

https://dspace.org/

Bone turnover markers in children living with HIV remaining on ritonavirboosted lopinavir or switching to efavirenz

Stephanie Shiau; MIchael Yin; Renate Strehlau; jing Shen; E Abrams ...

https://hdl.handle.net/10539/36561
WOS

Downloaded from a DSpace repository. https://dspace.org/

Bone 138 (2020) 115500

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Bone turnover markers in children living with HIV remaining on ritonavir-boosted lopinavir or switching to efavirenz

Stephanie Shiau[a], Michael T. Yin[b], Renate Strehlau[c], Jing Shen[d], Elaine J. Abrams[e,f,g], Ashraf Coovadia[c], Louise Kuhn[d,e], Stephen M. Arpadi[d,e,f,g,*]

[a] Department of Biostatistics and Epidemiology, Rutgers School of Public Health, Piscataway, NJ, USA
[b] Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY, USA
[c] Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Department of Pediatrics and Child Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[d] Gertrude H. Sergievsky Center, College of Physicians and Surgeons, Columbia University, New York, NY, USA
[e] Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, USA
[f] Department of Pediatrics, College of Physicians and Surgeons, Columbia University, New York, NY, USA
[g] ICAP at Columbia, Mailman School of Public Health, Columbia University, New York, NY, USA

ARTICLE INFO

Keywords:
Osteocalcin
Bone turnover markers
Protease inhibitors
Bone

ABSTRACT

Introduction: We previously found lower bone mass but similar bone turnover in pre-pubertal children living with HIV (CLWH) on a ritonavir-boosted lopinavir (LPV/r)-based vs. efavirenz-based antiretroviral therapy regimen 2 years after switch. Here, we evaluate if bone turnover differed between the groups close to the time of switch.

Methods: Samples from 108 children remaining on LPV/r and 104 children switched to efavirenz were available for analysis 8 weeks post-randomization. Bone turnover markers, including C-telopeptide of type 1 collagen (CTx), procollagen type-I N-terminal propeptide (P1NP), and osteocalcin were measured. Markers of immune activation were also measured, including IL-6, TNF-alpha, soluble CD14 and high-sensitivity C-reactive protein (CRP).

Results: Eight weeks post-randomization, we did not detect differences in CTx (1.42 vs. 1.44 ng/mL, p = 0.85) or P1NP concentrations (622 vs. 513 ng/mL, p = 0.68) between treatment groups. At 8 weeks, the treatment groups also had similar levels of IL-6, TNF-alpha, soluble CD14 and high-sensitivity CRP. Osteocalcin (ng/mL) was higher in the LPV/r than efavirenz group both at 8 weeks (88.6 vs. 67.3, p = 0.001) and 2 years (67.6 vs. 49.8, p = 0.001).

Conclusions: Overall, we failed to detect difference in bone turnover by P1NP and CTx in virologically-suppressed CLWH on different regimens at a time point close to the switch. We did observe higher levels of total osteocalcin in children remaining on LPV/r compared to children switched to efavirenz. Future studies should focus on uncovering the mechanism and determining whether perturbation in undercarboxylated osteocalcin could explain some of the bone side effects noted with protease inhibitors.

1. Introduction

Children and adolescents living with HIV (CLWH) have deficits in skeletal development, including decreases in bone mass accrual and alterations in bone microarchitecture [1,2], which could increase risk of osteoporosis and fracture later in life [3]. We previously reported lower bone mineral content (BMC) in a group of South African CLWH who initiated antiretroviral therapy (ART) before 2–3 years of age and maintained excellent virologic control [4]. In particular, CLWH who remained on a ritonavir-boosted/lopinavir (LPV/r)-based ART regimen had lower bone mass compared to CLWH switched to an efavirenz-based regimen; however, unexpectedly there were no significant differences in measures of bone turnover levels between the two ART groups. From studies in adults on ART, it is well-established that increases in bone turnover marker (BTM) levels and bone loss are greater in the first year after initiating or switching ART than subsequent years [5]. Therefore, we hypothesized that between-group BMC differences would be reflected in differences in bone turnover within the first year

* Corresponding author at: Gertrude H. Sergievsky Center, Columbia University, 630 W. 168th Street, PH 19-114, New York, NY 10032, USA.
E-mail address: sma2@columbia.edu (S.M. Arpadi).

https://doi.org/10.1016/j.bone.2020.115500
Received 14 February 2020; Received in revised form 15 May 2020; Accepted 14 June 2020
Available online 23 June 2020
8756-3282/ © 2020 Published by Elsevier Inc.

Table 1
Characteristics and markers of inflammation and immune activation of children living with HIV (CLWH) randomized to remain on a ritonavir-boosted lopinavir (LPV/r) regimen or switch to an efavirenz-based regimen at 8 weeks and 2 years after randomization.

Characteristic or measurement	8 weeks			2 years		
	LPV/r (N = 108)	Efavirenz (N = 104)	p	LPV/r (N = 113)	Efavirenz (N = 106)	p
Male, N (%)	52 (48.2)	53 (51.0)	0.68	53 (46.9)	54 (50.9)	0.55
Age (years), mean (SD)	4.4 (1.0)	4.4 (0.9)	0.92	6.4 (1.3)	6.3 (1.2)	0.74
Weight-for-age Z-score, mean (SD)	−0.86 (0.9)	−0.74 (0.9)	0.33	−0.90 (0.9)	−0.76 (0.9)	0.25
Height-for-age Z-score, mean (SD)	−1.31 (0.9)	−1.37 (0.9)	0.62	−1.36 (0.9)	−1.45 (0.9)	0.48
Tanner stage, N (%)						
1	108 (100.0)	104 (100.0)	1.0	113 (100.0)	102 (96.2)	0.053
2	0 (0.0)	0 (0.0)		0 (0.0)	4 (3.8)	
BMC Z-score, mean (SD)	–	–	–	−1.20 (0.8)	−0.68 (0.8)	< 0.0001
IL-6 (pg/mL), mean (SD)	2.90 (2.1)	2.73 (1.80)	0.65	2.17 (4.8)	1.25 (1.4)	0.059
IL-6 (pg/mL), median (IQR)	2.08 (1.39, 4.07)	2.15 (1.57, 3.44)	0.97	0.96 (0.61, 1.61)	0.81 (0.53, 1.33)	0.047
IL-6 (pg/mL) ≥9.96, N (%)	0 (0.0)	0 (0.0)	1.0	4 (3.5)	0 (0.0)	0.12[F]
Missing	42	53		0	0	
TNF-alpha (pg/mL), mean (SD)	3.67 (1.27)	3.56 (1.27)	0.52	2.40 (1.33)	1.89 (1.34)	0.005
TNF-alpha (pg/mL), median (IQR)	3.38 (2.85, 4.62)	3.34 (2.79, 3.91)	0.66	2.09 (1.60, 2.75)	1.64 (1.29, 2.18)	< 0.01
TNF-alpha (pg/mL) ≥4.71, N (%)	23 (22.8)	16 (17.2)	0.33	4 (3.5)	2 (1.9)	0.68[F]
Missing	7	11		0	0	
Soluble CD14 (ng/mL), mean (SD)	1915 (516)	2000 (568)	0.26	1428 (535)	1480 (566)	0.50
Soluble CD14 (ng/mL), median (IQR)	1970 (1650, 2198)	2049 (1652, 2382)	0.22	1312 (1062, 1651)	1360 (1152, 1669)	0.44
Soluble CD14 (ng/mL) ≥2300, N (%)	24 (22.6)	28 (26.9)	0.47	10 (8.9)	8 (7.6)	0.61
Missing	2	0		0	1	
High-sensitivity CRP (mg/dL), mean (SD)	2.29 (3.58)	2.30 (2.88)	1.0	4.75 (12.6)	3.20 (7.5)	0.28
High-sensitivity CRP (mg/dL), median (IQR)	0.80 (0.40, 2.69)	0.61 (0.37, 2.75)	0.97	0.66 (0.30, 2.48)	0.83 (0.31, 2.29)	0.44
High-sensitivity CRP (mg/dL) ≥0.5, N (%)	21 (67.7)	18 (62.1)	0.65	63 (56.8)	64 (62.1)	0.49
Missing	77	75		2	3	

Abbreviations: LPV/r – ritonavir-boosted lopinavir; IL-6 – interleukin-6; TNF-alpha – tumor necrosis factor alpha; CRP – C-reactive protein. [F]Fisher's exact test; all others are Chi-squared tests.

after ART switch.

In this study, we evaluated BTM levels in pre-pubertal CLWH on LPV/r-based and efavirenz-based regimens at 8 weeks post-randomization in a clinical trial of treatment options, and compared them to previously published results from 2 years post-randomization [6].

2. Methods

2.1. Study population

This analysis includes 219 CLWH who were participants in a non-inferiority randomized clinical trial evaluating the safety and efficacy of preemptive switching to efavirenz compared with remaining on LPV/r (Neverest 3) and subsequently enrolled into an observational study to evaluate bone health (CHANGES Bone Study) [4,7]. In the trial, 113 CLWH were randomized to remain on LPV/r and 106 were randomized to switch to efavirenz after achieving viral suppression. CLWH were also receiving two nucleoside reverse transcriptase inhibitors including lamivudine and either abacavir, zidovudine, or stavudine; none had exposure to tenofovir. Additionally, none received corticosteroids or antiepileptic medications.

2.2. Measurements

Plasma samples from the 8-week time point were analyzed at the Biomarkers Core Laboratory at Columbia University Medical Center in New York, NY. The bone resorption marker was C-telopeptide of type-1-collagen (CTx) (ELISA; Immunodiagnostic Systems, Scottsdale, AZ). Bone formation markers included procollagen type-I-N-terminal-propeptide (P1NP) (RIA; Immunodiagnostic Systems, Scottsdale, AZ) and osteocalcin (ELISA; Immunodiagnostic Systems, Scottsdale, AZ). Inflammatory biomarkers were also analyzed, including pro-inflammatory, pro-resorptive cytokines IL-6 (ELISA; R&D Systems, Minneapolis, MN) and TNF-alpha (ELISA; R&D Systems, Minneapolis, MN), as well as soluble CD14 (ELISA; R&D Systems, Minneapolis, MN), a marker of monocyte activation, and high-sensitivity C-reactive protein (CRP) (Cobas Integra 400 Plus; Roche Diagnostics, Indianapolis, IN), an acute phase reactant and marker of general inflammation. Of note, sample volume was limited and we prioritized assays in the following order: CTx, P1NP, osteocalcin, soluble CD14, TNF-alpha, IL-6, and high-sensitivity CRP. These same assays were utilized for the analysis of BTMs 2 years post-randomization [6].

Bone mineral content (BMC) in grams of the whole body and lumbar spine were determined by dual-energy X-ray absorptiometry (DXA) using a Hologic Discovery Wi bone densitometer with Apex software version 3.4 (Hologic Inc., Bedford, MA) at the 2-year visit as previously presented [4]. Demographic and clinical data, including historical plasma HIV-RNA levels and CD4 counts and percentages were extracted from study databases. The study was approved by the Institutional Review Boards of Columbia University and the University of the Witwatersrand.

2.3. Statistical analysis

Chi-squared or Fisher's exact tests were used to compare proportions, *t*-tests to compare means, and Wilcoxon rank-sum test to compare medians. Relationships between change in BTMs from the 8 week visit to the 2-year visit and bone outcomes (whole body BMC and lumbar spine BMC) were evaluated using Spearman correlations. All p-values are 2-tailed and p-values < 0.05 were considered statistically significant. Analysis was performed using SAS 9.4 (Cary, North Carolina, USA).

3. Results

3.1. Characteristics

Characteristics of the study participants at the 8 week visit are shown in Table 1 along with data from the 2-year visit. Samples were only available for 108 children on LPV/r (48.2% male) and 104 children on efavirenz (51.0% male). Children were between 3.2 and 7.3 years of age (mean 4.4 years). The mean duration on ART was

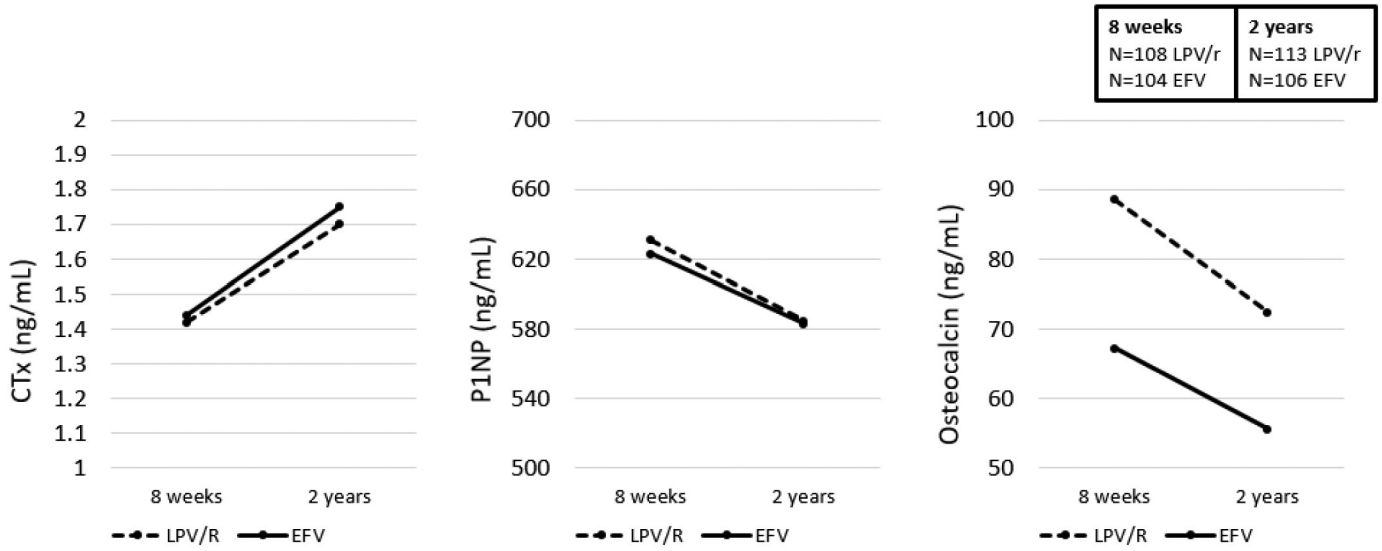

Fig. 1. Bone turnover markers of children living with HIV (CLWH) randomized to remain on a ritonavir-boosted lopinavir (LPV/r) regimen or switch to an efavirenz-based regimen at 8 weeks and 2 years after randomization.
Abbreviations: LPV/r – ritonavir-boosted lopinavir; EFV – efavirenz; P1NP – procollagen type I N-terminal propeptide; CTx – C-telopeptide of type 1 collagen (CTX).

3.6 years (1.3–6.1 years) and this visit occurred an average of 8.1 weeks after randomization in the trial.

3.2. Bone turnover markers

Mean CTx concentration was not different between LPV/r and efavirenz groups at 8 weeks (1.42 vs. 1.44 ng/mL, p = 0.85) or 2 years after randomization (1.70 vs. 1.75 ng/mL, p = 0.53). Mean P1NP concentration also did not differ between LPV/r and efavirenz groups at 8 weeks (622 vs. 513 ng/mL, p = 0.68) or 2 years after randomization (585 vs. 583 ng/mL, p = 0.94). In contrast, osteocalcin was significantly higher in the LPV/r than efavirenz group both at 8 weeks (88.6 vs. 67.3 ng/mL, p = 0.001) and 2 years (67.6 vs. 49.8 ng/mL, p = 0.001). As shown in Fig. 1, from 8 weeks to 2 years after randomization, mean CTx concentrations significantly increased in both groups (p < 0.001). In contrast, mean P1NP (p = 0.008) and osteocalcin concentrations significantly decreased in both groups from 8 weeks to 2 years after randomization (p = 0.008 and p < 0.001, respectively). There were no significant correlations between absolute change or percent change in CTx (absolute r = 0.01, p = 0.91; percent r = −0.00, p = 0.98), P1NP (absolute r = −0.04, p = 0.57; percent r = −0.06, p = 0.40), or osteocalcin (absolute r = 0.08; p = 0.27; percent r = 0.03, p = 0.69) and whole body BMC. Similarly, there were no significant correlations between absolute change or percent change in CTx (absolute r = 0.02, p = 0.77; percent r = 0.02, p = 0.81), P1NP (absolute r = −0.03, p = 0.66; percent r = −0.05, p = 0.46), or osteocalcin (absolute r = 0.10, p = 0.15; percent r = 0.06, p = 0.40) and lumbar spine BMC. Correlations were similar when stratified by LPV/r and efavirenz groups.

3.3. Inflammation and immune activation

Mean IL-6 concentration did not differ between children remaining on LPV/r and those switched to efavirenz 8 weeks post-randomization (2.90 vs. 2.73 pg/mL, p = 0.65), but was higher in the LPV/r than efavirenz group 2 years post-randomization (2.17 vs. 1.25 pg/mL, p = 0.059). Similarly, TNF-alpha concentration did not differ between the groups 8 weeks post-randomization (3.67 vs. 3.56 pg/mL, p = 0.52), but was higher in the LPV/r than the efavirenz group 2 years post-randomization (2.40 vs. 1.89 pg/mL, p = 0.005). In both treatment groups, mean IL-6 and TNF-alpha decreased from 8 weeks to 2 years; however, the between-group difference was significant only for TNF-alpha, with a greater decrease in the efavirenz than the LPV/r group (−45% vs. -29%, p = 0.005). Mean high-sensitivity CRP and soluble CD14 did not differ between groups at 8 weeks or 2 years post-randomization.

4. Discussion

Overall, we failed to detect differences in bone turnover as measured by CTx and P1NP concentrations among virologically-suppressed CLWH 8 weeks after switching to efavirenz or remaining on a LPV/r-based regimen. In contrast, we found that osteocalcin levels were higher in the LPV/r than efavirenz group at both time points.

Closer to the time of switch, levels of circulating pro-inflammatory, pro-resorptive cytokines (IL-6 and TNF-alpha) were similar, whereas children remaining on LPV/r had elevated levels compared to children switched to efavirenz at the 2-year time point; this lends further support for the role of protease inhibitors in inducing some inflammatory responses [8]. We did not detect differences between treatment groups in measures of monocyte activation (soluble CD14) or high-sensitivity CRP near the time of regimen switch. Apart from high-sensitivity CRP, markers of inflammation and immune activation declined over time in both treatment groups. Differences in BMC observed 2 years following regimen change do not appear to be related to changes in BTMs or markers of inflammation and immune activation from 8 weeks to 2 years. The lack of correlations may be due to the sampling intervals selected, or the dynamic interaction between bone resorption and bone formation during childhood periods of growth.

Overall, CTx increased from the 8 week time point (mean age 4.4 years) to the 2-year time point (mean age 6.4 years), while osteocalcin and P1NP decreased. Bone turnover markers vary throughout childhood and follow different patterns compared to adults [9,10]. Although reference ranges have not been established in South Africa, studies in Caucasian European children have generally reported highest levels of osteocalcin and P1NP in the first 4 years of life, with levels decreasing and remaining stable until coinciding with pubertal growth spurts [9,10]. The decrease in osteocalcin and P1NP observed in our study may coincide with this pattern. Similarly, the increase in CTx observed in our study may coincide with the increase in CTx seen in study of Caucasian European children from ages 0 to 10 [9].

Our new measure of osteocalcin at both time points is of interest,

particularly the discrepancy between the dynamics of the two bone formation markers, P1NP and osteocalcin. The total osteocalcin assay that we utilized includes both carboxylated and undercarboxylated osteocalcin. Other groups have also found higher levels of total and undercarboxylated osteocalcin in patients on protease inhibitor-containing ART regimens. Kinai et al. found higher levels of osteocalcin as well as lower spine bone mineral density (BMD) in protease inhibitor users than non-protease inhibitor HIV-infected patients [11]. Similarly, Hirakawa et al. reported higher levels of undercarboxylated osteocalcin in patients treated with protease inhibitors compared to those switching to integrase strand transfer inhibitors [12]. In the same study, the group found impaired mechanical properties of bone associated with higher levels of undercarboxylated osteocalcin levels in mice treated with protease inhibitors. Lastly, Tan et al. found higher total osteocalcin levels in CLWH on protease inhibitors ages 2 to 12 years than controls [13]. The underlying mechanism is unclear, since circulating levels of total osteocalcin and undercarboxylated osteocalcin have been found to be inversely associated with components of metabolic syndrome (e.g. abdominal obesity, dyslipidemia, hypertension, hyperglycemia/insulin resistance) and other cardiovascular risk in the general population [14–18]. Therefore, we would expect that with protease inhibitor use, which is associated with both metabolic syndrome and low BMD, the osteocalcin levels would be lower. Future studies should focus on uncovering mechanisms and determining whether perturbation in osteocalcin or undercarboxylated osteocalcin metabolism could explain some of the bone side effects noted with protease inhibitors. In addition, obtaining repeated measurements to assess the timing of changes in osteocalcin, and measuring the active form of undercarboxylated osteocalcin in future studies may contribute further information.

In conclusion, we investigated longitudinal changes in immune activation and BTMs in relation to BMC in CLWH randomized to two ART regimens. Our study is limited by lack of data from uninfected children and some missing IL-6 and high-sensitivity CRP data due to low sample volumes at the early time point. We did not observe differences in P1NP or CTx, but we did find higher levels of circulating total osteocalcin in children randomized to remain on a protease-inhibitor regimen. Future studies to explore the role of osteocalcin in bone and other non-AIDS comorbidities in CLWH may be of interest.

CRediT authorship contribution statement

Stephanie Shiau: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Michael T. Yin**: Conceptualization, Investigation, Funding acquisition, Writing - review & editing. **Renate Strehlau**: Investigation, Writing - review & editing. **Jing Shen**: Investigation, Writing - review & editing. **Elaine J. Abrams**: Investigation, Writing - review & editing. **Ashraf Coovadia**: Investigation, Funding acquisition, Writing - review & editing. **Louise Kuhn**: Investigation, Funding acquisition, Writing - review & editing. **Stephen M. Arpadi**: Conceptualization, Investigation, Funding acquisition, Writing - review & editing.

Declaration of competing interest

Stephanie Shiau, Michael T. Yin, Renate Strehlau, Jing Shen, Elaine J. Abrams, Ashraf Coovadia, Louise Kuhn, and Stephen M. Arpadi declare that they have no conflict of interest.

Acknowledgements

This study received financial support from the National Institute of Child Health and Human Development (HD 073977, HD 073952).

References

[1] D.L. Jacobson, J.C. Lindsey, C.M. Gordon, et al., Total body and spinal bone mineral density across Tanner stage in perinatally HIV-infected and uninfected children and youth in PACTG 1045, AIDS 24 (2010) 687–696.
[2] S. Mora, I. Zamproni, S. Beccio, R. Bianchi, V. Giacomet, A. Vigano, Longitudinal changes of bone mineral density and metabolism in antiretroviral-treated human immunodeficiency virus-infected children, J. Clin. Endocrinol. Metab. 89 (2004) 24–28.
[3] S. Shiau, E.C. Broun, S.M. Arpadi, M.T. Yin, Incident fractures in HIV-infected individuals: a systematic review and meta-analysis, AIDS 27 (2013) 1949–1957.
[4] S.M. Arpadi, S. Shiau, R. Strehlau, et al., Efavirenz is associated with higher bone mass in South African children with HIV, AIDS 30 (2016) 2459–2467.
[5] D.A. Wohl, L. Bhatti, C.B. Small, et al., The ASSURE study: HIV-1 suppression is maintained with bone and renal biomarker improvement 48 weeks after ritonavir discontinuation and randomized switch to abacavir/lamivudine + atazanavir, HIV Med. 17 (2) (2015) 106–117.
[6] S. Shiau, M.T. Yin, R. Strehlau, et al., Decreased bone turnover in HIV-infected children on antiretroviral therapy, Arch. Osteoporos. 13 (2018) 40.
[7] A. Coovadia, E.J. Abrams, R. Strehlau, et al., Efavirenz-based antiretroviral therapy among nevirapine-exposed HIV-infected children in South Africa: a randomized clinical trial, JAMA 314 (2015) 1808–1817.
[8] L. Chen, S. Jarujaron, X. Wu, et al., HIV protease inhibitor lopinavir-induced TNF-alpha and IL-6 expression is coupled to the unfolded protein response and ERK signaling pathways in macrophages, Biochem. Pharmacol. 78 (2009) 70–77.
[9] M. Rauchenzauner, A. Schmid, P. Heinz-Erian, et al., Sex- and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years, J. Clin. Endocrinol. Metab. 92 (2007) 443–449.
[10] M. Bayer, Reference values of osteocalcin and procollagen type I N-propeptide plasma levels in a healthy Central European population aged 0-18 years, Osteoporos. Int. 25 (2014) 729–736.
[11] E. Kinai, H. Gatanaga, D. Mizushima, et al., Protease inhibitor-associated bone mineral density loss is related to hypothyroidism and related bone turnover acceleration, J. Infect. Chemother. 23 (2017) 259–264.
[12] H. Hirakawa, H. Gatanaga, H. Ochi, et al., Antiretroviral therapy containing HIV protease inhibitors enhances fracture risk by impairing osteoblast differentiation and bone quality, J. Infect. Dis. 215 (2017) 1893–1897.
[13] B.M. Tan, R.P. Nelson Jr., M. James-Yarish, P.J. Emmanuel, S.J. Schurman, Bone metabolism in children with human immunodeficiency virus infection receiving highly active anti-retroviral therapy including a protease inhibitor, J. Pediatr. 139 (2001) 447–451.
[14] Y. Bao, M. Zhou, Z. Lu, et al., Serum levels of osteocalcin are inversely associated with the metabolic syndrome and the severity of coronary artery disease in Chinese men, Clin. Endocrinol. 75 (2011) 196–201.
[15] A.A. Alfadda, A. Masood, S.A. Shaik, H. Dekhil, M. Goran, Association between osteocalcin, metabolic syndrome, and cardiovascular risk factors: role of total and undercarboxylated osteocalcin in patients with type 2 diabetes, Int. J. Endocrinol. 2013 (2013) 197519.
[16] M. Zhang, Z. Ni, W. Zhou, J. Qian, Undercarboxylated osteocalcin as a biomarker of subclinical atherosclerosis in non-dialysis patients with chronic kidney disease, J. Biomed. Sci. 22 (2015) 75.
[17] I. Kanazawa, T. Yamaguchi, M. Yamamoto, et al., Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus, J. Clin. Endocrinol. Metab. 94 (2009) 45–49.
[18] S.C. Moser, B.C.J. van der Eerden, Osteocalcin-a versatile bone-derived hormone, Front. Endocrinol. 9 (2018) 794.