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Biomarkers of Bone Turnover: Potential, Challenges and Pitfalls from the Laboratory Point of view

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Abstract

The present review aim to describe the most commonly used serum bone formation and resorption biochemical markers, discuss their advantages and disadvantages and give practical information on their use and result interpretation in the laboratory and clinical settings according to current recommendations from International Scientific Societies.

Keywords: Osteoporosis; Biochemical markers; Pathophysiology

Introduction

Bone turnover markers (BTM) may give information on bone formation and resorption, risk of fracture and response to treatments [1]. BTMs have been extensively studied as markers in the diagnosis and monitoring of osteoporosis (OP), and resulted potentially useful as tools to evaluate the estimation of fracture future risk, although their significance was essentially demonstrated helpful to monitor efficacy of anti-OP treatments [2]. Other possible application includes the prediction rate of bone loss, the identification of secondary OP, the improvement of targeted treatments and patient compliance, although other data are needed in such areas [3]. However, they are influenced by a number of pathophysiological factors, and by analytical aspects, still need to be overcome to extend their application and significance in the clinical practice [1]. Thus, BTMs practical use requires careful awareness of their advantages as well as their limitations to interpret results produced by the laboratory.

The present review aim to describe the most commonly used serum bone formation and resorption biochemical markers, discuss their advantages and disadvantages and give practical information on their use and result interpretation in the laboratory and clinical settings according to current recommendations from the International Osteoporosis Foundation (IOP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), as well as the US National Bone Health Alliance (NBHA) [4,5].

Bone Remodelling

Bone is a dynamic tissue, characterized by a continuously renewed through processes of bone removal parallel to bone formation and replacement, which occur in the so-called basic multicellular units (BMU). Main cells in the BMU are osteoblasts, deputed to bone formation, and osteoclasts, to bone resorption.

Osteoblasts not only release a variety of factors, which regulate bone formation, but also drive osteoclast maturation, which requires stimulation by RANKL expressed on osteoblasts [3,6]. The different proteins or released degradation products can be measured in blood or urine samples, representing reliable tools to assess the dynamic nature of bone tissue (Table 1).

Abbreviation	Name	Origin	Index
OC	Osteocalcin	Matrix protein	Generally formation
BALP	Bone-specific alkaline phospatase	Osteoblast enzyme	Formation
P1NP	propeptide of type I collagen	Collagen formation products	Formation
P1CP	propeptide of type I collagen	Collagen formation products	Formation
DPD	Deoxypyridinoline	Collagen degradation product	Resorption
PYD	Pyridinoline	Collagen degradation product	Resorption
NTX	N-terminal crosslinked telopeptide of type I collagen	Collagen degradation product	Resorption
CTX	C-terminal crosslinked telopeptide of type I collagen	Collagen degradation product	Resorption
TRACP	Tartrate-resistant acid phosphatase	Osteoclast enzyme	Resorption
CatK	Catepsin K	Osteoclast enzyme	Resorption
PN	Periostin	Osteoblast-specific factor	Formation
OPG	Osteoprotegerin	Osteoblast-osteoclast regulating biomarkers	Bone resorption regulation
SOST	Sclerostin	Osteocyte product	Bone formation inhibition

FGF-23	Fibroblast growth factor-23	Osteocyte-osteoclast product	Modulation of phosphate and calcitriol levels

Table 1: Traditional, most actually used and new proposed bone turnover biomarkers.

Bone resorption markers include collagen breakdown products, but also the less used pyridinoline rings, or osteoclast-specific enzymes (tartrate-resistant acid phosphatase) [3,6]. Conversely, bone formation markers include non-collagenous matrix proteins, such as osteocalcin (OC), precursor molecules of collagen type I synthesized by osteoblasts, and osteoblast-specific enzyme [3, 6].

However, as of today the most utilized BTMs in the clinical settings included OC, bone specific alkaline phosphatase (BALP), procollagen type I N propeptide (P1NP), and carboxy-terminal cross-linking telopeptide of type I collagen (CTX) [3,6]. In particular, serum PINP (s-PINP) and serum CTX (s-CTX) have been suggested by main International Scientific Societies (IOF, IFCC, and NBHA) as reference biomarkers of bone formation and resorption respectively in OP, although further data are needed to expand their laboratory and clinical use [4-6].

Biological and pre-analytical variability

Recent literature confirms that serum BTMs are still subjected to a variety of pre-analytical sources of variability that included uncontrollable as well as controllable variables (Table 2). The first group includes aging, gender, menopausal status, pregnancy and lactation, diseases that impact bone health (as diabetes, liver disease, and renal impairment), and recent fracture and immobility (Table 2). Conversely, controllable factors include sampling time (circadian variability) and sample processing (collection, handing and storage), fasting status and exercise (Table 2).

Biomarker	Pre-analytical issues	Analytical issues	Post-analytical issue
Osteocalcin	High biologial variability/Circadian rythm	Presence of intact protein and fragments	Reference ranges not well established
	Influenced by renal function	Many assays available, with high between assay variability	Need for common measurement units
	Tissue-specific biomarker	High variability between laboratories using the same method	Need for common reporting name/ abbreviation
	Small diet influence		Need for EQAS
	Sample instability :		
	-avoid lipemic samples (links to lipids)		
	- avoid hemolized sample (erythrocyte hydrolase degradation)		
	-avoid Freeze/Thaw Cycles		
	Aging-related increase		
	Diseases or drugs with impact on bone metabolism		
	Exercise, immobility and fractures		
Bone-specific alkaline phospatase	Long half-life (1-2 days), small circadian effects	More assays available, between assay variability	Reference ranges not well established
	No significant renal function effects	High variability between laboratories using the same method	Need for common measurement units
	Cross-reactivity with liver isoform		Need for common reporting name/ abbreviation
	Increased enzyme activity at room temperature		Need for EQAS
	Aging-related increase		
	Diseases or drugs with impact on bone metabolism		
	Exercise, immobility and fractures		
N-terminal crosslinked telopeptide of type I collagen	Stability at room temperature/Small circadian rythm	Intact (trimeric form; IDS-iSYS , Immunodiagnostic Systems, UK)	Reference ranges not well established
	Influenced by renal function	and total (trimer and monomer; Roche Elecsys, Roche diagnostics, Germany)	Need for common measurement units
	Mostly derived from Type I collagen	High variability between laboratories using the same method	Need for common reporting name/ abbreviation
	Food intake dependence		Need for EQAS
	Seasonal rythm		
	Variation during menstrual cycle		
	Age and menopausal status		
	Diseases or drugs with impact on bone metabolism		
	Exercise, immobility and fractures		

C-terminal crosslinked telopeptide of type I collagen	Circadian and seasonal rythm	Standard well characterized (8 aminoacid peptide)	Reference ranges not well established
	Influenced by liver and renal function	Manual and automated (Beta CrossLaps Roche Elecsys, Roche diagnostics, Germany	Need for common measurement units
	Mostly derived from Type I collagen	and CTx IDS-iSYS , Immunodiagnostic Systems, UK) assays available	Need for common reporting name/ abbreviation
	Food intake dependance	High variability between laboratories using the same method	Need for EQAS
	Variation during menstrual cycle		
	Age and menopausal status		
	Diseases or drugs with impact on bone metabolism		
	Exercise, immobility and fractures		

Table 2: Main characteristic and sources of variability of the most utilized BTM.

In particular, during the menopause transition, a decline in ovarian function beginning about 2 years before the final menstrual period is follow by an increase in bone resorption and subsequently by bone loss [7]. For it concerns variation related to menstrual cycle, CTX resulted highest at the start of the cycle (when oestrogens are lowest), decreased significantly from T 0 to T 26 (when oestrogens are highest, in the pre-ovulatory period, and when progesterone activity is highest, in the advanced luteal phase) and then increased again from T 26 to T 01 [8]. Accordingly, other recent data suggested that during 17-β estradiol administration CTX levels decreased, whereas P1NP concentration increased [9]. Serum OC and BALP seem not to significantly vary during the menstrual cycle [10, 11].

Physiological bone turnover has a circadian rhythm, and OC as well as P1NP and CTX are higher in early morning [6]. Postprandial suppression of bone resorption is considered one of the main contributors in the circadian rhythm of bone turnover markers. Accordingly, after oral glucose load, a reduction was observed for BTM, especially CTX (46.9% for β-CTX, 7.9% for P1NP, and 8% for OC) [12].

Seasonal variation may account for a significant percentage of BTMs variability, almost in part related to vitamin D decreased in winter, as evidenced by recent data in children and adults [13-15]. In particular, 25(OH) vitamin D was found higher and CTX lower during summer when compared to winter, especially in black children (15). In fact, although black children reported less sunscreen use and less travel to more sunny locations during holidays compared to whites, being exposure to sunlight (UV irradiation) the major source of vitamin D is derived from, skin pigmentation may significantly reduce UVB effects [16].

Elite athletes (who performs at a high level in a competitive sport, or professional athletes) showed an higher bone turnover than sedentary subjects, although a single session of exercise is insufficient to modify BTM concentration, and effects largely depends on the type, intensity and duration of physical activity, with bone formation markers more sensitive than bone resorption markers [17-20]. Conversely, immobility exacerbates bone loss and increased bone resorption and CTX levels, as reduced mobility accelerates the bone loss due to aging and increase CTX and P1NP levels in elderly subjects (n=111) [21-22]. Moreover, BTMs are increased following a fracture, and subjects with increased frailty risk had significantly high levels of PINP, β-CTX and PTH as well as low levels of 25(OH)D [23, 24].

Increasing data underlie the relevance of bone metabolism and turnover biomarker concentration on type 2 diabetes mellitus (T2DM) pathophysiology [25-27]. In particular, OC has been significantly related to glucose metabolism, through the increase of insulin release and sensitivity and energy expenditure, and reduction of visceral fat [24-28,29]. In a general population, an inverse relationship was found between OC and fasting plasma glucose, fasting insulin, and homeostasis model assessment-estimated insulin resistance (HOMA-IR) [30]. In patients with T2DM, serum OC levels resulted negatively related to glucose and fat mass, and atherosclerosis surrogates (brachial-ankle pulse wave velocity and carotid intima-media thickness, IMT), and positively to total adiponectin levels [30, 31]. In this context, two very recent meta-analyses confirm that OC results significantly lower in T2DM patients than controls, and it is inversely associated with the development of T2DM [32, 33].

Other sources of variability are related to sample handling. In particular, OC decrease with hemolysis and if left to room temperature even for few hours or underwent successive freeze-thaw cycles, subjected to rapid degradation in sample often characterized by presence of heterogeneous OC fragments, and can be present in undercarboxylated form [4, 6]. This form results mainly associated with enhanced cell function, while the carboxylated form appears more involved in improved insulin sensitivity [34]. Renal function can also affect OC levels, although OC evaluation retains important advantages as biomarker, as it is widely used, retains high tissue specificity, and relatively low within-subject variation [4].

The measurements of BALP still retain cross-reactivity with the liver form, which can be significant in liver disease patients [4]. Moreover, BALP half-life is long (1-2 days), which renders this biomarker less dependent to circadian rhythm than other BTMs [4]. In any case, samples must be stored at -20°C, because the activity of the enzyme increased at room temperature [4].

Serum CTX and P1NP retain high specificity, because mostly derived from bone, but CTX may vary in patients with liver or renal abnormality, whereas P1NP presents significant biological variability [4, 35].

Analytical variability

The lack of standardization is still a problem, and between-laboratory variability may be significant, rising question on the validity of comparing results from different laboratories [36]. For osteocalcin, for which several immunoassays are available, the measurement is complicated by the presence in variable amount of several fragments, which may negatively influence the reproducibility of repeated sampling (Table 2). However, the difference between BTM results may be significant even if laboratories used the same method (Table 2). For

this reason, one reference laboratory to monitor serial samples for the same patients is advocated.

Scientific Society recommendation and guidelines proposed by IOF, IFCC, and NBHA, have recommended the use of serum P1NP and CTX as the reference standard for bone formation and resorption, respectively, and advocate efforts for sample collection, standardization, establishment of reference intervals, and need for External Quality Assurance Schemes (EQAS) [1-6]. Both biomarkers retain high specificity, and for CTX the standard is well characterized as an eight-aminoacid peptide that permits the development of reference standard [4-6]. Sources of variability are well known, as well as procedures for sampling, and storage [4-6]. For it concerns method, s-P1NP is available as RIA (Orion) or automated immunoassay (Total P1NP, Roche Diagnostics, Germany; Intact P1NP, IDS-iSYS, UK), and s-CTX as ELISA (IDS, UK) or automated immunoassay (Roche Diagnostics, IDS-iSYS) [1-6].

Result reporting and interpretation

An example of available data on reference values for main BTMs is reported in (Table 3) [37-50]. BTM reference ranges are not established and may vary according to general population or patient cohort tested (Table 3). In particular, there is a great variability between individual subjects, and these values are more variable in postmenopausal women, and clearly reference ranges differ according to the method used (Table 3). Moreover, there is heterogeneity in reporting age intervals, biomarker name or abbreviation, and measurements units, which render more complex the interpretation and comparison of results.

BAP	OC	CTx	P1NP	Sex	Age (years)	Number	Reference
	10.2-41.0 µg/L (Roche)	117-740 ng/L (Roche)	18-129 µg/L (Roche)	Men	70-89	4248	37
				Men		1107	38
7.4-27.7 ng/mL (IDS-iSYS)		0.12-0.83 ng/mL (IDS-iSYS)	31.1-95.9 ng/mL (IDS-iSYS)	Men	25-29		
7.6-24.4		0.05-0.58	15.7-68.1	Men	75-79		
6.0-22.7		0.05-0.67	19.3-76.3	Premenopausal women	30-54	382	
8.1-31.6		0.09-1.05	18.2-102.3	Post-menopausal women	50-79	450	
6.0-13.6 µg/L (ELISA, IDS)	8.0-23.0 µg/L (IRMA, CisBio)	137-484 ng/L (Roche)	22.7-63.1 µg/L (Roche)	women	35-45	184	39
		109-544 ng/L (IDS-iSYS)	21.8-65.5 µg/L (IDS)	women	35-45	184	
		100-600 ng/L (Roche)	15-80 µg/L (Roche)	Men	25-70		40
		100-750	15-115	Men	>70		
		150-800	15-70	Premenopausal women	20-48		
		50-800	15-90	Post-menopausal women	50-70		
7.2-27.6 ng/mL (IDS-iSYS)	12.7-47.4 ng/mL (Roche)	0.1-1 ng/mL (IDS-iSYS)	18.3-94.1 ng/mL (IDS-iSYS)	Premenopausal women	30-39	158	41
5.2-18.6	8.8-36.4	0.05-0.63	4.2-74.5	Post-menopausal women	55-80		
			15-80 µg/L (Roche)	Men	25-70	1143	42
		170-600 ng/L (Roche)		Men	25-40		
		130-600		Men	40-60		
		100-600		Men	>60		
		150-800	25-90	Women	<30	1246	
		100-700	15-80	Women	30-39		
		100-600	15-60	Women	40-49		
		100-700	15-75 or more	Women	>50		

	4.91-3.90 ng/mL (Roche)	0.112 -0.210 ng/mL (Roche)	13.72-32.90 ng/mL (Roche)	Women	35-45	406	43
	5.58-16.57	0.100-0.378	16.89-42.43	Men	35-45	226	
5.15-15.32 ng/mL (Beckman)		0.114-0.628 ng/mL (Roche)	16.3-78.2 ng/mL (Roche)	Premenopausal women	30-39	637	44
	6.8-26.5ng/mL (ELISA, Nordic Bioscience Diagn)	0.1-0.62 ng/mL (Roche)	16.2-60.9 ng/mL (Roche)	Premenopausal women	35-45	153	45
	11.3-36.3 µg/L (Roche)	0.144-0.4 µg/L (Roche)	28-80 µg/L (Roche)	Men	40-59	33	46
	9.1-37.3	0.112-0.565	16.1-57.8	Premenopausal women	35-49	130	
	20.2-162	0.154-1.14	20-162	Post-menopausal women	48-81	56	
		0.12-0.62 mg/L (Roche)		Premenopausal women	21-39	33	47
5.8-17.5 ng/mL (Beckman)		0.111-0.791 ng/mL (Roche)	17.3-83.4 ng/mL (Roche)	Premenopausal women	35-39	194	48
	1.91-4.87 ng/mL (Roche)	0.07-0.61 ng/mL (Roche)	14.6-63.5 ng/mL (Roche)	Premenopausal women	45-50	534	49
5.4-16.4 mg/L (Beckman)		113-675 pg/mL (Roche)	21-85 mg/L (Orion)	Premenopausal women	28-45	118	50
Data are expressed as 95% Reference Intervals							

Table 3: Reference intervals for serum BTM in adults.

Guidelines suggested the use of "least significant changes" in the follow-up under anti-osteoporotic treatment to render more meaningful the clinical decision [2]. In particular, reduction of at least 30% for serum bone turnover biomarkers has been recommended, even in OP postmenopausal women with BTM levels in the pre-menopausal range at the start of treatment [2].

Conclusion

Scientific organizations have recently recommended the measurements of sPINP and sCTX as markers of bone formation and bone resorption, respectively [4-6]. In any case, this advice do not exclude the use of other BTM, as OC or BALP, but possibly used in parallel with P1NP and CTX, when clinicians are more familiar with the other BTM or if there are previous patient data obtained by using the other BTM. Moreover, these four biomarkers have effectively different characteristics, as BALP presents cross-reactivity with the liver form and so it is not advised in patients with liver disease, but it can be preferred in patients with renal function because less influenced by this factor respect to the other biomarkers (Table 2).

Standardization on patient condition (fasting status, etc.) and control of sample timing, handling and storage are important aspects to decrease controllable variability and increase the accuracy by which BTM may reflect the rate of bone remodelling (Table 2). Significant intra-method as well as inter-assay differences exists for BTM (Table 2). This analytical variability can be controlled referring to the same laboratory for serial assays, and standardization of methods for the reference BTM is advocated. Also a need for international reference standard, as well as the identification of reference intervals for the general population or relevant patient cohort remains a high priority target (Tables 2 and 3).

In this scenario, new possible additive biomarkers are continuously proposed, including periostin, a matricellular protein preferentially localized in the periosteal tissue, sphingosine 1-phosphate, a lipid mediator mainly involved in osteoclastogenesis, and sclerostin, an osteocyte factor, because both P1NP and CTX retain limitations related to lack of absolute specificity for bone tissue, and incapacity to reflect osteocyte activity or periosteal apposition [51].

Moreover, genome-wide studies identified genetic variants associated with bone mineral density and fracture risk, that could identify new additive biological pathways underlying bone metabolism, and provide new possibility of OP intervention and treatment [52-57].

Traceability, validation, harmonization, standardization, external quality assurance programs need to be improved to expand BTM clinical applicability. In any case, despite high variability, BTM changes are greater enough to identify subjects at high risk for bone loss and subsequent fracture, or to monitor the efficacy of OP therapies, due to BTM capacity to rapidly respond to treatments. In particular, reduction in BTMs after 3 months- 6 months of anti-resorptive therapy predict successive reduction in fracture risk, whereas the changes BMD in patients receiving therapy, particularly under anti-resorptive therapy, are not closely related to the fracture risk reduction [58]. It has been suggested that the increase in bone strength following anti-resorptive treatment may be partly explained by a reduction in trabecular perforations that might be captured in the measurement of BTMs, but not by BMD [59]. However, only clinicians aware of BTMs benefits and limitations may find in these biomarkers a useful tool in

association with other clinical, instrumental and laboratory parameters for the management of their OP patients.

References

1. Hlaing TT, Compston JE (2014) Biochemical markers of bone turnover - uses and limitations. Ann Clin Biochem 51: 189-202.
2. Lee J, Vasikaran S (2012) Current recommendations for laboratory testing and use of bone turnover markers in management of osteoporosis. Ann Lab Med 32: 105-112.
3. Brown JP, Albert C, Nassar BA, Adachi JD, Cole D, et al. (2009) Bone turnover markers in the management of postmenopausal osteoporosis. Clin Biochem 42: 929-942.
4. Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, et al. (2011) IOF-IFCC Bone Marker Standards Working Group. 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.
5. Bauer D, Krege J, Lane N, Leary E, Libanati C, et al. (2012) National Bone Health Alliance Bone Turnover Marker Project: current practices and the need for US harmonization, standardization, and common reference ranges. Osteoporos Int. 23: 2425-2433.
6. Bergmann P, Body JJ, Boonen S, Boutsen Y, Devogelaer JP, et al. (2009) Evidence-based guidelines for the use of biochemical markers of bone turnover in the selection and monitoring of bisphosphonate treatment in osteoporosis: a consensus document of the Belgian Bone Club. Int J Clin Pract 63: 19-26.
7. Sowers MR, Zheng H, Greendale GA, Neer RM, Cauley JA, et al. (2013) Changes in bone resorption across the menopause transition: effects of reproductive hormones, body size, and ethnicity. J Clin Endocrinol Metab 98: 2854-2863.
8. Mozzanega B, Gizzo S, Bernardi D, Salmaso L, Patrelli TS, et al. (2013) Cyclic variations of bone resorption mediators and markers in the different phases of the menstrual cycle. J Bone Miner Metab. 31: 461-467.
9. Limonard EJ, Veldhuis-Vlug AG, van Dussen L, Runge JH, Tanck MW, et al. (2015) Short-Term Effect of Estrogen on Human Bone Marrow Fat. J Bone Miner Res 30: 2058-2066.
10. Chiu KM, Ju J, Mayes D, Bacchetti P, Weitz S, et al. (1999) Changes in bone resorption during the menstrual cycle. J Bone Miner Res 14: 609-615.
11. Gass ML, Kagan R, Kohles JD, Martens MG (2008) Bone turnover marker profile in relation to the menstrual cycle of premenopausal healthy women. Menopause 15: 667-675.
12. Yavropoulou MP, Tomos K, Tsekmekidou X, Anastasiou O, Zebekakis P, et al. (2011) Response of biochemical markers of bone turnover to oral glucose load in diseases that affect bone metabolism. Eur J Endocrinol 164: 1035-1041.
13. Darling AL, Hart KH, Gibbs MA, Gossiel F, Kantermann T, et al. (2014) Greater seasonal cycling of 25-hydroxyvitamin D is associated with increased parathyroid hormone and bone resorption. Osteoporos Int 25: 933-941.
14. Al-Daghri NM, Alkharfy KM, Al-Othman A, Yakout SM, Al-Saleh Y, et al. (2012) Effect of gender, season, and vitamin D status on bone biochemical markers in Saudi diabetes patients. Molecules 17: 8408-8418.
15. Rajakumar K, Holick MF, Moore CG, Cohen E, Olabopo F, et al. (2014) Impact of seasonal flux on 25-hydroxyvitamin D and bone turnover in pre- and early pubertal youth. Pediatr Int 56: 35-42.
16. Vassalle C, Pérez-López FR (2013) The Importance of Some Analytical Aspects and Confounding Factors in Relation to Clinical Interpretation of Result. In: "Vitamin D: Daily Requirements, Dietary Sources and Symptoms of Deficiency" Ed Nova Publisher, Chapter 4. ISBN: 978-1-62808-816-8.
17. Banfi G, Lombardi G, Colombini A, Lippi G (2010) Bone metabolism markers in sports medicine. Sports Med 40: 697-714.
18. Rogers RS, Dawson AW, Wang Z, Thyfault JP, Hinton PS (2011) Acute response of plasma markers of bone turnover to a single bout of resistance training or plyometrics. J Appl Physiol (1985) 111: 1353-1360.
19. Scott JP, Sale C, Greeves JP, Casey A, Dutton J, et al. (2010) The effect of training status on the metabolic response of bone to an acute bout of exhaustive treadmill running. J Clin Endocrinol Metab 95: 3918-3925.
20. Corsetti R, Perego S, Sansoni V, Xu J, Barassi A, et al. (2015) Osteocartilaginous metabolic markers change over a 3-week stage race in pro-cyclists. Scand J Clin Lab Invest 75: 523-530.
21. Kim W, Lee SJ, Yoon YK, Shin YK, Cho SR, et al. (2015) Adults with spastic cerebral palsy have lower bone mass than those with dyskinetic cerebral palsy. Bone 71: 89-93.
22. Chen JS, Cameron ID, Cumming RG, Lord SR, March LM, et al. (2006) Effect of age-related chronic immobility on markers of bone turnover. J Bone Miner Res 21: 324-331.
23. Alvarez-Ríos AI, Guerrero JM, García-García FJ, Rodríguez-Mañas L, Medrano-Campillo P, et al. (2015) Associations between frailty and serum N-terminal propeptide of type I procollagen and 25-hydroxyvitamin D in older Spanish women: The Toledo Study for Healthy Aging. Exp Gerontol 69: 79-84.
24. Chubb SA, Byrnes E, Manning L, Beilby JP, Ebeling PR, et al. (2015) Reference intervals for bone turnover markers and their association with incident hip fractures in older men: the Health in Men study. J Clin Endocrinol Metab 100: 90-99.
25. Booth SL, Centi AJ, Gundberg C (2014) Bone as an endocrine organ relevant to diabetes. Curr Diab Rep 14: 556.
26. Yeap BB, Alfonso H, Chubb SA, Gauci R, Byrnes E, et al. (2015) Higher serum undercarboxylated osteocalcin and other bone turnover markers are associated with reduced diabetes risk and lower estradiol concentrations in older men. J Clin Endocrinol Metab 100: 63-71.
27. Hofbauer LC, Lecka-Czernik B, Seibel MJ (2015) Sweet and brittle - Diabetes mellitus and the skeleton. Bone.
28. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, et al. (2007) Endocrine regulation of energy metabolism by the skeleton. Cell 130: 456-469.
29. Fernández-Real JM, Izquierdo M, Ortega F, Gorostiaga E, Gómez-Ambrosi J, et al. (2009) The relationship between serum osteocalcin concentration to insulin secretion, sensitivity, and disposal with hipocaloric diet and resistance training. J Clin Endocrinol Metab 94: 237-245.
30. Pittas AG, Harris SS, Eliades M, Stark P, Dawson-Hughes B (2009) Association between serum osteocalcin and markers of metabolic phenotype. J Clin Endocrinol Metab 94: 827-832.
31. Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Kurioka S, et al. (2009) Serum osteocalcin level is associated with glucose metabolism and atheroesclerosis parameters in type 2 diabetes mellitus. J Clin Endocrinol Metab. 94: 45-49.
32. Liu C, Wo J, Zhao Q, Wang Y, Wang B, et al. (2015) Association between Serum Total Osteocalcin Level and Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. Horm Metab Res 47: 813-819.
33. Kunutsor SK, Apekey TA, Laukkanen JA (2015) Association of serum total osteocalcin with type 2 diabetes and intermediate metabolic phenotypes: systematic review and meta-analysis of observational evidence. Eur J Epidemiol 30: 599-614.
34. Hwang YC, Jeong IK, Ahn KJ, Chung HY (2009) The uncarboxylated form of osteocalcin is associated with improved glucose tolerance and enhanced beta-cell function in middle-aged male subjects. Diabetes Metab Res Rev 25: 768-772.
35. Garnero P, Vergnaud P, Hoyle N (2008) Evaluation of a fully automated serum assay for total N-terminal propeptide of type I collagen in postmenopausal osteoporosis. Clin Chem 54: 188-196.
36. Cavalier E, Souberbielle JC, Gadisseur R, Dubois B, Krzesinski JM, et al. (2014) Inter-method variability in bone alkaline phosphatase measurement: clinical impact on the management of dialysis patients. Clin Biochem 47: 1227-1230.

37. Chubb SA, Byrnes E, Manning L, Beilby JP, Ebeling PR, et al. (2015) Reference intervals for bone turnover markers and their association with incident hip fractures in older men: the Health in Men study. J Clin Endocrinol Metab 100: 90-99.
38. Michelsen J, Wallaschofski H, Friedrich N, Spielhagen C, Rettig R, et al. (2013) Reference intervals for serum concentrations of three bone turnover markers for men and women. Bone 57: 399-404.
39. Guañabens N, Filella X, Monegal A, Gómez-Vaquero C, Bonet M, et al. (2015) LabOscat Study Group. Reference intervals for bone turnover markers in Spanish premenopausal women. Clin Chem Lab Med.
40. Vasikaran SD, Chubb SP, Ebeling PR, Jenkins N, Jones GR, et al. (2014) Harmonised Australian Reference Intervals for Serum PINP and CTX in Adults. Clin Biochem Rev 35: 237-242.
41. Gossiel F, Finigan J, Jacques R, Reid D, Felsenberg D, et al. (2014) Establishing reference intervals for bone turnover markers in healthy postmenopausal women in a nonfasting state. Bonekey Rep 3: 573.
42. Jenkins N, Black M, Paul E, Pasco JA, Kotowicz MA, et al. (2013) Age-related reference intervals for bone turnover markers from an Australian reference population. Bone 55: 271-276.
43. Hu WW, Zhang Z, He JW, Fu WZ, Wang C, et al. (2013) Establishing reference intervals for bone turnover markers in the healthy shanghai population and the relationship with bone mineral density in postmenopausal women. Int J Endocrinol 2013: 513925.
44. Glover SJ, Gall M, Schoenborn-Kellenberger O, Wagener M, Garnero P, et al. (2009) Establishing a reference interval for bone turnover markers in 637 healthy, young, premenopausal women from the United Kingdom, France, Belgium, and the United States. J Bone Miner Res 24: 389-397.
45. Glover SJ, Garnero P, Naylor K, Rogers A, Eastell R (2008) Establishing a reference range for bone turnover markers in young, healthy women. Bone 42: 623-630.
46. Claudon A, Vergnaud P, Valverde C, Mayr A, Klause U, et al. (2008) New automated multiplex assay for bone turnover markers in osteoporosis. Clin Chem 54: 1554-1563.
47. Pagani F, Bonetti G, Stefini F, Panteghini M (2000) Evaluation of a fully automated assay to measure C-telopeptide of type I collagen in serum. Clin Chem Lab Med 38: 1111-1113.
48. Eastell R, Garnero P, Audebert C, Cahall DL (2012) Reference intervals of bone turnover markers in healthy premenopausal women: results from a cross-sectional European study. Bone 50: 1141-1147.
49. Adami S, Bianchi G, Brandi ML, Giannini S, Ortolani S, et al. (2008) Determinants of bone turnover markers in healthy premenopausal women. Calcif Tissue Int 82: 341-347.
50. de Papp AE, Bone HG, Caulfield MP, Kagan R, Buinewicz A, et al. (2007) A cross-sectional study of bone turnover markers in healthy premenopausal women. Bone 40: 1222-1230.
51. Garnero P (2014) New developments in biological markers of bone metabolism in osteoporosis. Bone 66: 46-55.
52. Estrada K, Styrkarsdottir U, Evangelou E, Hsu YH, Duncan EL, et al. (2012) Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet 44: 491-501.
53. Zhang L, Choi HJ, Estrada K, Leo PJ, Li J, et al. (2014) Multistage genome-wide association meta-analyses identified two new loci for bone mineral density. Hum Mol Genet 23: 1923-1933.
54. Oei L, Hsu YH, Styrkarsdottir U, Eussen BH, de Klein A, et al. (2014) A genome-wide copy number association study of osteoporotic fractures points to the 6p25.1 locus. J Med Genet 51: 122-131.
55. Kemp JP, Medina-Gomez C, Estrada K, St Pourcain B, Heppe DH, et al. (2014) Phenotypic dissection of bone mineral density reveals skeletal site specificity and facilitates the identification of novel loci in the genetic regulation of bone mass attainment. PLoS Genet 10.
56. Oei L, Estrada K, Duncan EL, Christiansen C, Liu CT, et al. (2014) Genome-wide association study for radiographic vertebral fractures: a potential role for the 16q24 BMD locus; 59: 20-27.
57. Oei L, Estrada K, Duncan EL, Christiansen C, Liu CT, et al. (2013) Genome-wide Association Study for Radiographic Vertebral Fractures: A Potential Role for the 16q24 BMD Locus versus Lessons Learned from Challenging Phenotype Definition. .
58. Delmas PD, Seeman E (2004) Changes in bone mineral density explain little of the reduction in vertebral or nonvertebral fracture risk with anti-resorptive therapy. Bone 34: 599-604.
59. Bergmann P (2008) [Clinical use of biological markers of bone turnover in osteoporosis]. Rev Med Brux 29: 295-300.