

CAT
Critically Appraised Topic

The clinical utility of bone turnover markers measurements and their use in UZL Hospital

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CLINICAL BOTTOM LINE

Bone is a dynamic organ and is constantly undergoing remodeling, maintaining a dynamic balance between osteoclastic (bone-resorption) and osteoblastic (bone-forming) activity and the function of both of them controlled by the osteocytes. Remodeling is structurally important for eliminating old bone and bone that has suffered accumulated micro-damage. It also allows the body to change the shape or composition of bones to respond to different stresses on the bones. Woven bone remodeled through this process to become lamellar bone.

In healthy adult humans, the remodeling cycle lasts 6–9 months. A representative value for the average turnover (volume replacement) of bone is 10% per year, corresponding to a mean lifespan of about 10 years and a mean age of about 5 years, but there are large differences in turnover rate and mean age between different regions of the skeleton **(1)**.

Our goals

*To find which marker(s) are the best to use in specific clinical applications (osteoporosis, chronic kidney disease, and primary/secondary bone tumors) through literature review.

*the cost efficiency of these specific markers and the possibility to cancel/exchange or apply new bone marker.

Bone formation markers are

- **Serum osteocalcin (OC)** is a major non-collagen bone protein; also called bone Gla-protein (BGP), produced by osteoblasts during bone formation, and released in part into the circulation. The flux of osteocalcin towards the serum also can result from matrix resorption, thus it is not a pure osteoblast function marker. Intact molecule OC is unstable **(2)**, has a large interlab variation **(3)** and a short half-life of a few minutes **(4)**. Therefore, a morning sample (serum or EDTA plasma) should be taken after an overnight fast, preserved with ice to prevent the rapid degradation in serum which can be an important analytical problem **(5)**. Osteocalcin concentration is dependent on renal function; its concentration is increased when glomerular filtration decreases **(6)**. OC gene is regulated at transcriptional level by 1,25-OH₂ Vitamin D. Vitamin K is an essential co-factor for γ -carboxylation of OC resulting in increased affinity for Ca and hydroxyapatite **(7)**. This test is performed in UZLeuven laboratory (in house radio-immunoassay) and is covered by RIZIV reimbursement.

- **Procollagen type 1 amino terminal propeptide (PINP)/Carboxy-terminal propeptide (PICP)**: specific products of proliferating osteoblasts and fibroblasts. They are cleaved from type 1 procollagen by protease when the collagen deposits to form the bone matrix. Serum PINP releases into circulation as a trimeric form, which is unstable at 37 °C and converts rapidly to its monomeric form. An automated electro chemiluminescent two-site immunoassay, on the Roche Elecsys analyzer, using monoclonal antibodies raised against both the monomeric and trimeric forms of the peptide, measures total serum PINP **(8)**. For PICP, which is a single protein these assays yielded disappointing results in patients with osteoporosis **(9)**.

Serum PINP has several practical advantages including its low diurnal variability and stability at room temperature **(8)**. Food intake does not significantly influence the circulating concentration of PINP. Catabolism of PINP is under hormonal control, and concentration is not dependent on renal function **(10)**. In individuals with severe liver disease, PINP clearance from the circulation might be affected resulting in elevated PINP levels. UZL laboratory does not perform the PINP test (referred to UCL Saint-Luc laboratory) and is not covered by RIZIV.

- **Serum bone specific alkaline phosphatase (BALP)**: enzyme present in osteoblast plasma membrane, responsible for the degradation of mineralization inhibitor (pyrophosphate) at alkaline pH. There are multiple methodologies to measure mass or activity **(11)**. BALP test is performed in UZL laboratory in form of electrophoresis (serum or heparin plasma sample pretreated with the lectin-'wheat-germ 'agglutinin) to differentiate alkaline phosphate isoenzymes. This test is stable, has low intra-individual variability <10%, is not affected by renal function **(12)** and there is a little effect by food **(13)**. The BALP is less sensitive to circadian rhythm because of its relatively long half-life (1–2 days) **(5)**. Unfortunately, there is a significant cross-reactivity (\pm 15%) with the liver form **(14)**. This test is a RIZIV covered test.

Bone resorption markers are

- **Beta-CrossLaps (B-CTX)**: beta isomerized carboxyl-terminal telopeptides of type I collagen. It is cleaved from type one collagen by cathepsin-k during bone resorption. It can be measured in urine, serum and EDTA plasma using immunoassays. Due to matrix variability of a urine-based test and the additional variance introduced by the creatinine adjustment, urine based tests have been replaced by serum assays **(2)**. Disadvantage of serum B-CTX is the large circadian variation and strong influence by food intake **(2)**. B-CTX is more stable in EDTA plasma than in serum **(11)**. It is measured by an electrochemiluminescence immunoassay method. It is riziv reimbursed.

- **Crosslinked N-telopeptide of Type I Collagen (NTX)**. It is cleaved from type one collagen by cathepsin-k during bone resorption. Urinary telopeptide fragments are stable in urine for days, and samples can be stored at – 20 °C for years without significant deterioration **(15)**. It's concentration is influenced by liver and renal function **(11)**. Dietary influence is small but there is significant diurnal variation (~ 20%) and standardized sampling (i.e., second morning void, fasting) is important **(16)**. It is measured by an electro chemiluminescence Immunoassay method.

-**Urinary pyridinoline (PYD) and urinary free deoxypyridinoline (DPD)**: These crosslinks (aldehyde links between lysine or hydroxylysine residues) are formed between collagen molecules and they stabilize the conjunctive tissue. They are released into the circulation and excreted into the urine when collagen catabolized. These two urinary markers are measured by immunoassays.

-**Urinary hydroxyproline**: Hydroxyproline is one of four amino acids that are the main components of the bone protein (collagen). It is formed through hydroxylation of the amino acid proline in the gastrointestinal tract. It is characterized by high diurnal variation and high dietary influence. Patients should be on a non-protein diet to avoid false positive results **(Frances T Fischbach RN, BSN, MSN, A Manual of Laboratory and Diagnostic Tests.7th ed. Lippincott Williams & Wilkins Publishers, July 2003)**.

-**Tartrate-resistant acid phosphatase-isoform 5b (TRAP5b)**: an isoform of acid phosphatase enzyme produced by osteoclasts during bone resorption. It cleaves type 1 collagen in to fragments. Kidney function or liver function do not influence TRAP5b concentration **(17)**. It is unstable at room temperature **(4)** **(18)**. Circadian variability increase by exercise **(19)**. It is measured by difficult and costly specific immunoassays.

There are also osteocyte activity markers such as Receptor Activator of Nuclear factor Kappa B Ligand (RANKL), Osteoprotegerin (OPG), Dickkopf-related protein 1 (DKK1), Sclerostin (SCL). They are considered as research methods only.

In this **CAT**, we started with the question: which labs in Flanders measure the bone markers? Which markers do they measure? Then we have done query requests in to the database information system of UZL Hospital for a recent period 01-01-2015 to 01-01-2016 for the markers (B-CTx, OC, P1NP and BALP) to

In summary: The measurement of bone turnover markers (BTMs) is complicated by large random within-patient variability, biologic variability (age, gender, body mass index BMI, circadian, and menstrual variation). These issues have confounded their widespread use in clinical practice.

see how many tests were performed per year and which the most frequent requesting departments are.

CLINICAL/DIAGNOSTIC SCENARIO

	University hospital Leuven	University hospital Brussel	University hospital Gent	University hospital Antwerpen	University hospital Saint-Luc	General hospital Sint- Jan	Jessa hospital
OC	YES	YES	X	YES	YES	YES	YES
P1NP	YES*	X	YES	X	YES	YES	X
B-CTx	YES	YES	YES	YES	YES	X	YES
NTX	X	YES	X	X	X	X	YES
DPD	X	X	X	YES	X	X	X
ISO-ALP	YES	YES	X	YES	X	YES	YES

Table 1

Overview of some laboratories in Flanders where bone markers tests are performed.

YES : Available, X : Not available. * : referred to UCL Saint-Luc laboratory.

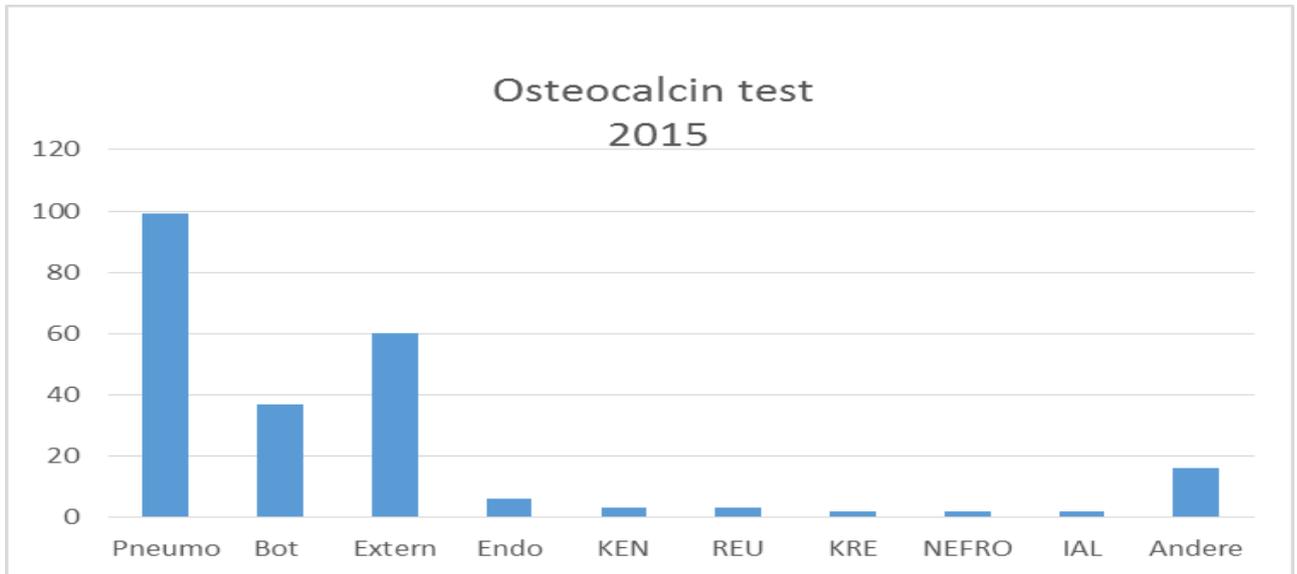


Figure 1

All osteocalcin tests performed in UZL hospital in 2015 (totally 230 test per year), divided per discipline. Bot: Multidisciplinary osteoporosis consultation, KEN: Pediatric Endocrinology, KRE: Pediatric Rheumatology, IAL: Internal medicine.

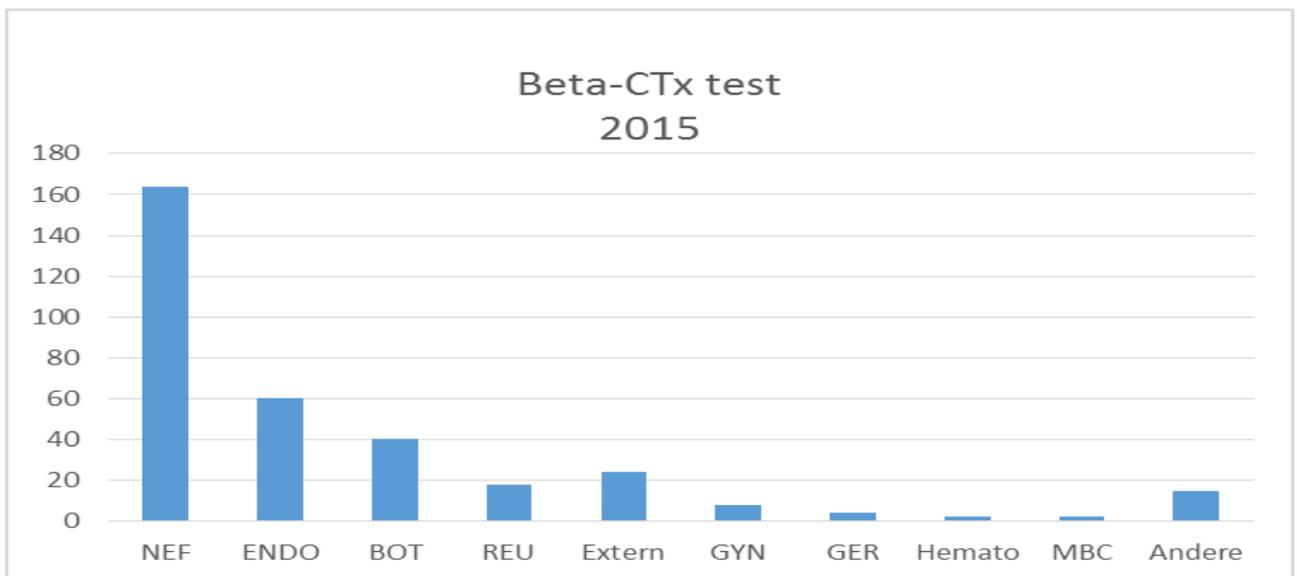


Figure 2

All Beta-CrossLaps (B-CTx) tests performed in UZL hospital in 2015 (totally 337 test per year), divided per discipline. MBC: Multidisciplinary Breast Carcinoma.

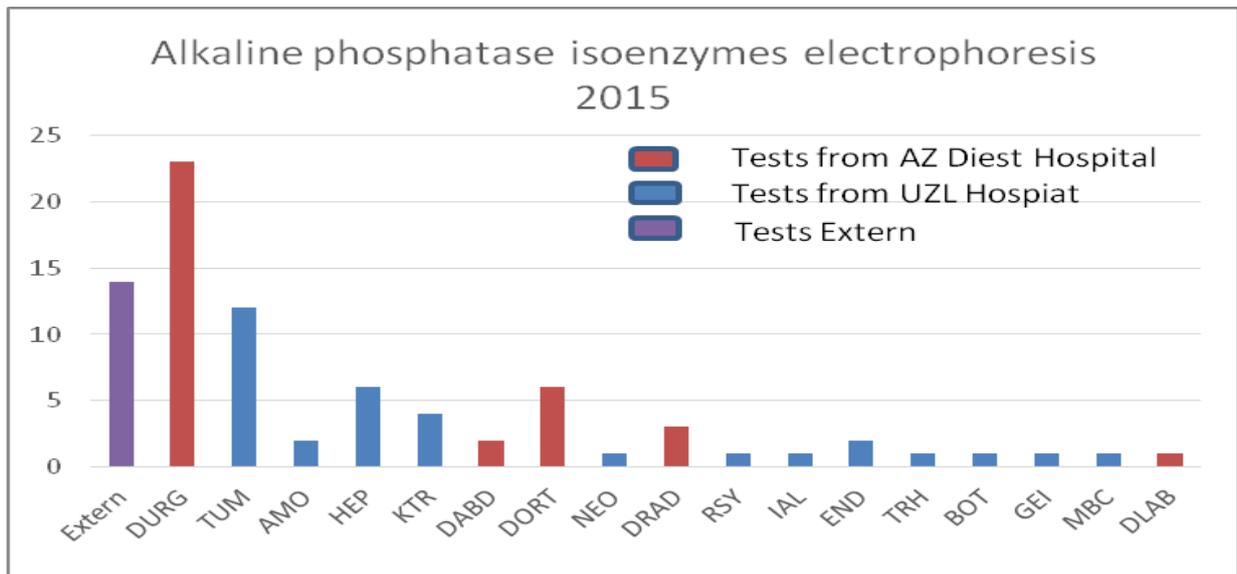


Figure 3

All alkaline phosphatase isoenzymes electrophoresis tests performed in UZL hospital in 2015 divided per discipline (totally 82 test per year).

*URG: Urgency, *TUM: Orthopaedic Tumors, *AMO: General Oncology, *KTR: Transplantation paediatrics, RSY: Rheumatoid Systemic disease, TRH: Trumatological Surgery, *MBC: Multidisciplinary Breast Carcinoma.

When looking at results of queries, we find that most OC and Beta-CTx tests requests came from specific disciplines such as Pneumology, Multidisciplinary osteoporosis consultation, Endocrinology, and Nephrology. The indication in the first three was follow-up management osteoporosis. The indication in the latter was monitoring of bone mineral status in chronic kidney disease patients.

When looking at results of query ALP iso-enzymes, we find that most test requests came from Urgency, Orthopedics and Radiology departments in AZ Diest for patients with traumatic fractures or for patients with non-specific symptoms. The determination of ALP iso-enzymes for each one of these traumatic patient was just for one time. Thus, biochemical monitoring of the healing process was not the intention, although there is no proved clinical usefulness from such monitoring could be found in the literature (**see Comments**).

Other common requesting departments for ALP iso-enzymes are: Orthopedic tumors (TUM) (12 patients) for patients with osteosarcoma, Hepatology department (6 patients) for follow-up patient after liver transplantation, kids transplantation (KTR) (three patients) for evaluation of transient alkaline hyperphosphatemia after organ transplantation. What is striking that only two ALP iso-enzymes test-requests came from general medical oncology (AMO) for follow up in patients.

We conclude from the results of these queries that the most common indications for determining bone turnover markers in UZL hospital are: osteoporosis, chronic kidney disease, malignancy and post transplantation in pediatric patients.

Methodology

Bone markers are assessed in blood (serum or plasma) or in urine. Measurement of BTMs in urine requires correction for creatinine level in the same sample. Immunoradiometric assays (IRMA), radioimmunoassay's (RIA) methods, as well as enzyme-linked immunosorbent assays (ELISA) and chemiluminescence assays, are available for the most of bone markers including OC, PICP, PINP, B-CTX, NTX, TRAP5b. BALP may be assessed by using colorimetric methods, IRMA, RIA and electrophoresis for qualitative and quantitative identification of the Alkaline Phosphatase isoenzymes. Some assays measure the "Ostase", i.e. the mass of BALP present in the serum (expressed as µg/L), whereas other assays measure the activity of the BALP enzyme (expressed as U/L). Results obtained by both types of assays, unfortunately, are not interchangeable **(20)**.

PYD and DPD are excreted in urine and can be measured by ELISA kits or chromatographic techniques such as high-pressure liquid chromatography.

QUESTION(S)

- 1) Which marker(s) is (are) the most sensitive indicator(s) of bone turnover in follow up osteoporosis patient?
- 2) What is the value of bone specific alkaline phosphatase in monitoring the bone mineral status in patients with malignancy or post organ transplantation?
- 3) Which bone marker(s) is/are useful to assess bone metabolic states in CKD patients?

SEARCH TERMS

- 1) *MeSH Database (PubMed): MeSH term: [Osteoporosis, Postmenopausal/blood*](#)
[Osteoporosis, Postmenopausal/drug therapy*](#)
[Drug Monitoring*](#)
[Bone Neoplasms/diagnosis](#)
[Biomarkers, Tumor*](#)
[Alkaline Phosphatase/blood*](#)
[Osteosarcoma/diagnosis*](#)
[Hyperphosphatemia/etiology*](#)
[Chronic Kidney Disease-Mineral and Bone Disorder/diagnosis](#)*
- 2) *PubMed Clinical Queries (from 1966; <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>): Systematic Reviews; Clinical Queries using Research Methodology Filters (diagnosis + specific, diagnosis + sensitive, prognosis + specific)*
- 3) *Pubmed (Medline; from 1966), SUMSearch(<http://sumsearch.uthscsa.edu/>), National Guideline Clearinghouse (<http://www.ngc.org/>), Institute for Clinical Systems Improvement (<http://www.icsi.org/>), The National Institute for Clinical Excellence (<http://www.nice.org.uk/>), Cochrane (<http://www.update-software.com/cochrane>), Health Technology Assessment Database (<http://www.york.ac.uk/inst/crd/htahp.htm>)*
- 4) *National Committee for Clinical Laboratory Standards (NCCLS; <http://www.nccls.org/>), International Federation of Clinical Chemistry (IFCC; <http://www.ifcc.org/ifcc.asp>), American Diabetes Association (ADA; <http://www.diabetes.org/home.jsp>), National Diabetes Information*

Clearinghouse (NDIC; <http://diabetes.niddk.nih.gov/>), Westgard QC (<http://www.westgard.com>),
Clinical Laboratory Improvement Amendments (CLIA; <http://www.cms.hhs.gov/clia/>)
5) UpToDate Online version 12.2 (2016)

RELEVANT EVIDENCE/REFERENCES

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5) Reference Works, Handbooks and Databases

6) Posters, "grey literature", presentations

1) Which marker(s) is (are) the most sensitive indicator(s) of bone turnover in follow up osteoporosis patient?

Osteoporosis, is a common disease that characterized by low bone mass and altered bone architecture leading to an increased susceptibility to fractures **(21)**, mostly in postmenopausal women, women with eating disorder, men over 60 years, or glucocorticoid induced osteoporosis. Bone mineral density measurement by dual energy x-ray absorptiometry (**DXA**) on the spine or hip is used for diagnosis, and for follow-up of osteoporosis. More than 2.5 SD below the normal peak values of DXA for young adults (T-score < -2.5) (WHO criteria) or by the occurrence of a low trauma fracture is enough to establish the diagnosis. BTMs are neither used for diagnostic means nor for triggering therapy but they may have potential predictive value for response to treatment, dosing bisphosphonates and prognosis.

The treatment of osteoporosis is mainly based on

- * Calcium and vitamin D: they are generally prescribed together with bisphosphonate therapy.

- * Bisphosphonates: they are considered as the first line therapy for osteoporosis. They are antiresorptive agents. They attach to the sites of active resorption, reduce recruitment and activity of osteoclast and increase their apoptosis **(22)**.

- * Strontium ranelate: salt of ranelic acid, has a dual action. It stimulates bone formation by increasing osteoblast precursor replication and inhibits bone resorption by decreasing osteoblast differentiation and resorption activity **(23) (24)**.

- * Raloxifene and bazedoxifene: selective estrogen receptor modulators (SERM), has antiresorptive effect. SERMs exert estrogenic activity in target tissue for example bone and spare breast and endometrium from undesirable stimulation.

- * Parathyroid hormone: Teriparatide (PTH 1–34) and PTH (the full-length peptide, PTH 1–84). They are recombinant parathyroid hormone used as bone formation agent that increases bone turnover and mass.

- * Denosumab: human monoclonal antibody against RANKL (Receptor Activator of Nuclear Ligand); It decrease the bone resorption by blocking osteoclast activity.

The efficacy of antiresorptive therapy can be assessed in three ways: fracture reduction, BMD measurement, and BTMs assessment. Fracture is relatively uncommon event and cannot be totally avoided by the use of antiresorptive therapy. In the current practice, DXA measurements are frequently used to assess the bone mineral density response to bisphosphonate. Although most of the efficacy of bisphosphonates in fracture reduction is not captured by DXA because it is slowly changed, (1 year at least is necessary to detect a significant

change) and there is no consensus on the optimal frequency of monitoring and the preferred site to monitor. Therefore, markers of bone turnover may be helpful in select circumstances as adjuvant to DXA for work-up and follow up of osteoporosis.

BTMs will decrease rapidly after initiation of bisphosphonate treatment. Prolonged inhibition of bone turnover through Bisphosphonate or SERM will eventually increase the degree of matrix mineralization that is expected to lead to increased bone mass and bone stiffness **(25)**.

Strontium ranelate, teriparatide are bone forming agents. Their therapeutic effect depends on the opposite changes of bone formation and bone resorption. Data obtained from the antiresorptive drugs (bisphosphonate) cannot be extrapolated on bone forming agents (hormones) because the mechanism of action is different. In the bisphosphonate itself, despite the structural similarity of the different generations of bisphosphonates, they differ in the potency and toxicity. The first generation of bisphosphonate include etidronate and tiludronate. The more potent second generation of bisphosphonate include alendronate, risedronate, and ibandronate. These nitrogen-containing bisphosphonate are incorporated into nonmetabolized analogs of adenosine triphosphate (ATP) **(26) (27) (28)**.

The International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine recommend that PINP and B-CTX are used as reference analytes in clinical osteoporosis studies, because of their robust nature and dynamic response to treatment. Serial measurement of one marker of bone formation and one marker of bone resorption help to monitor the biologic effect of osteoporosis therapies. Both will be significantly and parallelly reduced by anti-remodeling agents and increased by osteoanabolic agents given the proportional coupling of bone formation to resorption.

Significant response is determined usually in two manners: the **classic approach** is the least significant change (LSC) response. LSC calculations include pre-analytical and analytical variations and, by using standard statistical methods, provide a range of values that a given test must exceed to conclude that a true biologic effect has occurred as opposed to that of measurement error. The LSC is calculated using the coefficients of variation (CV) for analytical precision (CVa) and intra-individual (biological) variability (CVb).

$LSC = 1.96 \times \sqrt{2} \times \sqrt{(CV_a^2 + CV_b^2)}$ For the control group.

The CVa is established in each laboratory running the test, can be adjusted according to current laboratory performance The CVb is more difficult to personalize, and is most often collected from reported values in the literature, hopefully reflective of the appropriate target population.

Another approach is that the target for treatment is to decrease the BTM to the lower half of the premenopausal reference interval. However, the premenopausal reference interval (RI) is derived from the literature and may not adequately reflect the population that you are monitoring. However, not all women are above the reference interval mean before starting treatment. This

overlap of results between premenopausal and menopausal women is one of the limitations of the RI approach.

For BTMs markers to be useful in clinical practice, there should be a consensus on LSC calculation and robust RIs established using data from large populations **(29) (See attachments: table 2).**

We have screened many documents (EBLM based search) over the use of bone turnover markers in monitoring therapy of osteoporosis. After screening, 22 studies published between 1998 and 2016 were included. These studies might not be directly applicable to the question but they have assessed the potential role of a short-term change in specific bone markers (PINP, B-CTx, and OC) to monitor response to treatment.

Table 3 literature review of BTMs in osteoporosis

Study	Origin of population	Study design and statistical analysis	BTM and assay	Relation with BMD	Relation with fracture risk	Follow up months and treatment	Analysis and Results
Naylor, K.E et al.2016 (29)	50 postmenopausal osteopenic women from United Kingdom	Randomized controlled trial of raloxifene. LSC RI	B-CTx } PINP } By IDS-iSYS automated immunoassays	X	X	Raloxifene for 2 years	-The decrease in CTX was early, significant and below baseline by week 1 (-21%, $P < 0.001$), and the decrease in PINP from baseline was significant at week 4 (-17 % $P = 0.014$). -Change in B-CTx after 3 months (-39%) and in PINP (-32%) from baseline. -More responders by PINP than B-CTx after 3 and 12 months. -More responders by LSC method than by RI method for both BTMs.
Naylor, K.E et al.2016 (30)	172 postmenopausal osteoporotic women from United Kingdom	Randomized controlled trial of 3biphosphonates ANOVA LSC RI	B-CTx } PINP } OC } BALP } IDS-iSYS automated immunoassays	The percentage change in lumbar spine BMD at 96 weeks was greater in those reach the LSC target for B-CTx or PINP (not	X	Alendronate, ibandronate or risedronate for 2 years.	-B-CTx and P1NP had the greatest magnitude of change en highest number of responders. - The decrease in CTX was early, significant and below baseline by week 1, and the decrease in PINP below

				significant by RI responders).			baseline was significant at week 4. -Women with poor compliance did not reach the target for treatment by either LSC or RI approach for PINP or CTX.
Niimi,R et al.2014 (31)	154 postmenopausal women and men from Japan, diagnosed with osteoporosis.	Clinical trial of teriparatide Spearman correlation ROC analysis	PINP } RIA	Statistically significant correlations between absolute PINP changes at 1 month and spinal BMD changes at 12 months (r=0.36), p <0.01	X	Teriparatide for 12 months	Two thirds of patients with a PINP increase of >80 µg/l at 1 month after starting treatment showed a ≥10 % increase in spine BMD from baseline at 12 months.
Eekman, D.A et al.2011 (32)	31 patients from Netherlands diagnosed with osteoporosis.	Cohort Paired t test LSC RI	PINP } RIA B-CTx } Sandwich immunoassay on an Elecsys (Roche).	X	X	Bisphosphonates used were: Alendronate, risedronate, zoledronic acid ibandronate pamidronate	(81%) of patients had a decrease in levels of both markers with ≥ LSC after 3 months. -There were possible explanations in patients with a decrease ≤ LSC. -95% of chronically treated patients had values for both markers in the lower half of the reference range after? months.
Bruyere,O. et al. 2010 (33)	2373 women from Belgium diagnosed	Post hoc analysis for two randomized	BALP } PICP } RIA	3 months change B-CTx is not	The 3-month changes in	Strontium ranelate for 3 years	After 3 months of treatment with strontium ranelate,

	with osteoporosis.	controlled trials. Multiple regression	B-CTX Serum NTX } ELISA	correlated significantly with lumbar spine BMD change ($p < 0,001$). 3-month changes in PICP, BALP and CTX (not NTX) were significantly ($p < 0.001$) associated with 3-year BMD changes proximal femur.	BALP, PICP, B-CTX and serum NTX were not significantly associated with fracture incidence.		BALP increased by 9.6% (28.3), PICP by 9.9% (24.3), s-CTX decreased by 5.9% Changes in the tested BTMs explain less than 8% of the BMD changes
Hochberg Mark, C et al. 2010 (34)	323 postmenopausal women from ?, diagnosed with osteoporosis	Post hoc analysis for randomized controlled trial. Pearson correlation Linear regression	B-CTx No full text eligible	Significant association 3months change in B-CTx and 12 months change in spinal BMD Pearson= -0.19, ($p=0.0016$).	X	Ibandronate for 2 years	Short term change in BTMs can predict BMD changes $R^2= 0.61$, $p = 0.0007$
Collette,J. et al. 2010 (35)	6740 postmenopausal women from France and Belgium, diagnosed with osteoporosis	Post hoc analysis for two randomized controlled trials Tertiles	BALP } IRMA B-CTx } ELISA	X	No correlation between baseline BTMs and incidence of osteoporotic fracture.	Strontium ranelate for 3 years	Anti-fracture efficacy of strontium ranelate was independent of baseline BTMs levels
Bauer, D.C et al. 2006 (36)	119 postmenopausal women from USA diagnosed with osteoporosis.	Randomized, placebo-controlled trial of PTH (1-84).	PINP } B-CTx } Sandwich immunoassay on an	Stronger associations were observed between short-term change (1	The highest PINP tertile is associated with a decrease in	PTH (1-84) for 12 months.	After 1, and 3 months of PTH therapy, levels of PINP increased by 80 and 148%, respectively.

		Tertiles	Elecsys (Roche). BALP } microplate immunoassay (ostase assay) on Beckman	and 3 months) PINP and 1-year changes in lumbar spine DXA -Among compliant subjects, each 1 SD increase in the 1-month change in PINP was associated with a 4.3% increase in spine DXA BMD.	nonvertebral fracture risk.		B-CTx increased by 5% and 64%, respectively. The associations between changes in bone formation PINP and 1 year spine BMD were most apparent after 1 month of PTH treatment and after 3 months for bone resorption marker B-CT.
Bauer, D.C et al. 2006 (37)	6186 osteoporotic women from USA diagnosed with osteoporosis.	Posthoc analysis of randomized clinical trial of alendronate Tertiles	BALP } IRMA PINP } RIA B-CTx } ELISA	X	The highest baseline PINP tertile is associated with a decrease in nonvertebral fracture risk	Alendronate for 3 years	Alendronate nonspine fracture efficacy is greater among both osteoporotic and nonosteoporotic women with high pretreatment PINP.
Sarioglu, M. et al. 2006 (38)	50 postmenopausal women from Turkey, diagnosed with osteoporosis	Randomized controlled trial of 2 bisphosphonate Friedman test	OC } Urine DPD } Enzyme-amplified chemiluminescence assay BALP } Heat inactivation method	X	X	Alendronate or Risedronate for 12 months	DPD showed greater magnitude of change (57% from baseline) after 6months treatment than OC and DPD; Both BP have almost-similar effects in enhancing the BMD and in slowing bone turnover
Chen, P. et al. 2005 (39)	771 postmenopausal women from USA,	Retrospective study from Fracture	PINP } RIA PICP }	The increases in PINP at 3 months correlated best	No significant relationships between the	Teriparatide for 19 months	PINP and PICP were the best predictors of BMD response

	diagnosed with osteoporosis	Prevention Trial Spearman correlation	BALP } IRMA Urine DPD } Competitive enzyme immunoassay Urine NTX } ELISA	with increases in lumbar spine BMD at 18 months (0.61; $p < 0.05$).	changes in biochemical markers at any time-point and the overall fracture risk were found ($p > 0.05$).		to teriparatide in this analysis
Greenspan, S.L. et al. 2005 (40)	373 postmenopausal women from USA, diagnosed with osteoporosis.	Randomized controlled trial of alendronate ROC analysis Multiple regression	NTX } BALP } ELISA OC }	The greatest decrease in BTMs at 6 months had the greatest increases in spine and hip BMD at 3 years. NTX, $r = -0.428$ OC, $r = -0.219$; ($P < 0.001$).	X	Alendronate or HRT or combination for 3 years	The area under the receiver operator curve for a 6-month change in markers to predict a response in bone mineral density at 3 years was highest for urine NTX (range, 75-78%) and lowest for osteocalcin (range, 60-66%).
Kim, S.W. et al. 2005 (41)	138 postmenopausal women from Korea, diagnosed with osteoporosis.	Controlled trial of alendronate and HRT Regression analysis	OC } RIA NTX } ELISA	Percent change of NTx at 3 months correlated with the percent change of lumbar spine BMD at 12 months of treatment	X	Alendronate or HRT for 12 months	NTX decreased at 3 month (32% from baseline) while OC decreased gradually during 6 months with average 17%

Nenonen, A. et al. 2005 (42)	148 healthy postmenopausal women from Finland diagnosed with osteoporosis.	Randomized controlled trial of alendronate ROC curve Pearson correlation	B-CTX } Elecsys Immunoanalyser (Roche). TRAP5b } OC } In house immunoassays PINP } BALP } commercial immunoassays -U-DPD } high-performance liquid chromatography	Significant correlation between 3 months change in TRACP5b ($r=-.32$) and B-CTx (-0.24) with lumbar spine BMD change at 12 months. Correlation was non-significant for PINP.	X	Alendronate for 12 months	All markers had high clinical specificity. The highest value for B-CTX (98.6%) then PINP. The highest clinical sensitivities TRACP5b (82.7%), B-CTX (78.7%), and PINP (73.3%). The highest AUC values TRACP5b (0.879), B-CTX (0.868), PINP (0.862). The highest signal-to-noise ratios for S-TRACP5b (3.17), S-PINP (2.95), S-CTX (2.84).
Tahtela, R. et al. 2005 (43)	59 postmenopausal women from Finland diagnosed with osteopenia .	Extension study of a randomized controlled trial (all the subjects were treated). Pearson correlation LSC	TRAP5b } Immuno-extraction method PINP } RIA NTX } ELISA	1 year change in all BTMs correlated with 2 year change in lumbar spine and hip BMD $R=-0.33$ to $R=-0.45$.	X	Clodronate for 2 years	The most efficient marker for finding responders to treatment was PINP, which changed more than the LSC (32%) in 72% of the subjects at the 1-year time point and in 79% at the 2-year time point
Sebba, A. I et al. 2004 (44)	1053 postmenopausal women from USA, diagnosed with	Randomized controlled trial of alendronate or risedronate.	PINP } BALP } B-CTx } NTX }	X	X	Alendronate Risedronate for 1 year.	The percentage of patients achieving reductions in BTMs after 3 months treatment was the highest

	osteoporosis.	No full text eligible	No full text eligible				in B-CTx (60%) then PINP (50%). Significantly more alendronate- than risedronate-treated patients achieved predefined increases in BMD at 12 months and reductions in BTMs at 3 months
Bauer, D.C et al. 2004 (45)	6186 postmenopausal women from USA diagnosed with osteoporosis.	Randomized controlled trial of alendronate Linear regression	BALP] IRMA PINP] RIA B-CTx] ELISA	X	The relationships between 1-year change in all three markers of bone turnover and incident spine fracture were all statistically significant;	Alendronate for 4 years	Greater 1-year reductions in tested BTMs in response to alendronate were associated with a lower risk of spine, hip, and non-spine fracture. This relationship was most consistent for BALP, and to a lesser extent, for P1NP and B-CTX
Ravn, p. et al. 2003 (46)	212 early postmenopausal women from Denmark, have normal BMD.	Intervention cohort. ROC curve	OC] RIA en ELISA Urine CTx } ELISA NTX }	percent change from baseline at month 6 in the bone markers correlated with percent change from baseline at year 4 in BMD in the spine, total hip, and total body ($r = -0.40$ to $r = -0.53$, $P < 0.001$)	X	Alendronate for 3 years	NTX showed the highest diagnostic performance of a 6-month change for predicting change in spine BMD at 4 years (highest AUC value). Unexpected decrease in uNTX and total OC (RIA) in the placebo group during the course of the study
Bjarnason, N.H.	2622	Randomized controlled	BALP } IRMA	X	Greater 1-year	Raloxifene for 3 years	The strongest predictor for

et al,2001 (47)	postmenopausal women with osteoporosis (international study)	trial of raloxifene Tertiles Odds ratio	OC Urine CTx] ELISA		reductions in BALP and OC were associated with fewer incident vertebral fractures. (OR =0.75 and 0.69 respectively)		vertebral fracture was prevalent vertebral fracture even during therapy
Fink, E. et al. 2000 (48)	20 postmenopausal women from France diagnosed with osteoporosis.	Cohort LSC	OC] Automated immunoassay PINP] RIA PICP } BALP } ELISA B-CTx } NTX } DPD] HPLC	X	X	Alendronate	B-CTx then PINP had the greatest response of change and highest number of responders. -85% of patient had 70.8% decrease in B-CTx
Ravn, P. et al. 1999 (49)	1202 postmenopausal women, diagnosed with osteoporosis. (international study).	Randomized controlled trial of alendronate Regression analysis	OC] RIA NTX] ELISA	6 months change in NTX is significant correlated with 24 months change in all regions BMD R =-0, 31 (slightly higher than OC)	X	Alendronate for 1 year	NTX was beter indicator than OC for BMD response -Small decrease in both biochemical markers in the placebo group was observed!

Braga de Castro M.; A. et al. 1999 (50)	26 postmenopausal women from United Kingdom, diagnosed with osteoporosis	Randomized controlled trials of alendronate LSC	OC BALP PICP NTX PYD DPD } Method s not mentioned	No significant correlation between short term change in BTMs and 6 months change in spine and hip BMD	X	Alendronate for 6 months	NTX had the greatest magnitude of change and highest number of responders (68% decrease at 8 weeks). Biochemical markers performed better than DXA for monitoring alendronate therapy over 6 months.
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* Fracture risk is defined as number of patients with at least one new osteoporotic vertebral fracture.

Discussion

Of the 22 studies that were selected, only 15 focused on PINP and/or B-CTx.

Of the 15 studies, 2 post-hoc studies analysis of pooled data from previously carried out clinical trials. In those analysis, there was no correlation found between baseline (pre-treatment) serum B-CTx level and incidence of osteoporotic fracture. In contrast, high baseline PINP was associated with a decrease in fracture risk.

From the remaining 13 studies, 8 studies had evaluated the relation between short term changes in BTMs and long term changes in BMD in response to treatment. Of the 8 studies, only 2 showed no association between BTMs changes and BMD changes. One of them is a post-hoc analysis of a clinical trial of strontium ranelate (n=2373), which showed no significant correlation between 3 months change in serum B-CTx and 3 years change in lumbar spine BMD but well with proximal femur BMD. The other is a double blinded controlled trial of alendronate, which showed significant correlation between 12 months lumbar spine BMD changes with 3 months changes in serum B-CTx but not in PINP.

The other 6 studies showed significant negative correlations between 3 or 6 months B-CTx and/or PINP with 12 or 36 months lumbar spine BMD changes in response to bisphosphonate or teriparatide treatment.

Significant correlation was found between short term changes in B-CTx or PINP and fracture risk, this was shown in 2 prospective randomized clinical trials of bisphosphonate and of PTH. In contrast, 2 retrospective studies showed no correlation between short term changes in PINP or serum B-CTx and fracture risk. The strongest predictor for vertebral fracture during therapy was prevalent vertebral fracture.

In this review, 7 randomized clinical trials and one intervention cohort that focused on OC. All of them showed disappointing results what OC related. 6 prospective randomized clinical trials, 2 retrospective studies and 2 cohort showed promising results what B-CTx and PINP related.

The potential reasons of the discrepancies in the findings between studies could be due to differences in

- *Population characteristics: age (younger patients have more active bone metabolism in postmenopausal period than older ones), BMI, physical activity, smoking, alcohol drinking and dietary intake (protein, calcium, caffeine, vitamin D)

- *Diabetes, endocrine and metabolic factors (hyperthyroidism, hyperparathyroidism, renal dysfunction),

- *Bone measurement equipment.

- *Type osteoporosis therapy, difference in bioavailability and dose effect.

*Follow up months: 12 months may not be a long enough interval to observe a significant change in BMD.

* Some of the studies had no control subjects to compare.

Correlations between short-term BTMs changes and fracture risk reduction or long-term BMD changes mean that serial measurement of BTMs can predict treatment efficacy. It can also be an additive tool in improving therapy compliance because it identifies patients with poor response earlier than BMD. This will allow investigation for cause of poor response to be undertaken and changes in management to be instituted at an early stage in treatment.

It seems somewhat difficult from reviewing studies' results to draw definite conclusions concerning identification of ultimate responders or nonresponders to treatment. In general, the direction of the change in PINP and B-CTx levels (decrease or increase) will depend on the type of osteoporosis treatment. In patients taking bisphosphonates, PINP and B-CTx have been shown to decrease up to 70% from baseline after 3 to 6 months of therapy. Treatment with hormone replacement therapy also shows a decrease in PINP and B-CTx levels, but to a lesser degree than bisphosphonates therapy. Because of the remarkable antiresorptive effect of bisphosphonate and HRT, the decrease in B-CTx levels will be greater and earlier than the decrease in PINP levels in patients treated with either one of these two medications.

In patients treated with teriparatide (recombinant human parathyroid hormone), PINP levels increase early from baseline reflecting the stimulatory effect of teriparatide on osteoblasts and bone formation. PINP levels have been shown to significantly increase after 1 month, peaking at 3 to 6 months following teriparatide treatment. While B-CTx levels begin to increase significantly after 3 months of PTH treatment.

In patients treated with strontium ranelate, there is a small increase in bone formation markers and a small decrease in bone resorption markers. The role of BTM here is not clear because the changes in BTMs with this medication are small.

The two approaches (LSC) and (RI) do not fully overlap and may be complementary to each other. The benefit of (LSC) approach is that it takes into account the fact that patients may appear to have decrease in bone turnover as result of precision error. Therefore, we think that LSC approach, as a cut-off for a significant change to define a responder is better than any other approaches.

Bone Marker Standards Working Group anticipate that manufacturers will calibrate their PINP and B-CTx assays in future using an international reference standard to establish robust reference ranges in order to give clinicians more confidence in their use of bone turnover markers to help monitor osteoporosis treatment. The project is ongoing.

Conclusion

-PINP as a marker of bone formation and serum B-CTx as a marker of bone resorption are useful in monitoring osteoporosis treatment to confirm compliance with oral therapies, and efficacy of treatment.

- The pre-analytical advantages of PINP including a low diurnal, intra-individual variability, and stability at room temperature in addition to its dynamic response to treatment make PINP a good alternative to OC.

-BTMs in general, including PINP and B-CTx are independent predictors of fracture risk, their inclusion in fracture risk calculations will have to await further data to clarify their contribution to fracture risk and interactions with other risk factors

-Further studies with reference BTMs (PINP and B-CTx) are needed to clarify treatment targets for various therapies and optimal monitoring regimes.

Cost

Name of the test	Number of tests/year	Total cost per test	Incom RIZIV 100%B	
OC	230	14.51 euro	9 euro	Manual in house assay (no reagent cost but personnel cost)
PINP	2	24 euro	NO	Mainly reagent kit cost
B-CTx	337	8.02 euro	12 euro	Mainly reagent kit cost
BALP	82	182.53 euro	12 euro	Reagent kit cost+ personnel cost

Table 4

Costs of BTMs tests performed in UZL hospital in addition to the calculated assumed cost of total PINP test (Roche Diagnostics).

The cost of performing either OC or PINP are almost equal. The cost of osteocalcin is mostly personnel costs, while the cost of PINP is mostly reagent cost. At present time, there is no refund by RIZIV for P1NP, which makes it necessary to charge these costs to the patient. We remain tentative offer osteocalcin until PINP will be refunded (file is pending in ministry).

2) What is the value of the test alkaline phosphatase iso enzymes in monitoring the bone mineral status in patients with malignancy or after organ transplantation?

Osteosarcoma is the most common primary bone tumor affecting children and young adults; the peak age is between 13 and 16 years. It is characterized by the production of osteoid or immature bone by the malignant cells. The majority of patients with osteosarcoma present with localized pain. The most important finding on physical examination is a soft tissue mass, which is frequently large and tender to palpation. At presentation, between 10 and 20 percent have demonstrable metastatic disease, most often involving the lung. No specific laboratory tests for the diagnosis of bone sarcoma are available (**European Sarcoma Network Working Group ESMO guidelines**). Laboratory evaluation is usually normal, except for elevations in alkaline phosphatase (in approximately 40 percent) **(51)** lactate dehydrogenase (LDH, in approximately 30 percent) **(52)** and erythrocyte sedimentation rate. Laboratory abnormalities do not correlate with disease extent, although a very high LDH level is associated with a poor clinical outcome **(53)**. Conventional radiographs should always be the first investigation. The next imaging step is magnetic resonance imaging (MRI) or Computed tomography (CT) in case of doubt. Biopsy is required for definitive diagnosis. There is no added value of the BSALP in diagnosis or in follow up in patients with osteosarcomas and other primary bone tumors could be found in recent literature (**See Actions1**).

In conclusion of 2 very recent metaanalysis about prognostic significance of serum alkaline phosphatase in osteosarcoma (**Hao, H. et al. 2016**) (**Ren, H. Y. et al. 2015**). It was mentioned that total ALP could be a potential prognostic marker for osteosarcoma. Total ALP is more simple, rapid, and cost-effective in comparison with BALP.

Metastatic bone disease is a prominent source of skeletal complications and deterioration in quality of life. Skeletal complications include pain, pathologic fracture, hypercalcemia, and spinal cord compression, typically referred to as skeletal related events **SRE**. Bone metastases are most commonly seen in breast, prostate, lung and kidney cancer, as well as in multiple myeloma. It can be osteoblastic or osteolytic or mixed type. Skeletal imaging is the standard method for the diagnosis of bone metastasis and the choice of imaging usually guided by the clinical presentation and the underlying histologic tumor type. Dual-energy X-ray absorptiometry (DXA) scans should be carried out in patients at risk for cancer treatment-induced accelerated bone loss. Patients with bone metastasis receive initially an osteoclast inhibitor (bisphosphonate or denosumab) to reduce the risk of skeletal complications. However, the utility of bone biomarkers in monitoring therapy for cancer patients receiving an osteoclast inhibitor is controversial, and it is not recommended for routine clinical use (**ESMO Guidelines 2014**).

Studies have been selected to evaluate the significance of the bone markers including BALP as tools for the diagnosis of bone metastasis in cancer patients. These studies, however, have inconsistent and contrary results.

Some observational and retrospective studies supported the role of BALP in oncology setting. Some of them support the possible complementary role of BALP in the assessment of bone metastases in prostate cancer patients **(54)**. Other support the correlation of BALP with risk to SRE, elevated serum BALP levels are associated with a greater risk of adverse skeletal outcomes in men with metastatic prostate cancer **(55)**. Other support the correlation of BALP with the number of osteoblastic lesions **(56)** and mortality, when higher levels of serum BALP were associated with shorter overall survival **(57)**. However, there is no evidence support the role of BALP in detecting or predicting bone metastasis. Other Studies support the role of urinary NTX in predicting the clinical outcome in patients with metastatic solid tumors (breast, prostate, myeloma, non-small-cell lung, and other) during treatment with bone targeting therapy (zoledronic acid). They cannot directly address a causal link between decreases in urinary NTX and clinical outcome, but they do suggest that elevated baseline and on-study elevated urinary NTX have a twofold increased risk of developing SRE or bone disease progression when compared with those with normal levels. On the opposite, early NTX normalization is associated with a significant decrease in the risk of first SRE and death. There was insufficient evidence to suggest heterogeneity of effects by disease type ($P = 0.813$) **(58)**. Limited evidence suggest that measurement urine or serum NTX could be used to identify patient with bone metastasis from solid tumors **(59) (60) (61) (see attachments: figure 4)**.

Other studies suggested that PINP might have a promising role in oncology setting. PINP showed stronger association with the development of bone metastasis than did BALP in patients with metastatic prostate cancer **(62) (63)**. Other observational and retrospective studies support the diagnostic and prognostic value of PINP level for bone metastasis in patients with gynecological cancer **(64) (65)**. A small sample study ($n=36$) has suggested that PINP can help to diagnose bone metastasis and to evaluate the progress of chemotherapy in patients with renal cell carcinoma **(66)**.

Moreover, significant associations have been reported between elevated levels of serum B-CTx and progression of bone osteolysis in patients with multiple myeloma. NTx levels were not consistently elevated **(67)**.

Another study showed that total ALP and BALP have the same diagnostic value; area under the receiver operating-characteristic curve for ALP and BALP was exactly the same. Most of the markers (serum B-CTx, urinary NTX, urinary deoxypyridinoline, total ALP and BALP) had the same specificity in diagnosis of bone metastasis in prostate cancer patient, although B-CTx yielded better results **(68)**.

Another study suggested that the diagnostic performance of BTMs (total ALP, BALP, PINP, PICP, PYD, DPD, B-CTx, and TRAP5b) is not good enough in

comparison with bone scintigraphy for the diagnosis of bone metastasis in lung

Advantages of use of BTMs in oncology setting	Disadvantages of use of BTMs in oncology settings
Sample blood or urine are easily collected (not invasive)	Lack of tissue specificity for bone, as type I collagen is widely distributed in different organs and an inability to distinguish the metabolic activity of the different skeletal compartments
A variety of assays are available	Hormonal therapies for breast cancer and prostate cancer, as well as bone metastases, can increase all bone resorption marker levels (72) (73) .
May decrease the frequency of of imaging.	Urinary creatinine levels can also fluctuate in patients with advanced malignancies because of cachexia (74) .
In renal cell carcinoma are predominantly osteolytic lesions; in prostate cancer are predominantly osteoblastic lesions.	All other types of cancers are associated with elevation in both osteolytic and osteoblastic markers level (75) .

carcinoma patients. The areas under the receiver operating-characteristic curves were greatest for total ALP, followed by BALP and PINP. However, bone scintigraphy was clearly superior over BTMs due to its high sensitivity (100%) and acceptable specificity (76.4%)(**69**).

Other study encouraged the use of panels of BTMs in patients with breast, prostate and lung cancers to identify those with metastasis **(70)**.

In contrast, a study comparing a panel of BTMs between control and renal cell carcinoma patients, levels were almost similar between patients with nonbone and bone metastases **(71)**.

The most important limitation in most of these studies is: they were not restricted to men to exclude postmenopausal related increases of bone markers. There is a need for standardization of BTMs assays, selection of appropriate reference range. Then, further prospective trials that proof the standardized bone marker directed strategy of therapy. This will maximize benefits of BTMs use in clinical settings and decrease risks and costs.

Table 5

List of the possible advantages and disadvantage of the use of BTMs in oncology settings.

Transient hyperphosphatemia (TH) after liver/renal transplantation

Is a sudden, transient, benign increase in serum ALP, predominantly its bone or liver isoform, in either sick or healthy children under 5 years of age. It is frequently in excess of fivefold the upper limit of the normal ALP level for adults. It usually returns to normal within a few months without any treatment. The causal mechanism of TH remains unclear. Previous reports indicated that impaired clearance of ALP from the circulation might be the most probable mechanism, particularly in consideration of the hypothesized association between viral infection and subsequent excessive sialylation from virus **(76-78)**.

The prevalence of transient hyperphosphatemia is higher after organ transplantation **(79)**. Two possible reasons for this are as follows: (I) patients who undergo liver transplantation receive immunosuppressive agents, rendering them immunologically naive and vulnerable to infection by the aforementioned viruses; and (II) TH is typically discovered during “routine blood testing” and is more likely to be detected in those patients undergoing frequent detailed investigation **(19-20)**. We have screened European Liver Transplant Registry ELTR guidelines 2014 and American Association for the Study of Liver Diseases AASLD guidelines 2013. In both of them liver function test, which measures serum ALP, aspartate transaminase AST, alanine aminotransferase ALT, γ -glutamyl transferase GGT, total bilirubin, and direct bilirubin, was recommended as part of the routine monitoring and management after transplantation. Neither TH nor BSALP was mentioned.

The available literature over the importance of early recognition of TH after organ transplantation is not of high quality, they are mostly case reports or retrospective studies with small sized sample (maximum 6 patients), and agarose gel electrophoresis for ALP iso-enzymes was not performed in most of them **(see attachments: figure 5, table 6)**.

Isolated increase in the serum alkaline phosphatase level following transplantation should not be of concern in this population of patients and children with TH should be spared from excessive diagnostic procedures if it is not necessary **(80) (81) (82) (83)**. Experts have proposed a diagnostic approach to children with increased total ALP post-transplant:

- Evaluation of basic biochemical indices (serum levels of calcium, phosphate, creatinine, bilirubin, intact PTH, and activities of ALT and AST) from the same blood draw as the serum total ALP, if applicable.
- X-ray of the wrist.

Once all other results are normal, the TH is the most likely diagnosis and the total ALP can be monitored on a monthly basis; the diagnosis will be re-assessed after three months. If there is no tendency of high total ALP to decline, further diagnostic tests are recommended **(83)**.

Conclusion:

-No added value of measuring bone specific alkaline phosphatase post transplantation.

-No single marker has proven consistently reliable in monitoring bone mineral status in patient with malignancy.

-Several markers have shown promise to be used as adjunct tool to bone scintigraphy for detection of bone metastases such as urinary NTX in multiple myeloma and in breast cancer, total ALP, BALP or PINP in patients with prostate cancer **(Coleman, R. et al. 2011) (Ferreira, A. et al. 2015)**, LDH or total ALP in patients with osteosarcoma. To prove these hypotheses, large-scale prospective studies are warranted.

-BTMs in general, including BALP are not ready to be used in the malignant bone disease setting, neither for screening nor in the diagnosis of bone metastasis in place of established diagnostic techniques **(Cavalier, E. et al. 2016)**.

3) Which bone marker(s) is/are useful to assess bone metabolic states in chronic kidney disease patients?

Fracture is the most important clinical outcome of the bone disease that accompanies chronic kidney disease (CKD). This bone disease can be high or low bone-turnover disease. Both low and high bone-turnover disease may compromise bone quality which lead to increased risk of fractures **(84)**. Chronic kidney disease-mineral bone disease (CKD-MBD) in general is characterized by:

- secondary hyperparathyroidism; phosphate retention; decreased free calcium concentration and/or
- Abnormalities in bone turnover, mineralization, volume linear growth, or strength, and/or
- Extra skeletal calcification

High bone turnover disease characterized by Increase in both osteoblastic and osteoclastic activity due to secondary hyperparathyroidism. This often results in osteitis fibrosa.

Low bone turnover disease results most commonly from excessive suppression of the parathyroid glands. This often results in a dynamic bone disease, which is the most common CKD-related bone lesion among dialysis patients. If CKD patient has a low bone turnover disease in combination with abnormal mineralization, this may result in osteomalacia. Although this complication is uncommon since the decline in use of aluminum-containing phosphate binders. Mixed uremic bone disease also exist but less prevalent than adynamic bone disease and osteitis fibrosa.

Symptoms and/or signs due to the various bone disorders, such as fractures and bone pain, generally do not occur until the patient is already on maintenance dialysis **(85)** (see attachments: **Figure 6**).

The use of BMD (DXA) in chronic kidney disease patients is not recommended **(KDIGO Guidelines 2009)**. DXA can not nullify the association between CKD and increased fracture risk. Bone Biopsy is considered as the gold standard for quantifying bone turnover in CKD-MBD patient. It gives information not only over bone turn-over but also bone volume and mineralization, but it has disadvantages: invasive, expensive and require specific skills **(86)**. Therefore, non-invasive imaging techniques and bone turn-over markers have been suggested as surrogate of or adjuvant to bone biopsy to assess bone turn-over, to classify risk of bone loss and fracture and to guide therapeutic decision.

KDIGO guidelines 2009 suggested that measurements of serum PTH or **BALP** could be used to evaluate bone disease because markedly high or low values predict underlying bone turnover. Although, PTH is not a “true” bone biomarker. It acts on calcium metabolism and indirectly on bone.

PTH levels increase as kidney function deteriorates **(87)**. Chronic PTH excess is catabolic for cortical bone, causing subperiosteal and intracortical erosion, and can be anabolic for trabecular bone causing increased trabecular thickness and number **(88)**. Moreover, insufficiently increased PTH is often observed in end

stage renal disease patients. It has even harmful effect as the increased PTH levels, if not more.

PTH monitoring in advanced CKD is recommended every 3 months and alkaline phosphatase activity yearly, or more frequently if levels of PTH are elevated. However, the correlation between circulating PTH levels and bone turnover is rather weak unless at the extremes of the PTH concentration range (**KDIGO guidelines 2009**).

In patients with CKD stage 5D (D means on dialysis), **KDIGO guidelines** suggested maintaining iPTH levels in the range of approximately two to nine times the upper normal limit for the assay (2C). While The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (**NKF-KDOQI**) used 300 pg/mL as cutoff for iPTH.

The utility of PTH level in differentiating high bone turnover from nonhigh turnover using the iPTH cutoff for **NKF-KDOQI** (>300 pg/mL) and **KDIGO** (>9 times the upper limit of normal) had relatively high specificity, with use of the KDIGO value being slightly more specific. However, both guideline cutoff values had low sensitivity (**89**) (**see attachments: table 7**)

It was hypothesized that the simultaneous quantification of 1-84 PTH and 7-84 PTH fragments and calculation of their ratio would reveal superior predictive power regarding bone turnover. Unfortunately, this hypothesis could not be uniformly confirmed in bone biopsy studies (**90**). Due to analytical and clinical limitations of PTH measurement, such as low stability and high LSC (**91**), **KDIGO guidelines** recommend to measure a "true" bone biomarker (BALP) in nephrology setting.

The role of BALP as a biomarker of CKD-MBD has been investigated in patients with ESRD in several bone biopsy studies. Reported data from 2 bone biopsy studies showed that low BALP levels have positive predictive values for adynamic bone disease between 89% to 100% (**92**) (**93**). In another bone biopsy study applying Bayes' theorem, positive predictive value of BALP for adynamic bone disease was 75% (**94**). Another large bone biopsy study showed an AUC for BALP (0.76) for diagnosis of low bone turnover disease and AUC (0.71) for diagnosis of high bone turnover disease (**89**). These studies confirmed the benefit of BALP to assess mineral status in (CKD-MBD) patients. However, this test have some important limitations: BALP assays are not standardized yet, specificity is not perfect (**95**), in addition to the very high cost with low effectiveness.

Some data suggested that the combination of the two markers PTH and BALP is the best diagnostic tools for CKD-MBD patients (**96**) (**94**).

However, when there is a dissociation between both parameters, the sensitivity of BALP is higher than that of iPTH, and it is, therefore, a better indicator of bone remodeling. PTH level follow rapidly any acute change in calcium concentration in serum, while BALP level depend on bone remodeling which takes much longer time. Even so, bone biopsy appears to be still indispensable in making the

accurate diagnosis of CKD-MBD and in situations in which there is a discordance between plasma BALP and iPTH values **(97)**.

It should be stressed that an independent increase of either plasma iPTH or BALP may not always correspond to an increased bone turnover.

Other data have reported an association between total ALP levels and mortality in dialysis patients **(98) (99)** even after adjustment for liver function. The association between total ALP levels and risk of mortality was more linear and incremental than the association between PTH levels and mortality risk, this was showed in 2 apart cohort studies in dialysis and pre dialysis patients **(100) (101)**. Prospective controlled trials may be needed to test whether total ALP measurements could be used as a less costly alternative in CKD-MBD patients who are free from liver disease.

Other bone markers such as PINP and B-CTx, although not recommended by KDIGO, could be of interest in CKD-MBD patients. The literature over their use in this setting is very limited.

Data from a recent large cross-sectional bone biopsy study (n=492) in hemodialysis patients showed an AUC for PINP (Roche Elecsys) of 0.65 and 0.74 in predicting low and high bone turnover diseases, respectively **(89)**. It is important to know that PINP present in 2 forms in blood the monomeric and the trimeric forms. Some assays recognize both forms (total PINP), which available on Elecsys (Roche Diagnostic) while other assays recognize the trimeric form only (Intact PINP), which is available on IDS iSYS and RIA Iron Diagnostic). CKD patients have elevated proportion of monomeric form. Therefore, Intact PINP assays are not suitable for CKD patients because elevated monomeric PINP will not be captured in this assay **(Delanaye, P. et al. 2014)**.

B-CTx serum levels in patients undergoing hemodialysis are found to be five time more than the normal population due to the accumulation of B-CTx and due to the hyperthyroidism **(102)**. However, B-CTx measurement did not appear to be more effective at predicting clinical outcomes or bone histology than serum PTH or b-ALP yet **(Delanaye, P. et al. 2014)**.

Osteocalcin is thought to be superior to iPTH in a bone biopsy study (n=86) in patients with advanced CKD not yet in dialysis. In which OC showed a sensitivity, specificity and AUC of 83%, 67% and 0.80 respectively in detecting adynamic bone disease **(93)**. In another bone biopsy study (n=103), iPTH and BALP were superior to OC as a marker of adynamic bone disease in hemodialysis patients **(94)**. Due to the heterogeneity in the circulating OC (fragments, carboxylated versus uncarboxylated osteocalcin) and its dependence on renal function for its clearance. It is not surprising that osteocalcin is not a good biochemical marker for bone mineral status in chronic kidney disease patients, especially when the lower range of the determination is important such as in low bone turnover.

Other degradation products of the type-I collagen in urine such as PYD, DPD and NTX were also investigated. The available bone biopsy data in CKD patients suggested that they are may be useful in this setting. Urinary markers DPD and

PYD correlated significantly with histomorphometric parameters in hemodialysis patients **(103) (104)**. NTX level was significantly correlated with histomorphometric parameters in predialysis chronic renal failure patients but not in patients undergoing dialysis **(105)**.

There are a few bone biopsy studies over the diagnostic role of the recently automated bone marker TRAP5b and the osteocyte enzyme sclerostin. A good correlation of TRAP5b with histomorphometric and histodynamic parameters has been shown in 2 bone biopsy studies in hemodialysis patients **(103) (106)**.

Sclerostin correlate negatively with histomorphometric parameters of bone turnover in dialysis patients and thought to be superior to PTH in positive prediction of high bone turnover and number of osteoblasts **(107)**. This test is not automated yet. The results of commercially available ELISA are not encouraging **(108)** .

Conclusion

- No marker is able to predict or monitor accurately bone mineral status in chronic kidney disease patients especially mixed uremic disease or osteomalacia.
- Until now : no biomarker so far clearly proved to be superior to iPTH in predicting bone turnover in CKD. Therefore, PTH is still the most used biomarker.
- Osteocalcin is not the ideal bone turn over marker in clinical setting and in CKD patients in particular due to the high (pre)analytical variability, heterogeneity of circulating osteocalcin (fragments, carboxylated versus uncarboxylated osteocalcin) and its dependence on renal function for its clearance.
- The place of other bone markers such as PINP, B-CTx and TRAP5b in monitoring bone mineral status in CKD-MBD patients still need to be further studied.
- Inter-method variation of most, if not all BTMs is high. Further standardization and harmonization is needed.
- Additional large cross-sectional and longitudinal studies that compare BTMs (established BTMs or combination of BTMs) with the gold standard, i.e. bone histomorphometry are required.

Comments

Contact was made with the laboratory medicine department in AZD about the high number of "ALP isoenzymes" requests, from AZD. It has been found that this test belongs to a fixed application bilan "routine surgery / ortho / preoperation". This test is not always indicated, and the biologists have decided to remove it from this application.

To do/ACTIONS

- 1) Contact the test-requesting clinicians from the departments Orthopedics Tumors and pediatric transplantation for the possibility of cancelling BALP test in these clinical applications since it has no impact on the decision-making process.
- 2) Replace osteocalcin assay with PINP assay once PINP assay allowed to be refunded by RIZIV. The cost of both assays is comparable. The clinical utility of both serum B-CTx and PINP is therapy monitoring in osteoporosis patients.

ATTACHMENTS

Table 2

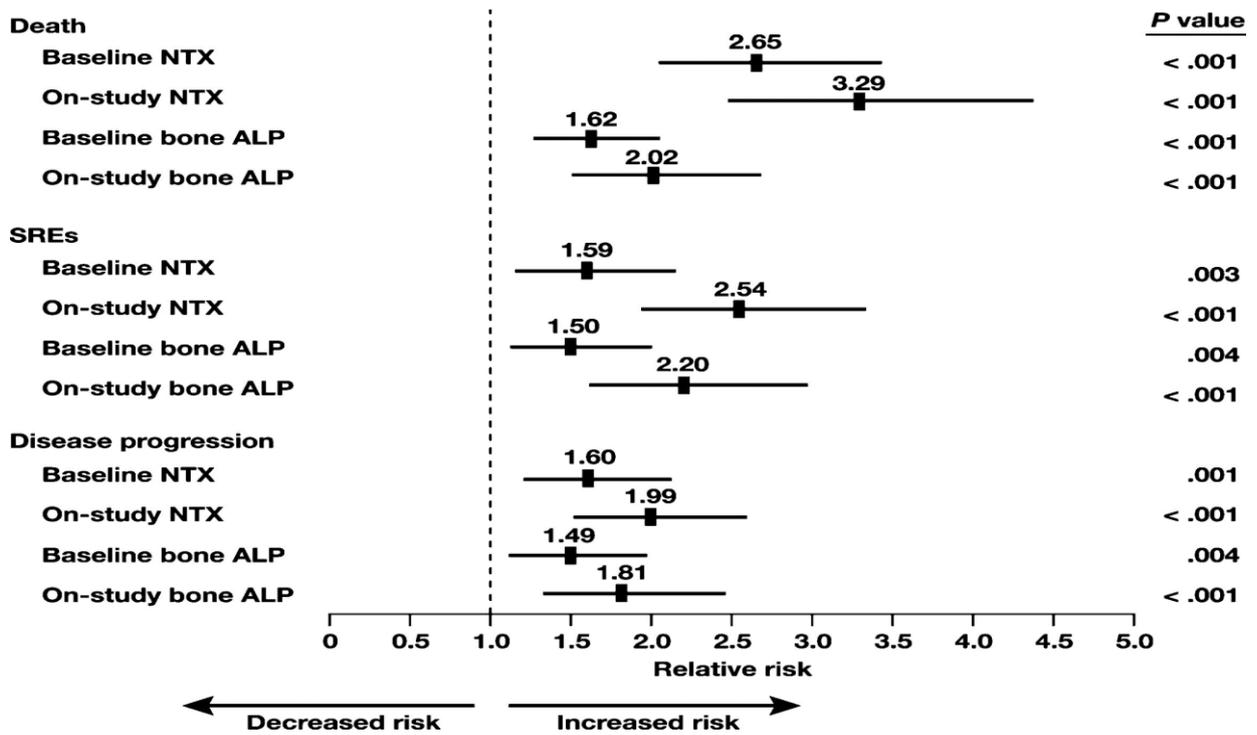
Responder analysis for least significant change (LSC) and reference interval (RI)

BTM	Visit	<i>n</i>	LSC (%)	LSC responders	Geometric mean (RI)	RI responders
CTX	Baseline	21	-45	–	0.32 µg/L (0.13 to 0.81)	3 (14 %)
	12 weeks	21		8 (38 %)		8 (38 %)
	48 weeks	20		12 (60 %)		8 (40 %)
PINP	Baseline	21	-27	–	28 µg/L (15 to 54)	2 (10 %)
	12 weeks	21		11 (52 %)		8 (38 %)
	48 weeks	20		13 (65 %)		9 (45 %)

Naylor, K. E. et al.2016

Figure 4

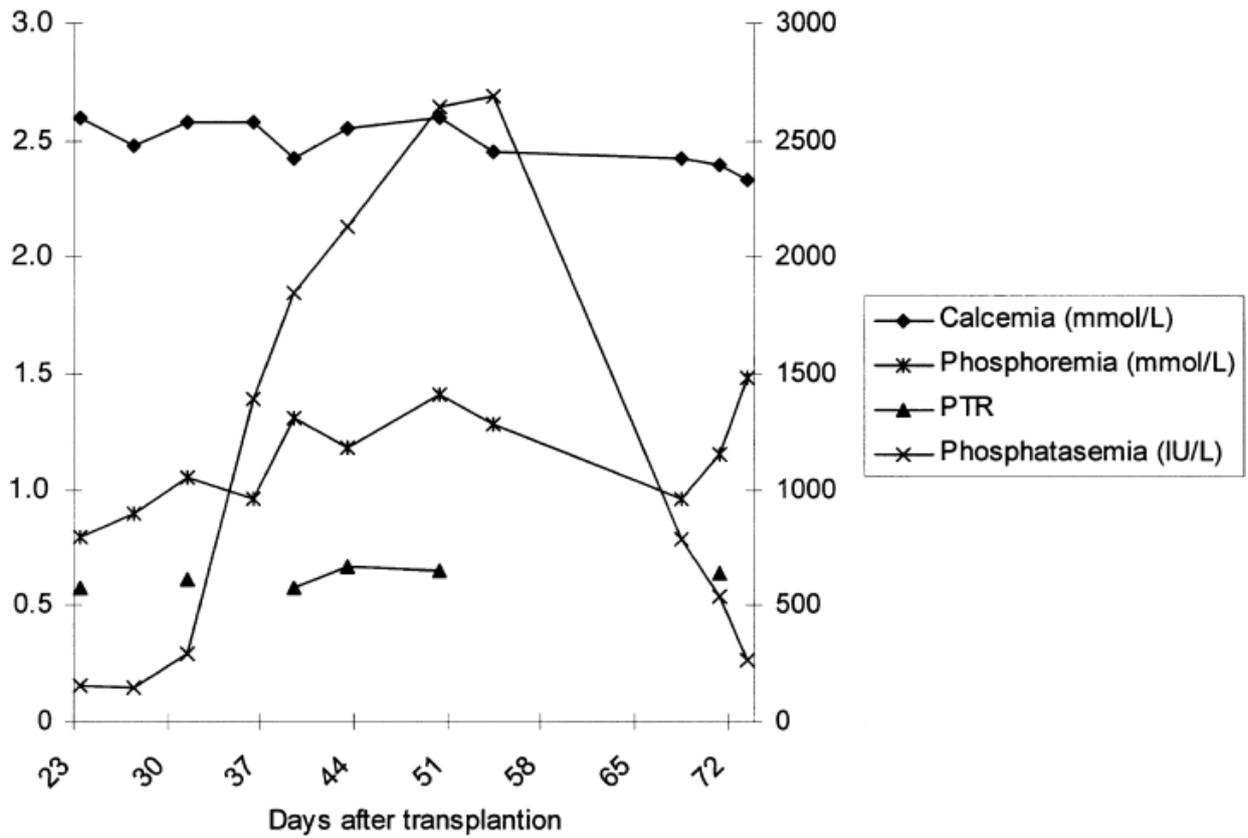
Correlation between elevated bone markers levels (NTX>100 nmol/mmol) and (BALP> 146 U/L) and clinical outcomes in patients with bone metsatasis from solid tumors who did not receive biphosphonate therapy.



Coleman, R. et al. 2011

Figure 5

Transient hyperphosphatasemia after organ transplantation in children



Ranchin B. et al. 2002

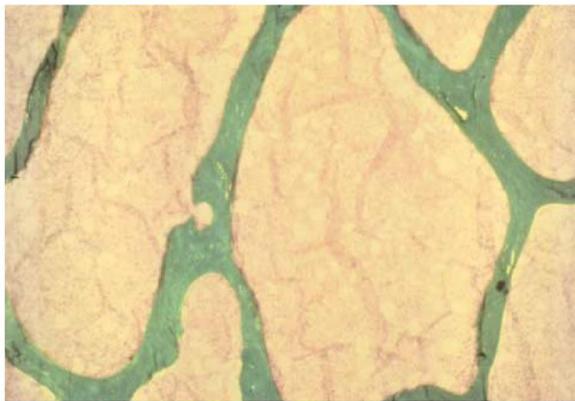
Table 6 Literature review of transient hyperphosphatemia after liver transplantation

Study, Year	No. patients	Incidence of TH (%)	Age range	Peak ALP (IU/L)	Duration of TH	Agarose gel electrophoresis	Liver biopsy	Estimated etiology of TH	IS	Steroid	Albumin	ST
1 Koneru <i>et al.</i> 1989 ⁸	6	2.2	2–6 years	NA	2 weeks–16 months	No	No	–	CsA	NA	NA	NA
2 Egawa <i>et al.</i> 1995 ⁹	4	2.9	15–32 months	7220	1–3 months	No	No	Growth acceleration after withdrawal of PSL	Tac	yes	NA	NA
3 Lachaux <i>et al.</i> 1996 ¹⁰	1	NA	9 months	4600	3 months	No	NA	-	CsA	yes	NA	NA
4 Ranchin <i>et al.</i> 2002 ¹¹	3	5.2	1–9 years	5574	42–204 days	No	No	-	CsA, Tac	Yes	NA	NA
5 O’Riordan <i>et al.</i> 2002 ¹²	6	4.3	11–198 months	13 740	6–77 weeks	Yes	Yes	Chronic cholangiopathy, CMV hepatitis	CsA, Tac	Yes	no	yes
6 Arikan <i>et al.</i> 2006 ¹³	2	2.8	2.6–4 years	21 110	18 days–8 months	No	Yes	Rotavirus	CsA, Tac	Yes	NA	yes
7 Hranjec <i>et al.</i> 2008 ¹⁴	1	NA	3 years	10 099	5 months	No	No	EBV	Tac, MMF	Yes	NA	NA
8 Present study	5	6.0	1–8 years	31 018	60–173 days	Yes	No	–	Tac, MMF	Yes	Yes	Yes

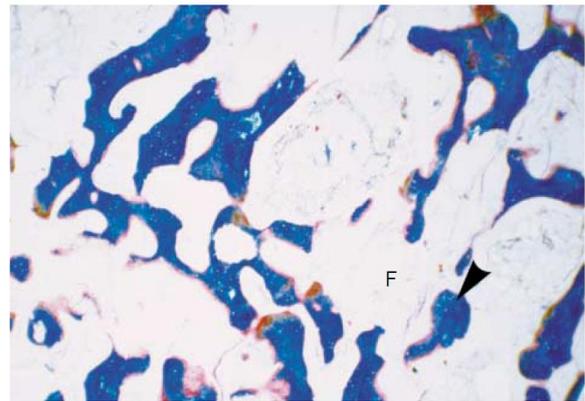
CMV, cytomegalovirus; CsA, cyclosporin A; EBV, Epstein–Barr virus; IS, immunosuppressive agent; LT, liver transplantation; MMF, mycophenolate mofetil; NA, not available; ST, sulfamethoxazole/trimethoprim; Tac, tacrolimus; TH, transient hyperphosphatemia.

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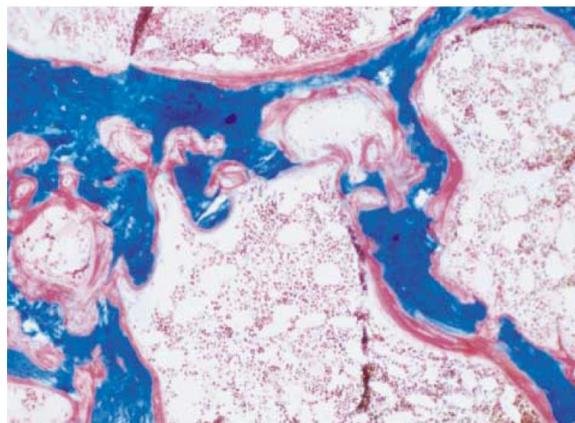
Figure 6 Specimens of trabecular bone. Panel A shows normal trabecular bone. Panel B shows osteitis fibrosa, loss of trabecular connections, and the appearance of bone islands (arrowhead). Panel C shows osteomalacia, osteoid=red. Panel D shows mixed disease, F=fibrosis. Panel E shows adynamic bone disease.



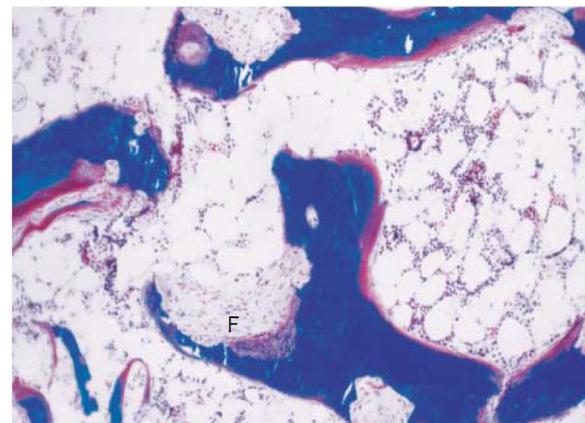
A



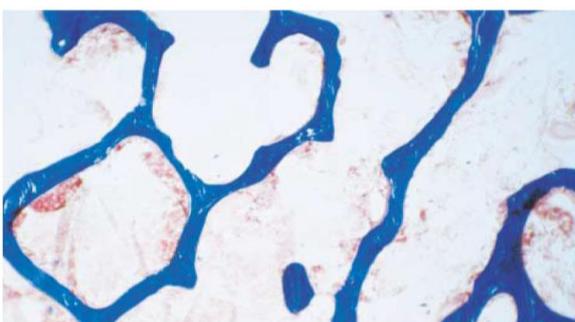
B



C



D



E

Keith, A. et al. 1995

Table 7 Utility of NKF-KDOQI and KDIGO iPTH thresholds for diagnostic decision-making

	NKF-KDOQI ^a				KDIGO ^b			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Differentiating low from nonlow turnover bone disease, or “When do I stop therapy?”	68.5%	61.2%	71.6%	57.7%	65.7%	65.3%	73%	57%
Differentiating high from nonhigh turnover bone disease, or “When do I start therapy?”	58.0%	77.7%	34.8%	90%	37.0%	85.8%	34.9%	86.9%

Abbreviations: iPTH, intact parathyroid hormone; KDIGO, Kidney Disease: Improving Global Outcomes; NKF-KDOQI, National Kidney Foundation–Kidney Disease Outcomes Quality Initiative; NPV, negative predictive value; PPV, positive predictive value.

^aUsing serum iPTH < 150 pg/mL for lower and >300 pg/mL for upper threshold.

^bUsing serum iPTH < 130 pg/mL for lower and >585 pg/mL for upper threshold (2× and 9× upper limit of normal for assay).