head'' analysis is currently recommended by the ISCD Official positions as a preferable option for clinical purposes. Worth emphasizing are the strengths of the study: the homogenous group of patients treated for IBD in the same centre during this two-year observation period, and a control group of randomly selected children matched by sex and age, who were representative for the population of young people.

Concluding, in the studied group BMD was found to be lowered in comparison to normative data both at baseline and follow-up, although no further deterioration was observed during 2-year follow-up period. It seems, therefore, that proper therapy according to the ECCO guidelines and monitoring of IBD children may allow to keep a similar trend in the development of bone tissue as in healthy children. The bone properties assessed by QUS method did not differ between patients and controls, despite the reduced

BMD values. This may suggest that QUS at hand phalanges appears not to be proper diagnostic tool for detection of bone alterations in IBD children. QUS measurements are probably insufficient to identify bone alterations in that specific group of patients. Nutritional status seems to have an important impact on bone status.

Disclosure of interest

The authors declare that they have no competing interest.

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