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The role of Insulin-like Growth Factor 1, Receptor Activator for Nuclear Factor κ B ligand — Osteoprotegerin system, Interleukin 6 and 1β in post-transplantation bone metabolic disease in childhood

Rola insulinopodobnego czynnika wzrostowego 1, systemu ligand aktywatora receptora jądrowego czynnika κ B — osteoprotegeryna, interleukina 6 i 1β w potransplantacyjnej metabolicznej chorobie kości u dzieci i młodzieży

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Abstract

Introduction: Bone disorders observed commonly after haematopoietic stem cells transplantation (HSCT) can be caused by several factors, but their detailed pathomechanism is still not well known, especially in childhood.

The aim of this study was to evaluate: IGF-I, RANKL-OPG system, IL-6, and IL-1 β levels and their association with bone mineral density (BMD) in children and adolescents after HSCT.

Material and methods: Thirty five patients after allogeneic (N = 21) and autologous (N = 14) HSCT, mean age 8.48 \pm 5.18 years, were included in the study. Blood samples were taken before HSCT, on the transplantation day, three and six months after HSCT, then each year after HSCT for 2–8 years. RANKL, OPG, and IL-1 β , IGF-1, and IL-6 were measured by immunochemistry. Total BMD was evaluated six months after HSCT using dual energy X-ray absorptiometry, then annually.

Results: All Z-core values for BMD were negative in all patients. It was significantly higher in patients after auto HSCT than after allo HSCT. Serum levels of IGF-1 and IL-6 significantly changed after HSCT. IGF-1 levels started to increase in the second year after transplantation. IL-6 increased up to 12 months after transplantation. Dynamic although not significant changes of OPG and RANKL levels were observed after HSCT. RANKL and IGF-1 values correlated with BMD. IL-6 correlated positively with IL-1 β but both did not correlate with BMD.

Conclusions: Our data indicates that factors influencing bone remodelling change dynamically in the post-transplantation period. It suggests that serum RANKL and IGF-1 levels could be markers of bone metabolism after HSCT in paediatric patients. (*Endokrynol Pol* 2013; 64 (4): 248–254)

Key words: RANKL-OPG system, IGF-1, interleukins, post-transplantation bone metabolic disease, children and adolescents

Streszczenie

Wstęp: Znanych jest kilka czynników mogących mieć wpływ na zaburzenia kostne obserwowane często po przeszczepieniu komórek krwiotwórczych (PKK). Ich dokładna etiopatogeneza pozostaje jednak wciąż nie znana, szczególnie u pacjentów po PKK przebyłym w dzieciństwie. Celem pracy była ocena surowiczych stężeń: IGF-1, RANKL, OPG, IL-6 i IL-1 β oraz ich korelacji z gęstością mineralną kości (BMD) u dzieci i młodzieży po PKK.

Materiał i metody: Do badań włączono 35 pacjentów w średnim wieku 8,48 \pm 5,18 lat po allogenicznych (N = 21) i autologicznych (N = 14) PKK. Próbkę krwi pobierano przed PKK, w dniu transplantacji, 3 i 6 miesięcy po PKK, a następnie co rok przez 2–8 lat. Stężenia IGF-1, RANKL, OPG, IL-1 β , IL-6 oznaczane były metodami immunochemicznymi. BMD całego ciała oceniane było metodą DEXA (dual-energy X-ray absorptiometry) 6 miesięcy po PKK, a następnie co rocznie.

Wyniki: Stwierdzono ujemne wartości Z-core dla BMD u wszystkich pacjentów. Były one znacząco wyższe u pacjentów po autologicznych niż po allogenicznych PKK. Surowicze stężenia IGF-1 i IL-6 znacząco zmieniły się w czasie po PKK. Stężenia IGF-1 wzrastały od 2. roku po PKK, zaś IL-6 narastały do 12. miesiąca po PKK. Obserwowano również dynamiczne, chociaż nieznamiennie zmiany stężeń OPG i RANKL po PKK. Wykazano korelacje RANKL i IGF-1 z BMD. IL-6 korelowała pozytywnie z IL-1 β , obie interleukiny nie korelowały z BMD.

Wnioski: Stwierdzono istotne i dynamiczne zmiany czynników mogących mieć wpływ na metabolizm kostny po transplantacji. Sugeruje się, że surowicze stężenia RANKL i IGF-1 mogą być markerami metabolizmu kostnego po PKK u dzieci i młodzieży. (*Endokrynol Pol* 2013; 64 (4): 248–254)

Słowa kluczowe: system RANKL-OPG, IGF-1, interleukiny, potransplantacyjna choroba metaboliczna kości, dzieci i młodzież



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Introduction

Since the end of the 20th century, the number of allogeneic and autologous haematopoietic stem cell transplantations (HSCT) performed for malignant and non-malignant disorders has increased steadily [1]. With less early mortality and more widespread use of HSCT, the number of long-term survivors will continue to grow. Likewise, many children are now surviving HSCT and require structured long-term follow-up care to evaluate risk factors of post-transplantation complications and to provide appropriate prevention and treatment. One of the most frequent long-term complications after HSCT is transplantation bone disease associated with bone turnover abnormalities [2].

Based on dual-energy X-ray absorptiometry, more than half of long-term survivors show bone loss after HSCT [3–5]. Some factors influencing bone remodelling after transplantation like hypogonadism, glucocorticoid exposure, use of calcineurin inhibitors, secondary hyperparathyroidism, hyperthyroidism, and calcium and/or vitamin D deficiency, are already known [6–8]. However, the detailed mechanism of bone metabolism disorders is not exactly understood, especially in children and adolescents. IGF-I, which dynamically changes during childhood, plays a role in increasing bone mass by influencing osteoblasts' maturation and function [9]. It has been shown that circulating IGF-1 directly regulates not only bone growth but also bone density [10]. Significant correlations between bone mineral density (BMD) and serum IGF-1 levels in adult patients after HSCT have been observed [11]. On the other hand, interleukins such as IL-6 and IL-1 are implicated in bone loss through osteoclastic activation [12, 13]. It has been reported that enhanced bone resorption following HSCT was related to the dose of administered steroids and the increase in IL-6 level. Recent studies have presented the OPG/RANKL/RANK system as the dominant, final mediator of osteoclastogenesis [14, 15]. RANKL is a member of the TNF ligand family. Osteoclast precursors express Receptor Activator of Nuclear Factor κ B (RANK), a TNF receptor family member, recognise RANKL through cell-to-cell interaction with osteoblast/stromal cells, and differentiate to pre-fusion osteoclasts. OPG is a soluble receptor for RANKL and acts as a decoy receptor in the RANK-RANKL signalling system [16, 17]. Some studies have shown that circulating osteoprotegerin and receptor activator of NF- κ B ligand system are associated with bone metabolism [18, 19]. It was also suggested that interleukins may play

an important role for post-BMT bone loss in adults [20], possibly via the RANKL pathway [21].

In children, normal values for BMD as well as interleukins levels and OPG/RANKL/RANK system have not yet been well established, although it is clear that loss of bone density and increased risk of fracture is a significant issue in children after HSCT.

The aim of this study was to assess the RANKL-OPG system, IGF-I, IL-6, and IL1 β levels in children and adolescents after HSCT, and to evaluate their association with bone mineral density.

Material and methods

Thirty five patients (15 girls and 20 boys, mean age 8.48 ± 5.18 years) treated in the Transplantation Centre in the University Hospital in Krakow between 2008 and 2010 were included in the study. The Local Ethical Committee of Jagiellonian University in Krakow approved the study. All participants and their parents gave their written, informed consent.

The patients were admitted due to different haematological (nine patients with acute lymphoblastic leukaemia, four with acute myeloid leukaemia, and two patients with aplastic anaemia), oncologic (14 patients with solid tumours and one with Hodgkin's disease) and non-malignant diseases (five patients with immunodeficiency syndromes).

Autologous haematopoietic stem cells transplantation were performed in 14 patients. High-dose chemotherapy before autologous HSCT consisted of busulphan and melphalan in eight patients, and cyclophosphamide, etoposide and melphalan in six patients.

Twenty one patients were recipients of allogeneic haematopoietic transplants. Fourteen received grafts from matched related donors, and seven from matched unrelated donors. A conditioning regime included total body irradiation and etoposide in 14 patients, high-dose chemotherapy consisting of busulphan and cyclophosphamide in six patients, and fludarabine and melphalan in three patients. All patients after allogeneic transplantation received cyclosporine A as graft versus host disease prevention for 3–6 months.

Anthropometrical measurements were made in all participants included in the study. Height was measured to the nearest centimetre using a rigid stadiometer. Weight was measured unclothed to the nearest 0.1 kg using a calibrated balance scale. Reference for Polish Children for the analysis of the obtained data were used [22]. The pubertal developmental stage of each patient was determined according to Tanner scale.

Mean height SDS of patients was near mid-parental height SDS (up to 1 SD) and patients' body mass was appropriate for their height (mean 89% of body mass

Table I. Mean data \pm SD of serum concentrations of interleukin 6 (IL-6), interleukin 1 β (IL-1 β), osteoprotegerin (OPG), Receptor Activator for Nuclear Factor κ B ligand (RANKL), and z-SDS for insulin-like growth factor 1 (IGF-1) in patients after haematopoietic stem cell transplantation (HSCT) (* $p < 0.05$)

Tabela I. Średnie surowicze stężenia wraz z odchyleniami standardowymi dla interleukiny 6, interleukiny 1 β , osteoprotegeryny, liganda aktywatora receptora jądrowego czynnika κ B oraz uwzględniające wiek i płeć odchylenia standardowe dla insulinopodobnego czynnika wzrostu 1 u pacjentów po przeszczepieniu komórek krwiotwórczych (* $p < 0.05$)

	Before HSCT	HSCT day	3-M after HSCT	6-M after HSCT	1-Y after HSCT	\geq 2-Y after HSCT
z-SDS IGF-1	-2.62 ± 1.3	-2.67 ± 1.2	-2.6 ± 1.0	-1.8 ± 1.0	-2.12 ± 0.9	$-0.89 \pm 0.9^*$
IL-6 [pg/mL]	9.7 ± 7.2	20.2 ± 15.2	16.1 ± 8.2	21.3 ± 8.3	18.7 ± 23.7	$8.27 \pm 4.6^*$
IL-1 β [pg/mL]	1.64 ± 3.0	0.2 ± 0.1	0.58 ± 1.2	0.2 ± 0.1	1.9 ± 4.5	1.3 ± 3.6
RANKL [pmol/L]	0.12 ± 0.2	0.12 ± 0.1	0.02 ± 0.01	0.02 ± 0.02	0.07 ± 0.1	0.04 ± 0.1
OPG [pmol/L]	4.66 ± 0.5	5.71 ± 2.9	6.62 ± 1.8	6.56 ± 2.1	4.9 ± 4.7	5.07 ± 1.3

* $p < 0.05$. HSCT — haematopoietic stem cell transplantation; z-SDS; IL-6 — interleukin 6; IL-1 β — interleukin 1 β ; RANKL — Receptor Activator for Nuclear Factor κ B ligand; OPG — osteoprotegerin

appropriate for the height). Nineteen patients were pre-pubertal, 12 were during puberty, and four had finished pubertal development before inclusion in the study.

Moreover a detailed analysis of all factors influencing bone remodelling such as other endocrine complications, renal function and drug administration was performed. One participant of this study was treated with recombinant growth hormone after diagnosis of growth hormone deficiency. Two girls received an oestrogen-progesterone replacement therapy due to hypergonadotrophic hypogonadism. Five patients received levothyroxine due to hypothyroidism and they were euthyrotic as were the remaining patients. All patients had normal serum levels of calcium and phosphorus. After HSCT they received vitamin D and calcium supplementation, however vitamin D level was not checked. All patients were encouraged to carry out regular physical activity, especially weight-bearing exercises. None of them was treated with bisphosphonates.

Two patients were treated with steroids because of GVHD development after HSCT. No chronic inflammations were observed in the presented group of patients.

Blood samples were taken from the antecubital vein in the fasting state, at 8am. After clotting, blood samples were centrifuged and serum samples were stored at -80°C until the measurement of required parameters. Blood samples for measurement of the above-mentioned parameters were taken in the following periods: before the HSCT procedure, on the transplantation day, three months after HSCT, six months after HSCT, then annually after the transplantation. RANKL (IDS, Germany), OPG (IDS, Germany), and IL-1 β (Diasource, Belgium) were measured by ELISA, IGF-I by RIA (Diasource, Belgium), and IL-6 by IRMA (Diasource, Belgium).

Total body bone mineral density (BMD) was evaluated using the dual energy X-ray absorptiometry

method using Delfi W. Hologic Densitometer (USA). The evaluation of BMD was performed before HSCT, six months after, and then annually after HSCT.

Statistical calculations were performed using Statistica program (StatSoft, Inc, USA). Results were expressed as mean \pm SD. SDS were used if necessary to remove age and gender dependence. Multiple regression, Student's t-test, and ANOVA with a post hoc Tukey's test were used for analysis. In the performed analysis $p < 0.05$ was considered statistically significant.

Results

The densitometry revealed negative Z-score values of total BMD of all assessed participants of the study. Z-score values of total BMD were significantly lower in patients with graft versus host disease (median -3.86) than others (median -1.16). Patients after autologous HSCT had significantly higher bone mineral density ($0.876 \pm 0.12 \text{ g/cm}^2$) than patients after allogeneic transplantation ($0.748 \pm 0.04 \text{ g/cm}^2$) ($p = 0.03$). Since the second year after autologous HSCT, bone mineral density ($0.991 \pm 0.05 \text{ g/cm}^2$) was significantly ($p = 0.05$) higher compared to BMD before this time ($0.761 \pm 0.06 \text{ g/cm}^2$). In allogeneic patients, the variability of BMD values during all observation times was not statistically significant ($p = 0.2$).

Mean values of serum concentrations of IL-6, IL-1 β , OPG, RANKL, and SDS for IGF-1 levels of all patients are presented in Table I. The serum levels of IGF-I and IL-6 significantly changed after HSCT. The concentration of IGF-I significantly increased since the second year after transplantation and it approached the normal values for a child of this age. IL-6 levels were significantly higher during the first 12 months after transplantation with a peak in the sixth month, compared to its values before HSCT and the values two years after the

Table II. Mean data \pm SD of serum concentrations of interleukin 6 (IL-6), interleukin 1 β (IL-1 β), osteoprotegerin (OPG), Receptor Activator for Nuclear Factor κ B ligand (RANKL), and z-SDS for insulin-like growth factor 1 (IGF-1) in patients six months after autologous and allogeneic haematopoietic stem cell transplantation (HSCT)

Tabela II. Średnie surowicze stężenia wraz z odchyleniami standardowymi dla interleukiny 6, interleukiny 1 β , osteoprotegeryny, liganda aktywatora receptora jądrowego czynnika κ B oraz uwzględniające wiek i płeć odchylenia standardowe dla insulinopodobnego czynnika wzrostu 1 u pacjentów w 6 miesięcy po autologicznych i allogenicznym przeszczepieniach komórek

	Autologous HSCT	Allogeneic HSCT	Normal range*	p
No of patients	14	21		
Age (years)	6.9 \pm 5.1	8.9 \pm 3.7		0.07
z-SDS IGF-I	-2.7 \pm 1.4	-1.8 \pm 0.8		0.012
IL-6 [pg/mL]	13.1 \pm 9.4	16.2 \pm 8.4	0-31 (3.27 \pm 3.1)	0.19
IL-1 β [pg/mL]	3.03 \pm 4.9	4.99 \pm 2.5	0-17 (2.6 \pm 5.3)	0.25
RANKL [pmol/L]	0.17 \pm 0.1	0.05 \pm 0.04	0-2 (0.43 \pm 0.13)	0.001
OPG [pmol/L]	5.7 \pm 1.5	5.9 \pm 1.5	0-30 (1.8 \pm 0.05)	0.38

*based on 2.5% to 97.5% percentiles. HSCT — haematopoietic stem cell transplantation; z-SDS; IL-6 — interleukin 6; IL-1 β — interleukin 1 β , RANKL — Receptor Activator for Nuclear Factor κ B ligand, OPG — osteoprotegerin

procedure. Some changes of OPG and RANKL levels were observed after HSCT. Although not statistically significant, there was a tendency toward a decrease of RANKL and an increase of OPG values between three and six months after transplantation. Six months after HSCT, the mean RANKL values were statistically higher in patients after autologous than after allogeneic transplantation. On the other hand, the serum IGF-1 levels in the autologous group were significantly lower than in allogeneic ones. The mean levels of IL-6, IL-1 β , OPG did not differ between patients after auto- and allogeneic HSCT for samples taken in the same time after HSCT (Table II).

IL-6 level correlated positively with IL-1 β concentration at every moment of the study (Fig. 1). Both interleukins did not correlate with BMD, as well as with RANKL and OPG values. For both studied allogeneic and autologous groups, BMD correlated significantly with serum levels of RANKL ($r = 0.624$, $p = 0.039$) as well as with OPG/RANKL ratio ($r = 0.683$, $p = 0.020$). For both groups, BMD correlated also with IGF-1 levels (Fig. 2).

Discussion

Several studies have tried to explain the pathomechanism of bone disorders in patients after HSCT, but the problem is still not completely understood. Although the peripheral mononuclear cells in the recipients are

of donor origin, the bone marrow stromal cells are of recipient origin after bone marrow transplantation (BMT). It is known that the differentiation of bone marrow stromal cells into osteoblasts is impaired after BMT, and this contributes to post-BMT bone loss [20]. The growth factors produced by osteoblasts, including IGF-1, play an important role in bone growth and osteogenesis [9]. A mouse model presented by Yakar et al. [10] demonstrated that a threshold concentration of circulating IGF-1 is necessary for normal bone growth and suggested that it plays a prominent role in the pathophysiology of osteoporosis. Moreover, IGF-1 correlated positively with total bone, spinal density and bone resorption markers [23]. Salerno et al. [24] documented that allogeneic HSCT restores IGF-1 production and linear growth in a SCID patient with abnormal growth hormone receptor signalling. This suggests that cells derived from haematopoietic stem cells may unexpectedly exert a significant role in IGF-1 production. In our study we observed an increase of IGF-1 concentration since the second year after HSCT, when the graft was stable. Similar to the report of Salerno et al. [24], our data also suggests that cells derived from haematopoietic stem cells are involved in IGF-1 production.

However, our observation could also suggest that at least two years are needed to rebuild bone marrow stromal cells which had been destroyed by a conditioning regime of high-dose chemo- and/or radiotherapy.

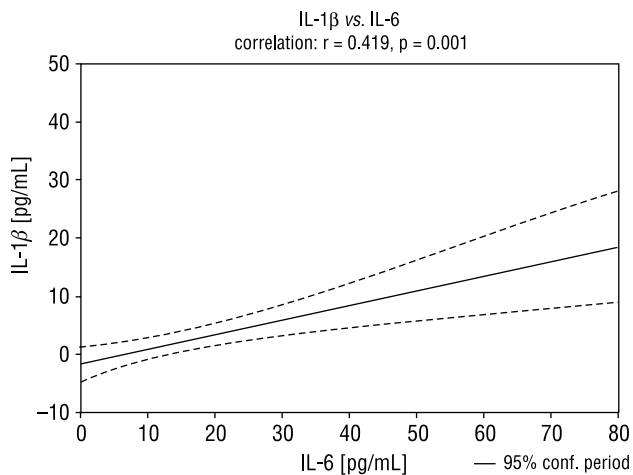


Figure 1. Correlation between serum interleukin 6 (IL-6) and interleukin 1 β (IL-1 β) in children and adolescents after haematopoietic stem cell transplantation

Rycina 1. Korelacja pomiędzy surowiczymi stężeniami interleukiny 6 i interleukiny 1 β u dzieci i młodzieży po przeszczepie komórek krwiotwórczych

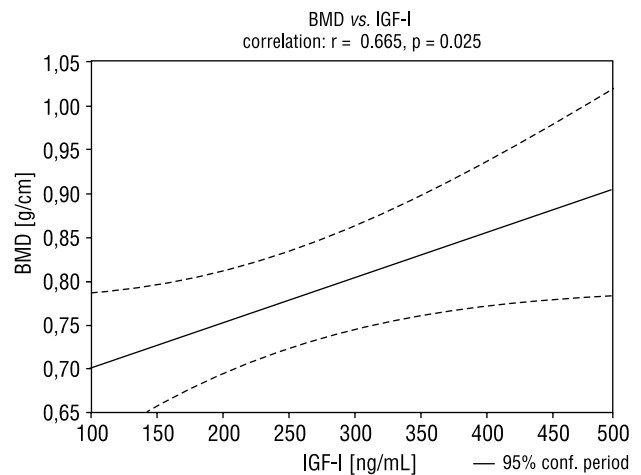


Figure 2. Correlation between serum insulin-like growth factor 1 (IGF-1) levels and bone mineral density (BMD) in children and adolescents after haematopoietic stem cell transplantation

Rycina 2. Korelacja pomiędzy surowiczymi stężeniami insulino-podobnego czynnika wzrostowego 1 i gęstością mineralną kości u dzieci i młodzieży po przeszczepie komórek krwiotwórczych

The positive correlation between IGF-1 and BMD in our study could suggest an important role of IGF-1 in osteogenesis and therefore in the pathophysiology of osteoporosis in childhood. This corresponds to previous reports regarding the data obtained in adults after HSCT [9, 21].

Some studies have indicated the possible role of the OPG/RANKL/RANK system in the pathophysiological evolution of post-transplantational osteoporosis. Increases in the mean value of sRANKL level and the sRANKL/OPG ratio could indicate a negative balance in bone metabolism following BMT [25]. Short-term changes of the biochemical markers' concentration in the post-transplantation period suggest a rapid impairment of bone formation and an increase in bone resorption [26]. Tae et al. [25] showed dynamic changes in the RANKL and OPG levels immediately during the post-BMT period, which were related to a decrease in bone formation and loss of lumbar spine bone mass density during the year following the BMT. In the present study, some changes of OPG and RANKL after HSCT has also been observed. Although not statistically significant, there was a tendency towards a decrease of RANKL and an increase of OPG levels between three and six months after transplantation. This finding is in agreement with the data presented by Gandhi et al. [27] who reported that bone-specific alkaline phosphatase activity showed a significant initial decline one month after transplantation, but the activity returned to pre-transplant levels by the third month. The changes of the serum RANKL and

OPG levels seem to precede the biological process of bone regeneration. Terpos et al. [28] showed that autologous stem cell transplantation normalises the abnormal bone resorption in multiple myeloma patients possibly through a decrease of RANKL/OPG ratio, while bone formation requires a longer period to return to normal [28].

The significant differences in IGF-1 and RANKL levels in patients six months after allogeneic HSCT, as compared to autologous, could imply that chemotherapy conditioning rather than radiotherapy affects these parameters. In patients after allogeneic HSCT also immunological effects of transplanted donor cells on these parameters cannot be ruled out. Fabrega et al. [29] showed that a significant amount of OPG and RANKL is released in the early post-transplant period of liver transplantation and they tried to explain this phenomenon by an activation of the immune system caused by allograft.

A quite interesting finding of our study is the significant correlation observed between RANKL concentrations as well as OPG/RANKL ratio with BMD. This could suggest that not only circulating IGF-1 concentration but also RANKL level reflects bone density metabolism in childhood, but this hypothesis requires further study on a larger population.

One of the factors responsible for post-transplantation osteoporosis is administration of glucocorticoids after HSCT. Long-term exposure to glucocorticoids results in a dose-dependent increase in serum RANKL concentration and the RANKL/OPG ratio, but not in the level of serum OPG [30]. Our patients treated with

steroids had the lowest Z-score values of total BMD. A definitive statement on the effect of glucocorticoids on bone metabolism cannot be made due to the small number of patients receiving such treatment after HSCT. However, similarly to cyclosporine A used in GVHD prevention, glucocorticoids might be responsible for lower BMD of allogeneic patients as compared to autologous ones [2].

In spite of immunosuppression, post-BMT bone loss is primarily related to gonadal dysfunction. Cytokines, especially interleukin-6, play an important role in the pathogenesis of postmenopausal osteoporosis related to oestrogen deficiency. Data on the pathogenetic role of cytokines in post-BMT bone loss is scarce. Lee et al. [31] reported an increase in bone marrow IL-6, which can induce bone resorption both alone and in concert with other bone-resorbing agents, during the immediate post-BMT period. There was a significant positive correlation between a serum bone resorption marker and bone marrow IL-6 levels following BMT, so it was suggested that the progressive increase in bone resorption is related to the increase in bone marrow IL-6 [31]. Polymorphism in the interleukin-6 gene is associated with bone mineral density in adolescents [32]. Two decades ago it was reported that interleukin-1 beta stimulates resorption and inhibits bone formation both *in vitro* and *in vivo* [13]. Several years later it was shown that interleukin-1 alpha and interleukin-1 beta blockade prevents cartilage and bone destruction [33]. IL-1 beta is one of the suggested factors involved in bone remodelling regulation, acting as local effectors, possibly under the control of PTH [34]. Interleukin-1 is essential for systemic inflammatory bone loss [35]. Interleukin-1 gene polymorphism is associated with bone mineral metabolism in rheumatoid arthritis [36]. We found a significant, positive correlation between IL-6 and IL-1 β levels, however this does not allow us to hypothesise the association between circulating levels of both interleukins and bone mineral metabolism. Lack of correlation between interleukin and RANK-RANKL-OPG system in our study could be explained by the age of the patient, as the majority of our patients were prepubertal so gonadal dysfunction did play any role in this period of life.

Our study is the first clinical report on the correlations between IGF-1, OPG/RANKL/RANK system, interleukins, and BMD after HSCT performed in children. Present data indicates that factors influencing bone remodelling undergo dynamic changes in the post-transplantation period in children and adolescents. It may be suggested that serum IGF-1 and RANKL could be predictors of bone metabolism after transplantation in paediatric patients, but these results require further study on a larger population.

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