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REVIEW PAPER

Application of bone turnover markers in skeletal and extra-skeletal conditions in children and adults

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ABSTRACT

The assessment of bone health is challenging. It comprises bone histomorphometry, imaging techniques and laboratory parameters including bone turnover markers (BTMs). The latter are bone-derived products which can be measured in biological fluids, including blood and urine. Considering the disadvantages and limitations of imaging techniques, BTMs serve as an additional, non-invasive source of information about the metabolic activity of the entire skeleton. BTMs have found applications as research and clinical tools in a wide range of skeletal and extra-skeletal conditions, including diagnosis and treatment of osteoporosis. Unfortunately, their utility in routine clinical practice, especially in children, is still limited due to heterogenous methodology, intra- and inter-patient variability, and a paucity of accurate reference ranges and approved guidelines. Therefore, there is a need for standardized clinical trials on currently available and new BTMs.

KEY WORDS:

clinical utility, bone turnover markers, bone metabolism, diagnostic tools.

INTRODUCTION

In human ontogenesis the bone tissue undergoes continuous transformation that includes growth and adaptation to changing conditions (modeling) and maintenance in a state of well-being (remodeling). The main purpose of the former is to shape bones and increase their mass. Hence, it occurs primarily during developmental age as a physiological adaptation to changing physical conditions and in adulthood as a mechanism of bone repair. The primary role of remodeling, on the other hand, is renewal of existing bone tissue and repair of microdamage. In addition, it enables the maintenance of calcium-phosphate homeostasis [1]. Bone modeling includes both bone formation as the result of the action of osteoblasts and resorption triggered by osteoclasts [1]. In children, bone formation predominates over resorption, and the bone turnover rate is up to three times higher than in adults [2].

The cycle of bone remodeling includes phases of activation, resorption, formation and resting. It is initiated by the activation of osteoclasts, mainly forced by natural apoptosis and microdamage of osteocytes. The disruption of cytoplasmic connections between the latter cells triggers the secretion of the receptor activator for nuclear factor κB ligand (RANKL). The binding of RANKL with the RANK receptor, which activates nuclear factor κB on the surface of osteoclast progenitor cells, is crucial for the induction of osteoclastogenesis [1]. The opposite effect is exhibited by osteoprotegerin (OPG), whose soluble form acts as a decoy receptor for RANKL. Bone formation is initiated by the migration of osteoblasts into the bone resorption cavity. The main regulator of this process is the canonical Wnt/β-catenin signaling pathway, which activates mesenchymal cells to differentiate into osteoblasts [1]. As soon as osteoid production and its initial mineralization are completed, osteoblasts undergo apoptosis or differentiation into osteocytes [1].

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Assessing bone condition is challenging. It comprises histomorphometry, imaging techniques and laboratory biomarkers. The transiliac biopsy with histomorphometry with double-labeled tetracycline is considered the gold standard for the evaluation of bone structure as well as dynamic processes of bone formation and resorption [2]. To determine bone turnover, the bone formation rate/bone surface (BFR/BS) measure is mostly used, but this parameter reflects directly only bone formation while the resorption rate is assumed indirectly [3]. Another limitation is related to the possible non-representativeness of a single sample obtained from the iliac crest biopsy [2].

Dual-energy X-ray absorptiometry (DXA) is the method of choice for assessing bone mineral density (BMD) and, to a lesser extent, fracture risk. The results of DXA in children may be influenced by motion artefacts and growth disorders, which may lead to both under- and overestimation of BMD. It is caused by DXA methodology, reflecting areal density and not volumetric, three-dimensional BMD [4].

Other imaging techniques proposed for bone evaluation, such as quantitative computed tomography (QCT), high-resolution QCT, magnetic resonance imaging (MRI), high-resolution MRI and bone quantitative ultrasound (QUS), are still not widely used in clinical practice [5]. Nuclear medicine is considered as a suitable tool in the evaluation of different bone conditions. Isotopic methods have applications mainly in bone metastases and traumas but also in metabolic diseases [2]. The uptake of radiotracer, usually technetium 99m-labeled bisphosphonates, by osteoblasts allows the identification of sites of increased metabolic activity and vascularization [6].

A novel sensitive method for the diagnosis of bone mineralisation disorders is the assessment of calcium isotope concentrations in serum and urine, although the results may be disturbed by factors such as dietary calcium intake [7].

Bone turnover markers (BTMs) are bone derived products which can be measured in blood and urine. Considering the disadvantages and limitations of imaging techniques, they provide an additional, non-invasive source of information about the metabolic activity of the entire skeleton. BTMs are traditionally classified into two groups, reflecting both bone formation and resorption. The former group mostly includes bone alkaline phosphatase (BALP), osteocalcin (OC) and procollagen type I propeptide (PINP), while the latter mostly includes C-terminal telopeptide of type I collagen (CTX-I) and tartrate-resistant acid phosphatase type 5b (TRAP5b). There is also a group of BTMs, mainly of osteocyte origin, whose role is complex: sclerostin (SCL), fibroblast growth factor 23 (FGF23), OPG, periostin. More recently, new BTMs have been proposed: cathepsin K and Dickkopf-1 (Dkk-1) (Table 1).

Historically, the first non-specific bone marker – serum alkaline phosphatase (ALP) –was found about a century ago, and the first bone-specific marker – OC – has been assayed since 1980s. BTMs are usually determined with enzyme-linked immunosorbent assay (ELISA) and validated against bone histomorphometry or DXA [2]. Although some of them are already recommended for evaluation of bone mineralization and their usefulness has been reported in many conditions, the proper interpretation of BTM values may be challenging, mainly due to their biological variability and methodological reasons [8]. BTMs exhibit a circadian rhythm, which is generally expressed to a greater extent for bone resorption than for formation markers [9]. Therefore they should be assessed in standard conditions, usually after overnight fasting, as food intake can significantly affect their levels in biological fluids [9]. BTMs are strongly age-dependent, usually reaching their highest values during adolescence [2]. In early childhood, serum BTM concentrations do not differ between the sexes, but after puberty, they are usually higher in males due to the higher bone mass [2, 9]. Overall, BTMs reach the lowest levels in the fourth decade in women and in the fifth in men. In the former, they increase significantly during menopause and remain relatively high thereafter [9]. Physical activity affects serum BTM levels [2, 9]. Therefore, refraining from intense exercise for 24 hours before assessment is recommended [9]. BTMs' evaluation may also be affected by medications, including antiepileptic drugs, hormonal contraception, glucocorticoids or antiosteoporotic agents [2].

BONE FORMATION MARKERS

BONE ALKALINE PHOSPHATASE AND ALKALINE PHOSPHATASE

BALP is an enzyme that is released from immature osteoblasts [2]. It is responsible for breaking down pyridoxal phosphate, which is an inhibitor of hydroxyapatite formation. Along with its hepatic and intestinal – as well as, in pregnant women, placental –isoforms, BALP accounts for the total ALP activity. Therefore, in contrast to BALP, ALP may only be treated as a bone marker in the case of intact liver and bowel function [2]. In early childhood, serum ALP and BALP concentrations are not sex-dependent. They reach a peak in early adolescence, which is more prominent in males [8]. In children, serum BALP is related to anthropometric factors and growth velocity [8]. Fasting and circadian rhythm do not significantly affect it. As BALP is not influenced by glomerular filtration rate (GFR), it is suitable in patients with impaired kidney function [8]. Similarly to ALP, serum BALP reference values for both adults and children have been established [10, 11].

In hypophosphatasia (HPP), a congenital metabolic bone disease, mutations of the *ALPL* gene encoding the tissue (liver/bone/kidney) non-specific ALP lead to a persistently low serum ALP concentration [12]. HPP is characterized by the accumulation of inorganic pyrophosphate, which is an inhibitor of bone mineralization. Therefore, HPP manifests as rickets or osteomalacia and

TABLE 1. Characteristics of selected bone turnover markers

	BTM	Main source	Importance for bone tissue	Elevated serum levels	Decreased serum levels
Bone formation markers	Bone alkaline phosphatase (BALP)	Osteoblasts	Impact on osteoid formation and mineralization. Indicator of osteoblastic activity	Osteoporosis, osteogenesis imperfecta, bone neoplasms	Hypophosphatasia
	Osteocalcin (OC)	Osteoblasts	Regulation of mineralization. Indicator of osteoblastic activity	Osteoporosis, fibrous dysplasia	DM, obesity
	Procollagen type I propeptide (PINP)	Osteoblasts	Peptide released during collagen type I synthesis. Indicator of proliferating osteoblasts, fibroblasts, and bone formation	Hyperparathyroidism, Paget's disease, bone metastasis	DM, osteomalacia, CKD
Bone resorption markers	C-terminal telopeptide of type I collagen (CTX-I)	Osteoclasts	Peptide released during collagen type I degradation. Indicator of bone resorption	Osteoporosis, bone metastasis	Hypoparathyroidism, hypothyroidism, malnutrition
	Tartrate-resistant acid phosphatase 5b (TRAP5b)	Osteoclasts	Specific enzyme of osteoclasts involved in bone matrix degradation	Osteoporosis, thalassemia, sickle cell anemia, osteosarcoma	Hypothyroidism
	Cathepsin K	Osteoclasts	Osteoclast released protease. Degradation of bone organic matrix	Osteoporosis, Paget's disease, osteoarthritis, atherosclerosis	Osteopetrosis, pycnodysostosis
Other BTMs	Dickopf-1 (DKK1)	Osteoblasts	Inhibits Wnt signaling pathway, affecting bone formation	Osteoporosis, CKD, MM, bone metastasis, atherosclerosis	Fresh fractures
	Fibroblast growth factor 23 (FGF23)	Osteocytes, osteoblasts	Regulates phosphate and vitamin D metabolism	ADHR, XLH, TIO, CKD	Hypoparathyroidism
	Osteoprotegerin (OPG)	Osteoblasts, osteocytes	Regulates bone resorption by inhibiting osteoclast activity	Arteriosclerosis, DM	Juvenile Paget's disease
	Sclerostin (SCL)	Osteocytes	Inhibits osteoblast activity by acting as a decoy receptor	Osteoporosis, DM, CKD	Van Buchem disease

ADHR – autosomal dominant hypophosphatemic rickets, CKD – chronic kidney disease, DM – diabetes mellitus, MM – myeloma multiplex, TIO – tumor-induced osteomalacia, XLH – X-linked hypophosphatemia.

dental hypoplasia [12]. In contrast, serum ALP is highly elevated in patients with Paget's disease, a condition leading to dysregulation of bone remodeling and serious skeletal deformities [13]. It is also used to monitor the effectiveness of treatment in this disease [13].

A higher serum BALP level is observed in osteoporosis [14] and is found to be significantly reduced by antiresorptive treatment [15]. For instance, in children with osteogenesis imperfecta, serum BALP concentration decreases upon therapy with intravenous bisphosphonate [16]. Moreover, it is observed in adults during anti-osteoporotic therapy and it is believed to be strongly correlated with a lower risk of vertebral fractures [15].

The level of BALP may have prognostic value in bone neoplasms [17, 18]. For instance, in children with osteosarcoma, high serum BALP concentrations may correlate with disease progression or recurrence and poor prognosis [17]. BALP is also a promising biomarker in the monitoring bone metastases in various neoplasms [19].

OSTEOCALCIN

OC is a small protein consisting of 49 amino acids. It is encoded by the *BGLAP* gene synthesized by osteoblasts, odontoblasts and hypertrophic chondrocytes [2]. Vitamin D and glucocorticoids are among the key modulators of its production. OC expression is also influenced by insulin level and glucose metabolism [2]. Gamma-carboxylation allows OC to bind to hydroxyapatite, enabling its incorporation into the bone matrix [20]. Only the circulating undercarboxylated form of OC functions as a hormone [20].

In both sexes, serum OC concentrations are highest during puberty and gradually decline after reaching a peak in early adulthood [21]. Its upper normal level at the age of 19 years is a positive predictor of BMD and bone size in young men [22]. Serum OC concentrations exhibit diurnal variation, with the peak during the night and the trough in the afternoon [23]. Efforts to define

reference values for OC have been undertaken in both adult and pediatric populations [10, 24].

In postmenopausal women, serum OC values are negatively correlated with BMD and its high concentration is considered as a risk factor of fractures [25, 26]. Therefore its decrease during antiosteoporotic treatment is expected [27]. In children with fibrous dysplasia, increased serum OC concentration is associated with disease progression [28].

Apart from its skeletal function, OC serves as an endocrine factor in various tissues. It promotes pancreatic β-cell proliferation, insulin secretion and its peripheral sensitivity. Therefore the role of OC in the pathogenesis of diabetes mellitus (DM) was analyzed [29]. It was found that children and adolescents with DM type 1 (DM1) show lower serum OC concentrations in comparison to non-diabetic controls and there is a negative correlation between serum OC and hemoglobin A_{1c} levels (HbA_{1c}) [30].

Moreover, OC enhances lipid metabolism in adipose tissue and in the liver. It affects the adiponectin expression in adipocytes and promotes the processes of lipolysis and programmed adipocyte necrosis [31]. Therefore, administration of uncarboxylated OC may be considered as a new therapeutic strategy for obesity and dyslipidemia [31]. There is a significant negative correlation between serum OC levels and obesity in children and adults [32, 33]. Moreover, decreased serum OC concentration predicts the progression of nonalcoholic fatty liver disease in obese children [34]. OC and its receptors are present in the brain, suggesting their potential roles in neurodevelopment and cognition. OC inhibits γ-aminobutyric acid production and increases the synthesis of some monoamine neurotransmitters, including serotonin, dopamine and norepinephrine [35]. Animal studies showed that OC crosses the placental barrier and enhances neurogenesis in the hippocampus of the embryo [35].

PROCOLLAGEN TYPE I PROPEPTIDE

PINP is released from collagen type I – the predominant bone protein – during a process of collagen synthesis in the bone extracellular matrix [2, 8]. In blood, PINP is present in two forms: a trimeric "intact" peptide, which is metabolized in the liver, and a monomeric peptide, excreted by the kidneys [2]. As the serum PINP is minimally dependent on the circadian rhythm and food intake, it is recommended by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) as a reference marker of bone formation [2]. To establish normative values for PINP, a few studies have been carried out in both adult and pediatric cohorts [35, 36].

In boys, an association between serum PINP concentration and bone mineral content was reported. Furthermore, this marker was non-significantly associated with reduced BMD in adolescent, amenorrheic girls [8].

PINP may be a sensitive marker in detecting osteoporosis in elderly women [38]. Elevated PINP levels may suggest excessive or dysregulated bone remodeling, potentially increasing fracture risk. However, PINP is not currently included in fracture risk calculators. Several metaanalyses based on adult studies showed a positive correlation between its serum level and the risk of fractures [39, 40]. Unfortunately, there are no data to confirm it in children.

Antiresorptive agents lower serum PINP levels, making it a suitable marker of treatment efficacy. It was observed that bisphosphonate therapy reduced PINP concentrations to the target goal in the majority of osteoporotic, postmenopausal women [41]. Furthermore, administration of the RANKL inhibitor denosumab and selective estrogen receptor modulators suppressed serum PINP concentrations as well [42]. In contrast, therapy with teriparatide, a recombinant PTH molecule, increased it in parallel to the improvement of BMD [43, 44]. Unfortunately, the role of PINP in evaluating the effectiveness of anti-osteoporotic treatment in the pediatric population has not been established yet.

Higher concentrations of serum PINP are also observed in some patients with hyperparathyroidism. As expected, they decrease shortly after parathyroidectomy [45, 46]. Furthermore, serum PINP level is elevated in Paget's disease and it seems to be superior to ALP in monitoring the disease activity [46]. Serum PINP concentration in children and adolescents with DM1 was significantly decreased [47, 48]. In addition, in newly diagnosed patients with DM2 it was found to be negatively correlated with HbA1c [49]. These observations provide some insights into the pathomechanism of increased risk of bone fractures in DM [50].

Serum PINP levels may be impaired by malignancies. Numerous adult studies have shown that PINP is a reliable marker of bone metastases [51]. In children with acute lymphoblastic leukemia (ALL), serum PINP levels are often reduced, which may be related to chemotherapy [52]. The same tendency was observed in children treated with systemic glucocorticosteroids [53].

Notably, the serum concentrations of total and monomeric forms of PINP are elevated in patients with advanced kidney failure. Therefore, only intact PINP can be used in an assessment of bone metabolism in this group [54].

BONE RESORPTION MARKERS

C-TERMINAL TELOPEPTIDE OF TYPE I COLLAGEN

CTX-I is released during the breakdown of type I collagen by the enzyme cathepsin K in the extracellular bone matrix [55]. Its serum levels strongly fluctuate in a circadian rhythm and should be assessed in a fasting status in the morning [9]. CTX-I serves as a valuable indicator of bone resorption activity in osteoporosis, which was emphasized in the IOF and IFCC recommendations [2, 8].

Its reference values in children and adults were recently published [56, 57].

In the pediatric population, serum CTX-I levels are fairly constant, with a slight peak at the onset of puberty, and are related to height [8]. It was found that in adults, elevated serum CTX-I concentration may indicate an increased fracture risk [40]. As its decrease is observed upon therapy with bisphosphonates and denosumab, CTX-I may be a useful parameter in the assessment of anti-osteoporotic treatment efficacy [41, 58]. In contrast, a significant increase of serum CTX-I concentration was observed in patients with juvenile idiopathic arthritis after 2 years of immunosuppressive treatment with tocilizumab [59]. Of note, serum CTX-I levels correlate only to a limited extent with joint damage [60].

As in Paget's disease bone turnover is very rapid, leading to bone matrix resorption before the process of beta-isomerization of CTX, only the nonisomerized ααCTX form may be used as a marker of the disease activity [61].

Serum CTX-I levels may be elevated in patients with bone metastasis in lung and prostate cancer [62, 63]. It was suggested that CTX-I monitoring may be even more widely used in assessing prognosis, evaluating treatment response and guiding therapeutic strategies in bone neoplasm [62, 63]. The data in pediatric patients are very limited. Children with ALL who underwent intensive chemotherapy were found to have higher serum CTX-I concentrations than healthy subjects [64].

TARTRATE-RESISTANT ACID PHOSPHATASE TYPE 5B

TRAP5b is an enzyme secreted by the osteoclasts. It is a 35 kDa protein, encoded by the *ACP5* gene [2]. TRAP5b is a hydrolase that cleaves phosphate from proteins such as osteopontin and bone sialoproteins [65]. Its activity is optimal in acidic pH environments, such as bone lacunae [65]. In children, serum TRAP5b levels are the highest at birth and then gradually decline [8].

Serum TRAP5b concentration is found to be elevated in patients with increased bone resorption and is used to monitor the effectiveness of anti-osteoporotic treatment in adults [66]. As the serum TRAP5b level is not affected by GFR and dialysis, it is proposed as an accurate BTM for patients with kidney failure [65].

Serum TRAP5b reference values in children were proposed [8]. Accordingly, elevated TRAP5b values have been reported in different pediatric conditions including steroid therapy and osteopathy due to sickle cell disease [67, 68]. Increased serum TRAP5b values were also observed in children with osteosarcoma, whereas high TRAP5b concentrations in the fluid of bone solitary cysts are thought to be associated with an increased risk of postoperative recurrence [69, 70]. By contrast, children with congenital hypothyroidism and low bone turnover show decreased serum TRAP5b levels [71].

OTHER BONE TURNOVER MARKERS

SCLEROSTIN

SCL is a glycoprotein encoded by the *SOST* gene and primarily produced by osteocytes [72]. SCL is mostly considered an inhibitor of bone formation, suppressing the activity of osteoblasts by antagonizing the Wnt/β-catenin signaling pathway [73]. In children, serum SCL concentrations are significantly higher in boys than in girls and decrease in late adolescence [74].

It was reported that serum SCL concentrations positively correlate with a higher risk of bone fractures [75]. Furthermore, they are positively associated with the bone cortical porosity index in children [74].

Of note, in van Buchem disease and sclerosteosis, the serum level of SCL is strongly decreased due to the *SOST* gene mutations in the latter or chromosomal deletion involving this gene in the former. In these conditions, reduced production or loss of function of SCL leads to excessive bone formation and abnormally high bone density [76].

It was reported that circulating SCL values are significantly higher in adult DM2 patients compared to healthy controls. In the former group a positive association between serum SCL level and subclinical atherosclerosis was noted [77]. Furthermore, increased serum SCL concentrations were found in children and adolescents with DM1 [78]. In pediatric patients with obesity, a negative correlation between SCL and HOMA-IR levels suggests its role in the pathomechanism of insulin resistance [79]. Serum SCL levels are elevated in adult patients with chronic kidney disease (CKD) [80]. Surprisingly, in children and adolescents, no association between serum SCL concentrations and stages of CKD was found [81].

SCL has become a potential therapeutic target for conditions associated with low bone mass. The effectiveness of romosozumab, an anti-SCL monoclonal antibody approved for the treatment of osteoporosis in postmenopausal women at high risk of fractures, was recently confirmed [82]. Its potential usefulness in children and adolescents has been discussed [83].

FIBROBLAST GROWTH FACTOR 23 AND A-KLOTHO PROTEIN

FGF-23 is a glycoprotein of 251 amino acids which is synthesized mainly in osteocytes and osteoblasts [84]. It regulates phosphate homeostasis by binding to the receptor complex, consisting of the FGF receptor (FGFR) and α-Klotho protein (α-KL), in renal proximal tubular cells. Thereby, FGF23 activates signaling pathways that lead to the downregulation of sodium-phosphate co-transporters, reduces tubular phosphate reabsorption and increases urinary phosphate excretion [85]. In parallel, FGF23 suppresses the synthesis and activation of calcitriol as well

as inhibiting the production and release of PTH [86]. Serum FGF23 concentrations in the pediatric population are age- and sex-independent [87].

Mutations in the *FGF23* gene result in dysregulated production and function of FGF23 [86]. In individuals with autosomal dominant hypophosphatemic rickets (ADHR), the mutated FGF23 protein is resistant to proteolytic cleavage leading to its overexpression, hypophosphatemia and disturbed bone mineralization [86]. Serum FGF23 levels are also elevated in X-linked hypophosphatemic rickets (XLH) caused by the *PHEX* gene mutation [88]. Similar symptoms are described in tumor-induced osteomalacia (TIO), a rare paraneoplastic syndrome caused by tumoral overproduction of FGF23 [89]. In this condition, serum FGF23 concentrations decrease after the surgical removal of the tumor. Non-operative management includes therapy with the anti-FGF23 monoclonal antibody burosumab, originally approved for the treatment of XLH in children and adults [88, 90].

Several studies have revealed a significant negative correlation between FGF23 levels and BMD in osteoporosis in adults, but other reports failed to find a similar association [91].

Elevated serum FGF23 levels are also observed in CKD [92]. They are inversely correlated with GFR [93]. In children and adults with CKD, the increase of serum FGF23 concentration appears even before the onset of secondary hyperparathyroidism, making it an early marker of calcium-phosphate disturbances [94]. In pediatric CKD patients, a high FGF23 level is positively correlated with the risk of left ventricular hypertrophy [95]. It is also a risk factor for the development of acute kidney injury in critically ill children and after cardiac surgery [96, 97].

Although α-KL protein is not a BTM, it plays an important role in the regulation of bone formation, both directly and indirectly as a co-receptor for FGF23 [98]. It is a transmembrane protein produced mainly by the distal renal tubular cells, but is also expressed in the brain, liver and blood vessels. Due to proteolytic cleavage, a soluble form of α-KL is released into circulation and may be detected in body fluids. Its biological role is not limited to being a co-receptor for FGFR [98]. α-KL is involved in the regulation of signaling pathways that are associated with aging [98]. Through its pleiotropic effects, it also influences the processes of neoplasia, cellular integrity, inflammation, oxidative stress and many others [98, 99]. Therefore, α-KL deficiency may lead to endothelial dysfunction, hyperphosphatemia, hypercalcemia, progressive aging and specific age-related disorders [100].

It has been reported that girls have higher serum α-KL levels than boys and their values are higher in both genders in the pubertal period. In children, there is also a strong positive correlation between serum α-KL and IGF-1 concentrations [101]. The average serum α-KL values decrease with age, and they negatively correlate with the all-cause mortality ratio [102]. Therefore, higher levels of serum α-KL are linked to an increased lifespan [103]. Strategies aimed at enhancing α-KL expression may hold promise for extending the healthy lifespan and delaying the onset of age-related disorders [104].

It was reported that α-KL plays a significant role in the regulation of bone formation [105]. The canonical FGF23/FGFR/α-KL signaling pathway is related to the regulation of bone extracellular matrix mineralization and calcium-phosphate homeostasis [105]. Moreover, α-KL upregulates osteoclastogenesis through activation of the NF-κB signaling pathway [106]. In adults, a correlation between low serum α-KL concentration and the occurrence of osteoporosis was found [107]. Unfortunately, there is a lack of similar data in the pediatric population.

OSTEOPROTEGERIN

OPG is a glycoprotein included in the tumor necrosis factor receptor (TNFR) superfamily [108] secreted by osteoblasts, but it is also expressed in other tissues, such as the liver, spleen and circulatory system [109]. OPG acts as a decoy receptor for RANK and prevents its binding to RANKL. Thus, OPG inhibits osteoclast differentiation and prevents excessive bone resorption [108]. Therefore the RANKL antagonist denosumab was used successfully in osteoporosis treatment [110]. The result of loss of function mutations in the *TNFRSF11B* gene encoding OPG is the juvenile Paget's disease. It leads to a very high bone turnover, which manifests as bone deformities, hearing loss, retinopathy and vascular calcification [111]. Several studies in adults and children have suggested an impact of *TNFRSF11B* polymorphisms on BMD disturbance [112, 113]. The data on serum OPG concentrations in women with postmenopausal osteoporosis are conflicting. While some indicate elevated values [114], others show the opposite results [115]. On the other hand, antiosteoporotic treatment caused an increase of OPG levels in this group [116]. Serum OPG concentrations seem to be positively correlated with the risk of arteriosclerosis and coronary artery disease [117]. They are also significantly higher in diabetic children than in healthy controls [118]. The RANK/RANKL/OPG system has a proven role in the pathogenesis of bone metastasis, particularly in breast and prostate cancer, and appears to be a potential treatment target [110].

NEW BONE TURNOVER MARKERS

Cathepsin K and Dickkopf-1 have been proposed for the evaluation of bone metabolism in adults [119]. The former is a catalytic enzyme that degrades type I collagen and may serve as bone resorption marker. Its serum concentrations have been found to be elevated in patients with osteoporosis, Paget's disease and some rheumatic disorders [119]. Its connection with cardiovascular diseases was also found [119]. Dkk-1 is a protein that acts as

an inhibitor of the Wnt/β-catenin signaling pathway. Its utility has been considered in evaluation of bone mineralization disorders and vascular calcification [119]. Recently, various circulating microRNA molecules such as miR-34a and miRNA-133a were proven to be involved in the regulation of bone homeostasis. Therefore, they have become proposed as biomarkers in several bone diseases, such as osteoporosis and osteogenesis imperfecta [120]. So far, research on new BTMs in the pediatric population is very limited [121].

CONCLUSIONS

The development of BTMs represents significant progress in the assessment of bone metabolism. Currently available BTMs have found applications as research and clinical tools in a wide range of skeletal and extra-skeletal conditions. However, their utility in routine clinical practice is still limited, mainly due to problems with the interpretation of results. It is caused by heterogenous methodology, intra- and inter-patient variability, and a paucity of accurate reference ranges and approved guidelines. Therefore there is a need for standardized clinical trials based on large groups of patients, both in adults and children. An obstacle to more widespread determination of BTMs is still the cost of assays, especially when they are performed manually. A search for new, more clinically useful BTMs would clearly be welcomed.

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