ACTA ABP BIOCHIMICA POLONICA

Epub: No 2018_2561
Vol. 65, 2018
https://doi.org/10.18388/abp.2018_2561

Regular paper

Is it possible to predict a risk of osteoporosis in patients with juvenile idiopathic arthritis? A study of serum levels of bone turnover markers

Marta Janicka-Szczepaniak[1], Krzysztof Orczyk[1,2], Katarzyna Szymbor[2], Danuta Chlebna-Sokół[3] and Elzbieta Smolewska[1,2]

[1]Department of Pediatric Cardiology and Rheumatology, Medical University of Lodz, Łódź, Poland; [2]Department of Pediatric Rheumatology, Medical University of Lodz, Łódź, Poland; [3]Department of Pediatric Propedeutics and Bone Metabolism Diseases, Medical University of Lodz, Łódź, Poland

Background: Low bone mineral density is a common finding in children with systemic connective tissue diseases, including juvenile idiopathic arthritis (JIA). The influence of the ongoing process of bone remodeling on the disease course merits further investigation. The aim of this study was to assess the clinical relevance of markers of bone turnover and their potential role as predictors of higher fracture risk and, by extension, risk of osteoporosis. Materials and Methods: Blood samples were collected from 59 patients diagnosed with JIA in order to determine serum levels of the following markers of bone turnover: Beta-Crosslaps, osteocalcin, bone alkaline phosphatase, osteoprotegerin and receptor activator for nuclear factor kappa-B ligand. The values were analyzed with laboratory parameters and results of dual X-ray absorptiometry (DXA). Results: Osteoprotegerin and bone alkaline phosphatase levels were age-dependent. Beta-Crosslaps values were significantly higher in patients with positive JADAS27 score (p=0.0410). Osteoprotegerin levels were higher in patients treated with biological agents than only with disease-modifying anti-rheumatic drugs (p=0.0273). There was no relation between markers of bone turnover and sex, DXA results, dosage of glucocorticosteroids and disease duration. Conclusions: The authors postulate performing DXA measurements every 6 months in patients with higher disease activity. The potential lower fracture risk in children with JIA within biological treatment needs further assessment. Age- and sex-adjusted reference rates of bone turnover markers need to be developed for Central European patients in order to assess individual values properly.

Key words: juvenile idiopathic arthritis, bone turnover, osteoporosis, glucocorticosteroids

Received: 30 January, 2018; revised: 11 March, 2018; accepted: 17 May, 2018; available on-line: 30 May, 2018

✉e-mail: e.smolewska@wp.pl

Abbreviations: ANA, antinuclear antibodies; BAP, bone alkaline phosphatase; bCTx, betaisomerized carboxy terminal telopeptide of type I collagen; BMD, bone mineral density; BMDCS, Bone Mineral Density in Childhood Study; CRP, C reactive protein; DMARD, disease-modifying anti-rheumatic drug; DM1, diabetes mellitus type 1; DXA, dual X-ray absorptiometry; ESR, erythrocyte sedimentation rate; GCS, glucocorticosteroids; GM-CSF, granulo-macrophage colony stimulating factor; IL, interleukin; ISCD, International Society for Clinical Densitometry; JADAS27, Juvenile Arthritis Disease Activity Score 27-joint Reduced Count; JIA, juvenile idiopathic arthritis; OC, osteocalcin; OPG, osteoprotegerin; RANK, receptor activator for nuclear factor kappa-B; RANKL, receptor activator for nuclear factor kappa-B ligand; RF, rheumatoid factor; S.D., standard deviation; SLE, systemic lupus erythematosus; TBLH, total body less head; TNF-α, tumor necrosis factor alpha; WBC, white blood count

BACKGROUND

Low bone mineral density (BMD) is an everyday finding in clinical practice of pediatric rheumatologist. Children diagnosed with systemic connective tissue diseases, including juvenile idiopathic arthritis (JIA), juvenile systemic lupus erythematosus (SLE), and systemic vasculitides are normally expected to develop a decrease in bone mass by both, the primary and secondary mechanisms. These include: negative influence of proinflammatory cytokines – specifically: tumor necrosis factor alpha (TNF-α), interleukin (IL) IL-1, IL-6, IL8, IL-12, IL15, IL-18 and granulo-macrophage colony stimulating factor (GM-CSF) – on bone metabolism (de Jager *et al.*, 2007; Jelusic *et al.*, 2007; Kim & Moudgil, 2008; Macaubas *et al.*, 2009); nutritional insufficiency of vitamin D3 and calcium; low physical activity, which is regularly caused by avoiding of every risk of trauma. Low bone mass may also constitute one of the side effects of pharmacotherapy, mainly usage of glucocorticosteroids (GCS). Inhibition of bone formation is the principal mechanism in which GCS affect bone tissue. Even moderate doses of GCS limit both, proliferation and maturation of osteoblast progenitor cells (osteoblastogenesis), and shorten the survival time of osteoblasts by induction of apoptosis (Weinstein *et al.*, 1998). These effects result in a significant depletion in the number of osteoblasts. GCS usage may also affect excretion of several cytokines and growth stimulating factors, including: IL-6, IL-11 (Weinstein *et al.*, 1998), insulin-like growth factors I and II (Delany *et al.*, 2001), and insulin-like growth factor binding proteins (Okazaki *et al.*, 1994). Consequently, synthesis of type I collagen and osteocalcin is impaired, which results in lower production of the osteoid (unmineralized portion of bone matrix). Additionally, GCS increase expression of collagenase-3 (matrix metallopeptidase 13) and thereby exacerbate osteoid deficiency (Canalis & Delany, 2002). By another mechanism, GCS suppress expression of the gene for osteoprotegerin (OPG) and in that way escalate osteoclastogenesis and breakdown of bone tissue (Hofbauer, 1999). Post-steroid insufficiency of estrogens, testosterone and suprarenal androgens caused by inhibition of the gonadotrope and corticotrope cells in the pituitary gland also results in increased bone resorption (Lukert & Raisz, 1994; Hampson *et al.*, 2002). Induction of apoptosis in osteocytes provoked by GCS may even lead to aseptic osteonecrosis (Weinstein *et al.*, 2000).

GCS-induced lowering of BMD may lead to secondary osteoporosis which is defined as impaired bone me-

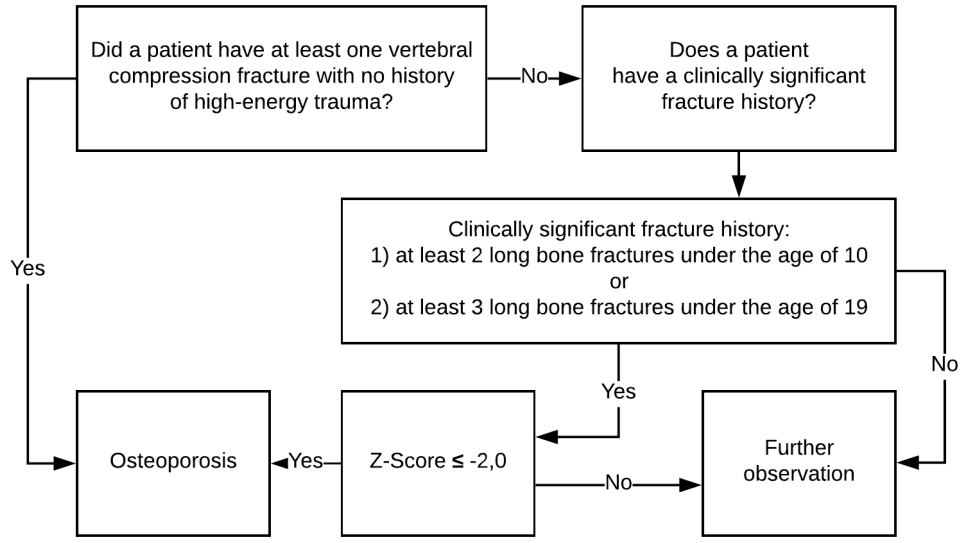

Figure 1. Diagnostic criteria of osteoporosis according to the International Society for Clinical Densitometry (ISCD) (Gordon *et al.*, 2014)

chanical resistance and elevated susceptibility to fractures caused by disrupted microarchitecture of the bone. According to diagnostic criteria developed by International Society for Clinical Densitometry (ISCD) (Gordon *et al.*, 2014), in the absence of vertebral compression fractures, both – a decreased BMD and a positive fracture history are necessary to make an osteoporosis diagnosis in children (Fig. 1). Dual X-ray absorptiometry (DXA) is a widely agreed method to assess BMD in both, the diagnostic process and monitoring of effectiveness of treatment. Because of continuous growth and alterations in ratios between tissues, age-adjusted reference rates of BMD and composition of soft tissues are essential in proper interpretation of single and repeated tests (Ward *et al.*, 2009). Lumbar spine and total body less head (TBLH) measurements are recommended to assess BMD in the pediatric population (Gordon *et al.*, 2014).

The human skeletal system undergoes continuous metabolic remodeling through processes of bone formation and its resorption. Alterations in serum concentrations of biochemical markers of bone turnover may indicate whether the bone metabolism has been dysregulated (Pereira *et al.*, 1999; Skowronska-Jozwiak & Lorenc, 2006). Van der Sluis and coworkers (Van der Sluis *et al.*, 2002) postulated that the measurements of these markers may constitute independent predictors of fracture risk. Betaisomerized carboxy terminal telopeptide of type I collagen, frequently referred to as BetaCrosslaps (bCTx), is released to the bloodstream within bone resorption as a product of collagen's degradation. Gorska and coworkers (Gorska *et al.,* 2008) reported elevated bCTx concentrations in JIA patients. Although bCTx is considered as a bone-specific marker, leukocyte lysate may decrease its serum concentration. Thus, serum should be separated from the whole blood immediately after coagulation and then stored until analysis (Herrmann *et al.*, 2002). Bone Alkaline Phosphatase (BAP) is a protein located on cellular membrane of osteoblasts and hypertrophic chondrocytes. BAP is secreted within bone mineralization, therefore it may be considered as a marker of bone formation (Vasikaran *et al.*, 2011). Caparbo and coworkers (Caparbo *et al.,* 2009) found a decrease in BAP levels in JIA patients. Osteocalcin (OC, also known as bone gamma-carboxyglutamic acid-containing protein, BGP) is one of non-collagenic proteins of bone matrix synthesized by osteoblasts, odontoblasts and hypertrophic chondrocytes (Vasikaran *et al.*, 2011). The function of OC is not fully elucidated yet. As Ducy and coworkers (Ducy *et al.*, 1996) observed increased bone formation in OC-deficient mice, OC is postulated to be considered as the marker of bone turnover rather than bone formation. There are conflicting findings in the literature on the OC level in JIA patients (Caparbo *et al.*, 2009; Gorska *et al.*, 2008). Active osteoblasts release a ligand protein (RANKL) for receptor activator for nuclear factor kappa-B (RANK) expressed on the membranes of preosteoclasts in order to promote their maturation to osteoclasts and stimulate bone resorption (Molines *et al.*, 2010). RANKL may also be expressed in activated T lymphocytes and synovial cells after their stimulation by proinflammatory cytokines (Boyle *et al.*, 2003). Osteoprotegerin (OPG) is a soluble decoy receptor for RANKL (Zdzisinska & Kandefer-Szerszen, 2006) released by osteoblasts in order to keep balance of the bone metabolism by neutralization of RANKL signaling pathway. OPG is postulated to prevent cartilage degradation through reduction of chondrocyte apoptosis (Shimizu *et al.*, 2007).

General objective of this study was to assess the clinical significance of measuring serum concentrations of the listed markers of bone turnover in patients with JIA. Specific aims included evaluation of GCS' influence on the bone metabolism and differentiation of patients with higher fracture risk and, by extension, risk of osteoporosis.

MATERIALS AND METHODS

Study participants. The study involved 59 patients already diagnosed with JIA, who were admitted to the Department of Pediatric Cardiology and Rheumatology, Medical University of Lodz, Poland between September 2014 and May 2017. The group included 40 girls and 19 boys between the ages of 5 and 18 years (mean age 12.7 ± 3.9 years). Patients' records were thoroughly reviewed in order to obtain demographic data (weight, height, body mass index, body surface area), previously assessed serological and genetic markers (rheumatoid factor, anti-cyclic citrullinated peptide autoantibodies, human leukocyte antigen B27) and treatment history (dosage and duration of the GCS

therapy, usage of disease-modifying anti-rheumatic drugs). Patients diagnosed with JIA were also assessed according to the Juvenile Arthritis Disease Activity Score 27-joint Reduced Count (JADAS27) and subtype of the disease (Petty, 2001; Bazso *et al.*, 2009).

Laboratory measurements. Fasting blood samples were collected before noon for the laboratory tests including: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood count (WBC), antinuclear antibodies (ANA) titer, serum concentrations of calcium, magnesium, phosphate, 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3, parathormone and thyroid-stimulating hormone. Samples of obtained sera were also stored at –80°C in order to determine concentrations of the listed parameters using ELISA Kits: bCTx (Cloud Clone, China), BAP (Quidel, USA), OC (Biovendor, Czech Republic), OPG (Biovendor, Czech Republic) and RANKL (Biovendor, Czech Republic).

Assessment of bone mineral density. Horizon-Wi Densitometer (Hologic Inc, Canada) was utilized for DXA examinations. All scans and analyses were performed by a physician with a certification in the field of bone densitometry and morphometry (MJ-Sz). The obtained results were interpreted according to the ISCD Official Pediatric Positions (Gordon *et al.*, 2014). Measurements of TBLH and lumbar spine BMD included in the database were expressed as Z-Score according to the Bone Mineral Density in Childhood Study (BMDCS) database provided by the manufacturer.

Statistical analysis. All statistical calculations were carried out using Statistica 13.1 software (Statsoft Polska, Krakow, Poland). The values were expressed as mean ± standard deviation (S.D.). The Shapiro-Wilk test was utilized to verify the normality of continuous variables. Spearman's rank correlation coefficients were calculated for variables not normally distributed. The Mann-Whitney U test was used for group comparisons. *P* values below 0.05 were considered significant.

The study was approved by the Bioethics Committee of the Medical University of Lodz (Approval No. RNN/520/14/KB).

RESULTS

Characteristics of the study group are shown in Table 1. All patients were previously diagnosed with JIA. The mean age at diagnosis was 9.0 ± 4.3 years and the

Table 1. Characteristics of the study group

Variables	Study group (n=59)
Female, n (%)	40 (67.8%)
JIA subtypes, n (%)	
Oligoarthritis	19 (32.2%)
RF-positive polyarthritis	2 (3.4%)
RF-negative polyarthritis	20 (33.9%)
Systemic-onset	9 (15.3%)
Enthesitis-related arthritis	9 (15.3%)
Age at diagnosis, years	9.0±4.3
Age at evaluation, years	12.7±3.9
Disease duration, years	3.7±3.3
JADAS27 score>0, n (%)	44 (74.6%)
Positive history of GCS usage, n (%)	54 (91.5%)
Cumulative GCS dose converted to prednisone-equivalent dose, mg/kg of body weight	9.8±6.0
Positive history of DMARDs usage, n (%)	
Methotrexate	55 (93.2%)
Hydroxychloroquine	10 (16.9%)
Sulfasalazine	10 (16.9%)
Cyclosporin A	2 (3.4%)
Current use of biological treatment, n (%)	20 (33.9%)
TBLH Z-Score, n	–0.86±1.25
Lumbar spine Z-Score, n	–0.86±1.28
Positive history of fractures, n (%)	
Vertebral compression fractures	1 (1.7%)
Other types of fractures	0 (0.0%)
Positive history of vitamin D3 intake, n (%)	59 (100.0%)
25-hydroxyvitamin D3 level, ng/ml	29.5±11.9
Vitamin D deficiency (<20 ng/ml), n (%)	15 (25.4%)
Vitamin D suboptimal status (20-30 ng/ml), n (%)	20 (33.9%)

Table legend: JIA – juvenile idiopathic arthritis, JADAS27 – Juvenile Arthritis Disease Activity Score 27-joint Reduced Count, RF – rheumatoid factor, GCS – glucocorticosteroids, DMARD – disease-modifying anti-rheumatic drug, TBLH – total body less head (Z-Score)

Table 2. Concentrations of markers of bone turnover – mean values

Markers	Mean values ± S.D.
OPG, pmol/L	5.38±1.10
RANKL, pmol/L	461.79±280.68
OC, ng/mL	6.06±5.34
BAP, U/L	63.0±34.9
bCTx, ng/mL	1.27±0.48

Table legend: S.D. – standard deviation, OPG – osteoprotegerin, RANKL – receptor activator for nuclear factor kappa-B ligand, OC – osteocalcin, BAP – bone alkaline phosphatase, bCTx – Beta-Crosslaps

mean disease duration was 3.7 ± 3.3 years (55.9% were diagnosed more than 2 years before evaluation). 54 patients (91.5%) had positive history of GCS intake. Cumulative GCS dose converted to prednisone-equivalent dose was 9.8 ± 6.0 mg/kg of body weight.

Regarding activity of JIA at evaluation, 74.6% of patients had positive JADAS27 score. CRP and ESR levels were elevated in 27.1% and 37.3% of patients, respectively. In respect to DXA results, mean TBLH Z-Score was –0.86 ± 1.25 (16.9% of patients with a result below 2.0), mean lumbar spine Z-Score was –0.86 ± 1.38 (20.3% of patients with a result below 2.0).

Mean values of the tested bone turnover markers are presented in Table 2. There was no association between concentrations of markers and DXA results or levels of the listed parameters: calcium, magnesium, phosphate, 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3, parathormone and thyroid-stimulating hormone. OPG and BAP levels were negatively correlated with age at evaluation (OPG: r=–0.295 p=0.0286; BAP: r=0.514 p=0.000044) and age at diagnosis (OPG: r=–0.389 p=0.00329; BAP: r=–0.311 p=0.0187). Correlation between OC and age at evaluation was marginally significant (r=0.260 p=0.0573). Concentrations of bCTx were independent of age. RANKL was excluded from further analysis as it turned out to be the only marker without any relation to the remaining ones.

Significant results of group comparisons are illustrated in Fig. 2. Concentrations of bCTx were higher in patients with positive JADAS27 score (p=0.0410). Concentrations of OPG were higher in patients treated with biological agents than those treated only with disease-modifying antirheumatic drugs (p=0.0273). Lumbar spine Z-Score was higher in a small group of patients without a history of GCS intake (p=0.0282). Although all of the remaining parameters included in the dataset were involved in group comparisons, they did not show statistically significant differences.

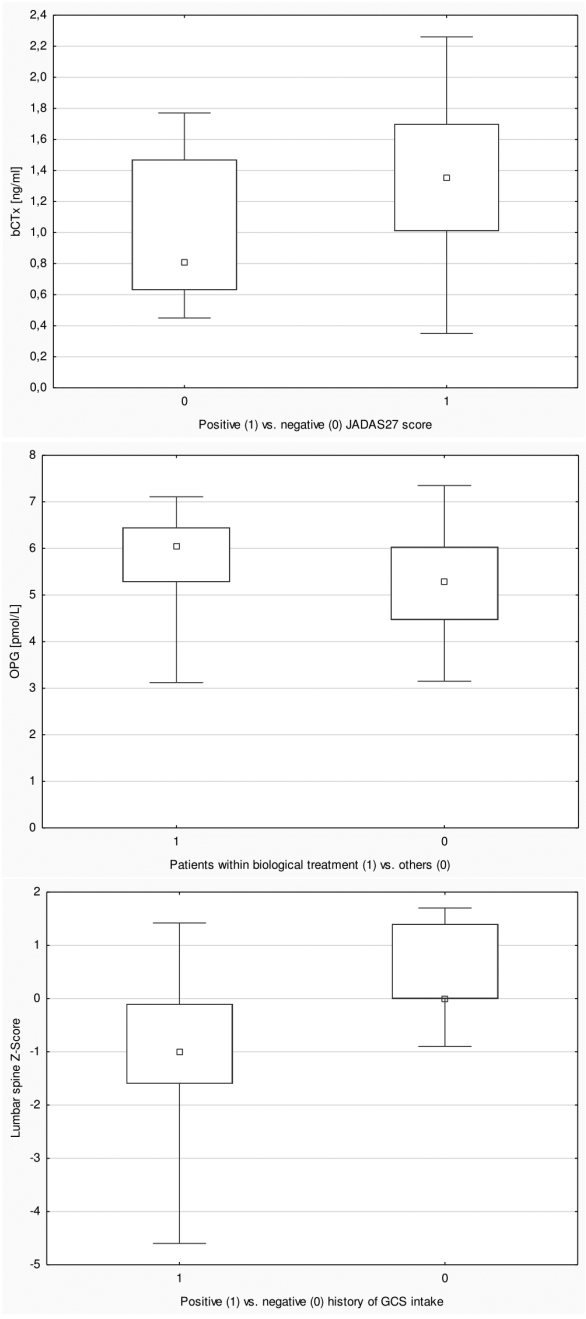

Figure 2. Graphs illustrating significant group comparisons
Figure legend: JADAS27 – Juvenile Arthritis Disease Activity Score 27-joint Reduced Count, bCTx – Beta-Crosslaps, OPG – osteoprotegerin, GCS – glucocorticosteroids

DISCUSSION

Risk of osteoporosis in children with JIA is difficult to assess. While 16.9% (TBLH) and 20.3% (lumbar spine) of patients had BMD Z-Score below –2.0, only one patient included in the study had a positive history of vertebral compression fracture and required further treatment with disodium pamidronate. Hence, serum concentrations of bone turnover markers were measured in the study group in order to evaluate their clinical relevance. In a study conducted by Masi and coworkers (Masi *et al.,* 2004), serum OPG levels were higher in children with polyarticular and extended oligoarticular JIA than in healthy controls and patients with oligoarticular-persistent disease. Conversely, OPG levels were lower in patients with early JIA (disease duration 19.4 ± 12.3 months) than in healthy individuals according to Lien and coworkers (Lien *et al.,* 2010). Comparing results of the recent study to the findings of Gajewska and coworkers (Gajewska *et al.*, 2006), who evaluated markers of bone turnover in healthy Polish children, JIA patients had higher concentrations of OPG than the cited reference group; postpubertal healthy children had lower bCTx levels than JIA patients. Lambrinoudaki and coworkers (Lambrinoudaki *et al.,* 2013) reported OPG and RANKL levels in Greek children, including patients with diabetes mellitus type 1 (DM1) and healthy controls. Both parameters were lower in these groups when compared to JIA patients included in the recent study. Levels of the measured parameters were independent of sex, however, previous reports found significantly higher levels of OC

in females than males in healthy children (Csakvary *et al.*, 2013) and DM1 patients (Napoli *et al.*, 2013).

Age of study participants was negatively correlated with OPG, BAP and nearly significantly correlated with OC. Such relations were also observed in DM1 patients and healthy controls (Lambrinoudaki *et al.*, 2013; Napoli *et al.*, 2013). However, markers of bone turnover are not specific for bone remodeling only. Their concentrations may be influenced by primary bone modeling as well (von Scheven *et al.*, 2014), therefore interpretation of the obtained results without proper age- and sex-adjusted reference rates may become challenging.

Concentrations of bCTx in the recent study were higher in patients with positive JADAS27 score, in other words, with higher disease activity. Similarly, bCTx levels were positively associated with disease activity in young male adults newly diagnosed with SLE (Guo *et al.*, 2017). Nevertheless, large population-based study of German children showed seasonal variation of bCTx levels (Thiering *et al.*, 2015), which was also approaching significance in the recent study group (data not shown). Therefore, clinical significance of bCTx measurements remains questionable.

Serum OPG levels were higher in children treated with biological agents – etanercept, adalimumab and tocilizumab – when compared to patients solely receiving disease-modifying anti-rheumatic drugs. Billiau and coworkers (Billiau *et al.,* 2010) observed a nearly significant (p=0.07) increase in OPG concentrations after 6 months of treatment with etanercept and methotrexate, unlike in patients treated only with methotrexate. These results may indicate efficient blocking of anti-osteoblastic effect of TNF-α and IL-6. When administered *in vitro*, TNF-α inhibits synthesis of type I collagen, OC expression and BAP activity (Corrado *et al.*, 2017), whereas IL-6 interacts negatively with the Wnt signaling pathway (Malysheva *et al.*, 2016). As higher OPG levels may prevent skeletal system from bone destruction through inhibition of osteoclast differentiation, activation and stimulation of their apoptosis (Romas *et al.*, 2002), a potential protective role of biological therapy needs to be further evaluated in the future.

Regarding OPG/RANKL axis, OPG is postulated to be a more relevant marker of the system activity (Lambrinoudaki *et al.*, 2013). Sarma and coworkers (Sarma *et al.,* 2008) suggested that RANKL levels increase in the early phase of JIA and persist elevated throughout the course of the disease. According to Lien and coworkers (Lien *et al.,* 2010) higher RANKL concentrations are predictors of a more severe disease. RANKL levels were also elevated in patients with active polyarticular JIA with bone erosions (Spelling *et al.*, 2008). Similarly to the recent study, RANKL levels were not associated with any factor in DM1 patients (Lambrinoudaki *et al.*, 2013).

Although all study participants had positive history of vitamin D intake, 59.3% of patients had serum 25-hydroxyvitamin D3 concentrations below 30 ng/ml. However, there was no association between markers of bone turnover and vitamin D. These findings are consistent with other authors' results (Schou *et al.*, 2003; Ginty *et al.*, 2004). Influence of vitamin D on bone development in adolescents may be dose-dependent, though. In a study of HIV-infected youth, decrease in the levels of markers of bone resorption was reported only in a group receiving monthly 120 000 IU (Eckard *et al.*, 2017).

In the study presented here, there were no associations between markers of bone turnover and DXA results. However, Masi and coworkers (Masi *et al.*, 2004) found positive correlation between BMD and OPG levels in JIA patients. Furthermore, BMD values were positively predicted by OC concentrations in healthy Hungarian children (Csakvary *et al.*, 2013).

Influence of using GCS on bone turnover needs to be further discussed. Wasilewska and coworkers (Wasilewska *et al.,* 2010) suggested that long-term use of GCS leads to a dose-dependent increase in the RANKL level. Although 91.5% of patients were treated with GCS, concentrations of markers of bone turnover were independent of the GCS dose in the study group. However, a GCS-associated reduction in the OC levels was observed in SLE patients (Dhillon *et al.*, 1990), and a decrease in OPG concentrations in rheumatoid arthritis patients treated with prednisolone was reported (Engvall *et al.*, 2013).

A lack of age- and sex-adjusted reference rates was the main limitation of our study. Age-dependent differences in the mentioned parameters might have been evaluated more accurately with Tanner stages. A small group of JIA patients who were not treated with GCS was not enough to perform a reliable analysis of GCS' influence on bone turnover.

CONCLUSIONS

Findings of this study suggest a potential association between the disease activity and a higher fracture risk. Therefore, the authors postulate performing DXA tests every 6 months in patients with higher disease activity in order to assess the dynamics of bone mass lowering. The potential lower fracture risk in children with JIA treated with biological agents needs further evaluation. A population-based study of healthy controls needs to be performed in order to assess concentrations of markers of bone turnover more properly.

Acknowledgements of Financial Support

This study was supported by the grant No.503/8-503-66-001 and 502-03/8-000-04/502-64-094 from the Medical University of Lodz, Poland.

REFERENCES

Bazso A, Consolaro A, Ruperto N, Pistorio A, Viola S, Magni-Manzoni S, Malattia C, Buoncompagni A, Loy A, Martini A, Ravelli A, Pediatric Rheumatology International Trials Organization (2009) Development and testing of reduced joint counts in juvenile idiopathic arthritis. *J Rheumatol* **36**: 183–190. http://doi.org/10.3899/jrheum.080432

Billiau AD, Loop M, Le PQ, Berthet F, Philippet P, Kasran A, Wouters CH (2010) Etanercept improves linear growth and bone mass acquisition in MTX-resistant polyarticular-course juvenile idiopathic arthritis. *Rheumatology (Oxford)* **49**: 1550–1558. http://doi.org/10.1093/rheumatology/keq123

Boyle WJ, Simonet WS, Lacey DL (2003) Osteoclast differentiation and activation. *Nature* **423**: 337–342. http://doi.org/10.1038/nature01658

Canalis E, Delany AM. Mechanisms of glucocorticoid action in bone (2002) *Ann N Y Acad Sci* **966**: 7381.

Caparbo VF, Prada F, Silva CA, Regio PL, Pereira RM (2009) Serum from children with polyarticular juvenile idiopathic arthritis (pJIA) inhibits differentiation, mineralization and may increase apoptosis of human osteoblasts "*in vitro*". *Clin Rheumatol* **28**: 71–77. http://doi.org/10.1007/s10067-008-0985-y

Corrado A, Maruotti N, Cantatore FP (2017) Osteoblast role in rheumatic diseases. *Int J Mol Sci* **18**: 1272. doi: 10.3390/ijms18061272

Csakvary V, Puskas T, Oroszlan G, Lakatos P, Kalman B, Kovacs GL, Toldy E (2013) Hormonal and biochemical parameters correlated with bone densitometric markers in prepubertal Hungarian children. *Bone* **54**: 106–112. http://doi.org/10.1016/j.bone.2013.01.040

de Jager W, Hoppenreijs EP, Wulffraat NM, Wedderburn LR, Kuis W, Prakken BJ (2007) Blood and synovial fluid cytokine signatures in patients with juvenile idiopathic arthritis: a cross-section-

al study. *Ann Rheum Dis* **66**: 589–598. http://doi.org/10.1136/ard.2006.061853
Delany AM, Durant D, Canalis E (2001) Glucocorticoid suppression of IGF I transcription in osteoblasts. *Mol Endocrinol* **15**: 1781–1789. http://doi.org/10.1210/mend.15.10.0704
Dhillon VB, Davies MC, Hall ML, Round JM, Ell PJ, Jacobs HS, Snaith ML, Isenberg DA (1990) Assessment of the effect of oral corticosteroids on bone mineral density in systemic lupus erythematosus: a preliminary study with dual energy x ray absorptiometry. *Ann Rheum Dis* **49**: 624–626
Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, Smith E, Bonadio J, Goldstein S, Gundberg C, Bradley A, Karsenty G (1996) Increased bone formation in osteocalcin-deficient mice. *Nature* **382**: 448–452. http://doi.org/10.1038/382448a0
Eckard AR, O'Riordan MA, Rosebush JC, Ruff JH, Chahroudi A, Labbato D, Daniels JE, Uribe-Leitz M, Tangpricha V, McComsey GA (2017) Effects of vitamin D supplementation on bone mineral density and bone markers in HIV-infected youth. *J Acquir Immune Defic Syndr* **76**: 539–546. http://doi.org/10.1097/QAI.0000000000001545
Engvall IL, Svensson B, Boonen A, van der Heijde D, Lerner UH, Hafström I, BARFOT study group (2013) Low-dose prednisolone in early rheumatoid arthritis inhibits collagen type I degradation by matrix metalloproteinases as assessed by serum 1CTP – a possible mechanism for specific inhibition of radiological destruction. *Rheumatology (Oxford)* **52**: 733–742. http://doi.org/10.1093/rheumatology/kes369
Gajewska J, Ambroszkiewicz J, Laskowska-Klita T (2006) Osteoprotegerin and C-telopeptide of type I collagen in Polish healthy children and adolescents. *Adv Med Sci* **51**: 269–272
Ginty F, Cavadini C, Michaud PA, Burckhardt P, Baumgartner M, Mishra GD, Barclay DV (2004) Effects of usual nutrient intake and vitamin D status on markers of bone turnover in Swiss adolescents. *Eur J Clin Nutr* **58**: 1257–1265. http://doi.org/10.1038/sj.ejcn.1601959
Gordon CM, Leonard MB, Zemel BS, International Society for Clinical Densitometry (2014) 2013 Pediatric Position Development Conference: executive summary and reflections. *J Clin Densitom* **17**: 219–224. http://doi.org/10.1016/j.jocd.2014.01.007
Górska A, Urban M, Bartnicka M, Zelazowska-Rutkowska B, Wysocka J (2008) Bone mineral metabolism in children with juvenile idiopathic arthritis – preliminary report. *Ortop Traumatol Rehabil* **10**: 54–62
Guo Q, Fan P, Luo J, Wu S, Sun H, He L, Zhou B (2017) Assessment of bone mineral density and bone metabolism in young male adults recently diagnosed with systemic lupus erythematosus in China. *Lupus* **26**: 289–293. http://doi.org/10.1177/0961203316664596
Hampson G, Bhargava N, Cheung J, Vaja S, Seed PT, Fogelman I (2002) Low circulating estradiol and adrenal androgens concentrations in men on glucocorticoids: a potential contributory factor in steroid-induced osteoporosis. *Metabolism* **51**: 1458–1462
Herrmann M, Pape G, Herrmann W (2002) Measurement of serum beta-crosslaps is influenced by proteolytic conditions. *Clin Chem Lab Med* **40**: 790–794. http://doi.org/10.1515/CCLM.2002.136
Hofbauer LC (1999) Osteoprotegerin ligand and osteoprotegerin: novel implications for osteoclast biology and bone metabolism. *Eur J Endocrinol* **141**: 195–210
Jelusic M, Lukic IK, Batinic D (2007) Biological agents targeting interleukin-18. *Drug News Perspect* **20**: 485–494. http://doi.org/10.1358/dnp.2007.20.8.1157617
Kim EY, Moudgil KD (2008) Regulation of autoimmune inflammation by pro-inflammatory cytokines. *Immunol Lett* **120**: 1–5. http://doi.org/10.1016/j.imlet.2008.07.008
Lambrinoudaki I, Tsouvalas E, Vakaki M, Kaparos G, Stamatelopoulos K, Augoulea A, Pliatsika P, Alexandrou A, Creatsa M, Karavanaki K (2013) Osteoprotegerin, soluble receptor activator of nuclear factor-κ B ligand, and subclinical atherosclerosis in children and adolescents with type 1 diabetes mellitus. *Int J Endocrinol* **2013**: 102120. http://doi.org/10.1155/2013/102120
Lien G, Ueland T, Godang K, Selvaag AM, Førre OT, Flatø B (2010) Serum levels of osteoprotegerin and receptor activator of nuclear factor-κB ligand in children with early juvenile idiopathic arthritis: a 2-year prospective controlled study. *Pediatr Rheumatol Online J* **8**: 30. http://doi.org/10.1186/1546-0096-8-30
Lukert BP, Raisz LG (1994) Glucocorticoid-induced osteoporosis. *Rheum Dis Clin North Am* **20**: 62950.
Macaubas C, Nguyen K, Milojevic D, Park JL, Mellins ED (2009) Oligoarticular and polyarticular JIA: epidemiology and pathogenesis. *Nat Rev Rheumatol* **5**: 616–626. http://doi.org/10.1038/nrrheum.2009.209
Malysheva K, de Rooij K, Lowik CW, Baeten DL, Rose-John S, Stoika R, Korchynskyi O (2016) Interleukin 6/Wnt interactions in rheumatoid arthritis: interleukin 6 inhibits Wnt signaling in synovial fibroblasts and osteoblasts. *Croat Med J* **57**: 89–98
Masi L, Simonini G, Piscitelli E, Del Monte F, Giani T, Cimaz R, Vierucci S, Brandi ML, Falcini F (2004) Osteoprotegerin (OPG)/RANK-L system in juvenile idiopathic arthritis: is there a potential modulating role for OPG/RANK-L in bone injury? *J Rheumatol* **31**: 986–991
Molines L, Darmon P, Raccah D (2010) Charcot's foot: newest findings on its pathophysiology, diagnosis and treatment. *Diabetes Metab* **36**: 251–255. http://doi.org/10.1016/j.diabet.2010.04.002
Napoli N, Strollo R, Pitocco D, Bizzarri C, Maddaloni E, Maggi D, Manfrini S, Schwartz A, Pozzilli P, IMDIAB Group (2013) Effect of calcitriol on bone turnover and osteocalcin in recent-onset type 1 diabetes. *PLoS One* **8**: e56488. http://doi.org/10.1371/journal.pone.0056488
Okazaki R, Riggs BL, Conover CA (1994) Glucocorticoid regulation of insulin-like growth factor-binding protein expression in normal human osteoblast-like cells. *Endocrinology* **134**: 126–32. http://doi.org/10.1210/endo.134.1.7506203
Pereira RM, Falco V, Corrente JE, Chahade WH, Yoshinari NH (1999) Abnormalities in the biochemical markers of bone turnover in children with juvenile chronic arthritis. *Clin Exp Rheumatol* **17**: 251–255
Petty RE (2001) Growing pains: the ILAR classification of juvenile idiopathic arthritis. *J Rheumatol* **28**: 927–928
Romas E, Sims NA, Hards DK, Lindsay M, Quinn JW, Ryan PF, Dunstan CR, Martin TJ, Gillespie MT (2002) Osteoprotegerin reduces osteoclast numbers and prevents bone erosion in collagen-induced arthritis. *Am J Pathol* **161**: 1419–1427. http://doi.org/10.1016/S0002-9440(10)64417-3
Sarma PK, Misra R, Aggarwal A (2008) Elevated serum receptor activator of NFkappaB ligand (RANKL), osteoprotegerin (OPG), matrix metalloproteinase (MMP)3, and ProMMP1 in patients with juvenile idiopathic arthritis. *Clin Rheumatol* **27**: 289–294. http://doi.org/10.1007/s10067-007-0701-3
Schou AJ, Heuck C, Wolthers OD (2003) Vitamin D supplementation to healthy children does not affect serum osteocalcin or markers of type I collagen turnover. *Acta Paediatr* **92**: 797–801
Shimizu S, Asou Y, Itoh S, Chung UI, Kawaguchi H, Shinomiya K, Muneta T (2007) Prevention of cartilage destruction with intraarticularosteoclastogenesis inhibitory factor/osteoprotegerin in a murine model of osteoarthritis. *Arthritis Rheum* **56**: 3358–3365. http://doi.org/10.1002/art.22941
Skowrońska-Jóźwiak E, Lorenc RS (2006) Metabolic bone disease in children: etiology and treatment options. *Treat Endocrinol* **5**: 297–318
Spelling P, Bonfá E, Caparbo VF, Pereira RM (2008) Osteoprotegerin/RANKL system imbalance in active polyarticular-onset juvenile idiopathic arthritis: a bone damage biomarker? *Scand J Rheumatol* **37**: 439–444. http://doi.org/10.1080/03009740802116224
Thiering E, Brüske I, Kratzsch J, Hofbauer LC, Berdel D, von Berg A, Lehmann I, Hoffmann B, Bauer CP, Koletzko S, Heinrich J (2015) Associations between serum 25-hydroxyvitamin D and bone turnover markers in a population based sample of German children. *Sci Rep* **5**: 18138. http://doi.org/10.1038/srep18138
van der Sluis IM, Hop WC, van Leeuwen JP, Pols HA, de Muinck Keizer-Schrama SM (2002) A cross-sectional study on biochemical parameters of bone turnover and vitamin D metabolites in healthy Dutch children and young adults. *Horm Res* **57**: 170–179. http://doi.org/10.1159/000058378
Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, Griesmacher A, McClung M, Morris HA, Silverman S, Trenti T, Wahl DA, Cooper C, Kanis JA, IOF-IFCC Bone Marker Standards Working Group (2011) Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int* **22**: 391–420. http://doi.org/10.1007/s00198-010-1501-1
von Scheven E, Corbin KJ, Stagi S, Cimaz R (2014) Glucocorticoid-associated osteoporosis in chronic inflammatory diseases: epidemiology, mechanisms, diagnosis, and treatment. *Curr Osteoporos Rep* **12**: 289–299. http://doi.org/10.1007/s11914-014-0228-x
Ward L, Petryk A, Gordon CM (2009) Use of bisphosphonates in the treatment of pediatric osteoporosis. *Int J Clin Rheumtol* **4**: 657–672. http://dx.doi.org/10.2217/ijr.09.58
Wasilewska A, Rybi-Szuminska A, Zoch-Zwierz W (2010) Serum RANKL, osteoprotegerin (OPG), and RANKL/OPG ratio in nephrotic children. *Pediatr Nephrol* **25**: 2067–2075. http://doi.org/10.1007/s00467-010-1583-1
Weinstein RS, Jilka RL, Parfitt AM, Manolagas SC (1998) Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. *J Clin Invest* **102**: 274–282. http://doi.org/10.1172/JCI2799
Weinstein RS, Nicholas RW, Manolagas SC (2000) Apoptosis of osteocytes in glucocorticoid-induced osteonecrosis of the hip. *J Clin Endocrinol Metab* **85**: 2907–2912. http://doi.org/10.1210/jcem.85.8.6714
Zdzisińska B, Kandefer-Szerszeń M (2006) The role of RANK/RANKL and OPG in multiple myeloma. *Postepy Hig Med Dosw (Online)* **60**: 471–482