Osteoporos Int (2011) 22:391–420
DOI 10.1007/s00198-010-1501-1

POSITION PAPER

Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards

S. Vasikaran · R. Eastell · O. Bruyère · A. J. Foldes · P. Garnero · A. Griesmacher · M. McClung · H. A. Morris · S. Silverman · T. Trenti · D. A. Wahl · C. Cooper · J. A. Kanis · for the IOF-IFCC Bone Marker Standards Working Group

Received: 8 November 2010 / Accepted: 9 November 2010 / Published online: 24 December 2010

Abstract
Summary The International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommend that a marker of bone formation (serum procollagen type I N propeptide, s-PINP) and a marker of bone resorption (serum C-terminal telopeptide of type I collagen, s-CTX) are used as reference analytes for bone turnover markers in clinical studies.
Introduction Bone turnover markers (BTM) predict fracture risk, and treatment-induced changes in specific markers account for a substantial proportion of fracture risk reduction. The aims of this report were to determine their clinical potential in the prediction of fracture risk and for monitoring the treatment of osteoporosis and to set an appropriate research agenda.
Methods Evidence from prospective studies was gathered through literature review of the PUBMED database between the years 2000 and 2010 and the systematic review of the Agency for Healthcare Research and Quality up to 2001.
Results High levels of BTMs may predict fracture risk independently from bone mineral density in postmenopausal women. They have been used for this purpose in clinical practice for many years, but there is still a need for stronger evidence on which to base practice. BTMs provide pharmacodynamic information on the response to osteoporosis treatment, and as a result, they are widely used for

S. Vasikaran
Department of Core Clinical Pathology and Biochemistry,
PathWest Laboratory Medicine, Royal Perth Hospital,
Perth, WA, Australia

S. Vasikaran
School of Pathology and Laboratory Medicine,
University of Western Australia,
Nedlands, WA, Australia

R. Eastell
Centre for Biomedical Research, Northern General Hospital,
Herries Road,
Sheffield, South Yorkshire, England S5 7AU, UK

O. Bruyère
Department of Public Health,
Epidemiology and Health Economics, University of Liège,
Liège, Belgium

A. J. Foldes
Osteoporosis Center, Hadassah University Hospital,
Mount Scopus,
Jerusalem 91240, Israel

P. Garnero
INSERM Unit 664,
Lyon, France

A. Griesmacher
Central Institute of Medical and Chemical Laboratory Diagnostics,
University Hospital of Innsbruck,
6080, Innsbruck, Austria

M. McClung
Oregon Osteoporosis Center,
5050 NE Hoyt Street, Suite 651,
Portland, OR 97213, USA

H. A. Morris
School of Pharmacy and Medical Sciences,
University of South Australia,
Adelaide, SA 5000, Australia

monitoring treatment in the individual. However, their clinical value for monitoring is limited by inadequate appreciation of the sources of variability, by limited data for comparison of treatments using the same BTM and by inadequate quality control. IOF/IFCC recommend one bone formation marker (s-PINP) and one bone resorption marker (s-CTX) to be used as reference markers and measured by standardised assays in observational and intervention studies in order to compare the performance of alternatives and to enlarge the international experience of the application of markers to clinical medicine.
Conclusion BTM hold promise in fracture risk prediction and for monitoring treatment. Uncertainties over their clinical use can be in part resolved by adopting international reference standards.

Keywords Bone makers · Bone turnover · Fracture risk · IOF · Monitoring treatment · Reference standards

Introduction

The burden of osteoporosis

Osteoporosis is a major health problem worldwide. It is defined as a disease characterised by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk [1]. The clinical consequences of osteoporosis reside in the fractures that arise, particularly hip fracture, which accounts for the major direct costs. In 1990, the number of osteoporotic fractures estimated in Europe was 2.7 million, with an estimated direct cost in 2004 of €36 billion (£24.5 billion), of which €24.3 billion (£16.6 billion) were accounted for by hip fracture. Costs are expected to rise to €76.8 billion (£52.4 billion) by the year 2050 [2]. Similar projections are made for many other regions of the world because of the increasing numbers of the elderly. In the USA, the annual cost of incident fractures due to osteoporosis or low bone mass is predicted to rise from $16.9 billion in 2006 to around $25.3 billion by the year 2025 [3].

Current approaches to diagnosis and treatment

Technological developments for the measurement of bone mineral density (BMD) have led to diagnostic criteria that are widely applied. The World Health Organization diagnostic criterion for osteoporosis is a BMD measurement equal to or more than 2.5 standard deviations (SD) below the young female (age 20–29 years) reference mean (T-score≤ −2.5 SD) [4, 5]. In addition, there have been major advances in the number and range of agents available for treatment, all with proven anti-fracture efficacy [6–8]. These agents have differing modes of action in protecting against fracture, and these need to be taken into account when developing monitoring strategies.

Gap analysis

Important gaps in the clinical armamentarium include the identification of individuals who would best benefit from intervention and, for those on treatment, the optimal manner in which response to treatment should be monitored. In this regard, there has been interest in the clinical potential of bone turnover markers (BTMs), both as tools to assess fracture risk and for monitoring treatment, to thereby aid intervention strategies [9–13]. Attractive features of these markers are that samples of blood or urine are easily collected, a variety of assays is available, sample collection is relatively non-invasive and results provide information that is complementary to BMD. In contrast to an extensive research base, there are uncertainties in their use for routine clinical application. Limitations variously include their biological variability (Table 1) [14] and, in some cases, the multiple methodologies used for the same analyte (e.g. the assays for osteocalcin) [15]. Laboratory variations become critical to

S. Silverman
Cedars-Sinai/University of California,
Los Angeles, CA, USA

T. Trenti
Patologia Clinica, Tossicologia e Diagnostica Avanzata,
Dipartimento di Patologia Clinica,
Ospedale Nuovo Sant'Agostino Estense,
via Giardini 1355,
4110, Modena, Italy

D. A. Wahl
International Osteoporosis Foundation,
Nyon, Switzerland

C. Cooper (✉)
MRC Lifecourse Epidemiology Unit,
Southampton General Hospital, University of Southampton,
Southampton SO16 6YD, UK
e-mail: cc@mrc.soton.ac.uk

C. Cooper
NIHR Musculoskeletal Biomedical Research Unit,
Institute of Musculoskeletal Sciences, University of Oxford,
Oxford OX3 7LD, UK

J. A. Kanis
Centre for Metabolic Bone Diseases (WHO Collaborating Centre),
University of Sheffield Medical School,
Beech Hill Road,
Sheffield S10 2RX, UK

Table 1 Uncontrollable and controllable sources of pre-analytical variability according to their importance

Source	Importance	Nature of effect
Uncontrollable sources		
Age	Very important	BTM increase with age in men and women
Menopausal status	Very important	BTM increase within a few months after the last menstrual period
Gender	Very important	BTM are higher in older women than older men
Fractures	Important—limits evaluation of case control studies	BTM increase after a fracture (maximal at 2 to 12 weeks, but effect lasts for up to 52 weeks)
Pregnancy and lactation	Important	BTM are increased during pregnancy; highest levels during third trimester, even higher postpartum
Drugs	Important: corticosteroids, anticonvulsants, heparin, GnRH agonists	BTM may be decreased (glucocorticoids) or increased (anticonvulsants)
Disease	Important: thyroid disease, diabetes, renal impairment, liver disease	BTM often increased (thyrotoxicosis, chronic kidney disease)
Bed rest/immobility	Important	Bone formation markers decrease and resorption markers increase
Geography	Somewhat important	Small changes amongst countries, usually explained by differences in lifestyle
Ethnicity	Not important	Small changes, such as lower OC in African Americans vs. Caucasians
Oral contraception	Not important, except in women over 35 years	Lower values for BTM
Controllable sources		
Circadian	Extremely important	Most striking for bone resorption markers; highest values in second half of night and on waking; lowest values in afternoon and evening
Fasting status	Important for specific markers	Feeding results in a decrease in BTM; for example, s-CTX decreases by 20% after breakfast
Exercise	Important—chronic and acute effects	Changes occur but depend on type of exercise and age of subjects
Menstrual	Not important	Small decreases in bone resorption and increases in bone formation during luteal phase
Seasonal	Not important for individual, but maybe for longitudinal studies	Small decreases in BTM over winter
Diet	Not important	Small reduction in BTM immediately following calcium supplementation

clinical care when measurements are done in a range of commercial and hospital settings.

These strengths and weakness of BTMs in clinical practice have been considered by a number of national societies and guideline development groups and have resulted in differing recommendations for their clinical use in risk assessment and in the monitoring of osteoporosis treatment (Table 2). Some advocate their routine use, others use more cautious language and others still, do not recommend their routine use.

Aim

These uncertainties prompted the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to convene the IOF–IFCC Bone Marker Standards Working Group. The aim of the group was to consider the research base available that was relevant to the application of BTMs to fracture risk assessment and monitoring of treatment and to provide recommendations on their clinical use. In the absence of clear recommendations, a research strategy was to be formulated. The present paper summarises the outcome of the review.

Methods

Evidence from prospective studies for the performance of BTMs in fracture risk prediction in untreated patients and for the performance of BTMs in monitoring therapy was gathered by searching the English published literature in PUBMED database between the years 2000 and 2010. The 2001 tabulated evidence from the Agency for Healthcare Research and Quality on BTMs [22], which was based on a MEDLINE database systematic review, provided the source

Table 2 Recent national guidelines on the utility of BTMs in the management of patients with osteoporosis

Country, title and year	Recommendations	Reference
Australia Clinical guideline for the prevention and treatment of osteoporosis in postmenopausal women and older men 2010	Monitoring therapy—"The role of bone turnover markers in the management of OP has not yet been fully investigated. In the absence of clear evidence of improved patient outcomes from their use and cost effectiveness data, routine use in patient monitoring in general practice is not currently recommended."	The Royal Australian College of General Practitioners [16]
Belgium Evidence-based guidelines for the use of biochemical markers of bone turnover in the selection and monitoring of bisphosphonate treatment in osteoporosis 2009	Fracture prediction and monitoring therapy—"Although the correlation between BMD and BTMs is statistically significant, BTMs cannot be used as predictive markers of BMD in an individual patient. Both are independent predictors of fracture risk, but BTMs can only be used as an additional risk factor in the decision to treat. Current data do not support the use of BTMs to select the optimal treatment. However, they can be used to monitor treatment efficiency before BMD changes can be evaluated. Early changes in BTMs can be used to measure the clinical efficacy of an anti-resorptive treatment and to reinforce patient compliance."	Belgian Bone Club [9]
Canada Bone turnover markers in the management of postmenopausal osteoporosis (review—endorsed by several national societies) 2009	"Potential clinical uses of BTMs include prediction of bone loss and fracture in untreated postmenopausal women, to monitor osteoporosis therapy, and perhaps to enhance adherence to therapy. BTMs should not be used to diagnose osteoporosis or to select the type of osteoporotic therapy is most appropriate." Possible algorithm: measure at baseline s-CTX (antiresorptive therapy) and Total s-PINP (anabolic therapy), other when available. Remeasure at 3–6 months. Significant change is measured by absolute percentages, >40% for bone formation markers, and 35–55% change in bone resorption markers	Multidisciplinary working group under the auspices of the Scientific Advisory Council of Osteoporosis Canada [11]
Europe European guidance for the diagnosis and management of osteoporosis in postmenopausal women 2008	Investigation of osteoporosis—non routine practice but acknowledge the use of markers of bone turnover for the investigation of osteoporosis, when available. Monitoring of treatment—"The most informative bone markers for the investigation of osteoporosis are osteocalcin and procollagen I N-terminal extension peptide (PINP) for assessing bone formation, and type I collagen—and C-telopeptide breakdown products (especially serum CTX) to assess bone resorption"	European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis [8]
Latin America Ibero-American consensus on osteoporosis 2009	Fracture prediction—"These markers are not useful to make the diagnosis, but they are useful to orient physicians about the dynamics of bone turnover in a particular patient. This will help physicians to identify patients with a higher fracture risk. The systematic determination of bone markers is not recommended in the evaluation of every patient with osteoporosis." Monitoring therapy—These markers are also useful to make an early evaluation of the response to treatment.	Iberoamerican Society of Osteology and Mineral Metabolism [17]
Poland Recommendations on the diagnosis and treatment of osteoporosis. Reducing the incidence of fractures through effective prevention and treatment 2007	Monitoring of therapy—BTMs (serum CTX, PINP, OC) are used to assess the efficacy of anticatabolic (bisphosphonates, raloxifene, hormone therapy, calcitonin) and anabolic (PTH) treatment in the short term. Baseline values before treatment are recommended. CTX levels at 3 months. PINP and OC at 6 months. Use of LSC to determine change efficacy.	Multidisciplinary Osteoporosis Forum [18]

Table 2 (continued)

Country, title and year	Recommendations	Reference
Singapore Singapore Ministry of Health: Clinical Practice Guidelines for Osteoporosis 2008	Monitoring therapy—"An alternative method for monitoring therapeutic response is evaluating bone turnover markers at baseline and at 3–6 month intervals. The use of most effective osteoporosis drugs has been associated with reductions from baseline of between 20% and 40% for bone formation markers such as osteocalcin and bone alkaline phosphatase, and 30–60% for bone resorption markers such as N-telopeptide, C-telopeptide and deoxypyridinoline. Because of significant biological variability, the timing and method of collection of blood or urine specimens should be consistent for serial measurements (second void for urine specimen and morning fasting for serum specimen)." Fracture prediction—"There is currently no role for bone turnover markers in the diagnosis of osteoporosis. However, bone turnover markers do aid in fracture risk assessment, the prediction of rates of bone loss, as well as in monitoring response to treatment."	Singapore Ministry of Health [19]
UK Osteoporosis—clinical guidelines for prevention and treatment, Executive Summary 2008	Fracture prediction and monitoring therapy—BTMs have the potential of aiding risk assessment as well as for monitoring therapy (level Ib). Further research in the field is recommended.	National Osteoporosis Guideline Group [20]
USA The National Osteoporosis Foundation Clinician's guide to prevention and treatment of osteoporosis 2008	Fracture prediction "Biochemical markers of bone remodelling (resorption and formation) can be measured in the serum and urine in untreated patients to assess risk of fracture. They may predict bone loss and, when repeated after 3–6 months of treatment with FDA approved antiresorptive therapies, may be predictive of fracture risk reduction." Monitoring of therapy "Suppression of biochemical markers of bone turnover after 3–6 months of specific antiresorptive osteoporosis therapies, and biochemical marker increases after 1–3 months of specific anabolic therapies, have been predictive of greater BMD responses in studies evaluating large groups of patients. Because of the high degree of biological and analytical variability in measurement of biochemical markers, changes in individuals must be large in order to be clinically meaningful. It is critical to appreciate the LSC associated with the biomarker being utilized, which is calculated by multiplying the "precision error" of the specific biochemical marker (laboratory provided) by 2.77 (95% confidence level). Biological variability can be reduced by obtaining samples in the early morning after an overnight fast. Serial measurements should be made at the same time of day and preferably during the same season of the year."	National Osteoporosis Foundation [21]

of relevant prospective studies up to the year 2001. To ensure the completeness of the search, key recent review studies were identified [9, 11–13, 23, 24], and all additional references were added.

For the assessment of fracture risk, we included only prospective cohort studies, which required that markers be assessed prior to a fracture event. The primary outcome was the first incident fracture in middle-aged or older men and women. We excluded cross-sectional and case–control studies and animal preclinical investigations and abstracts. We excluded studies that did not provide separate data for men and women [25] or did not provide separate data on hip fractures [26].

For the performance of BTM in monitoring therapy, we included studies that evaluated vertebral, non-vertebral or hip fractures. We excluded studies that examined mean changes in BTMs with mean changes in fracture risk, i.e. studies that did not perform the analysis at the individual level, e.g. [27].

Bone turnover markers

Markers of bone turnover are biochemical products measured usually in blood or urine that reflect the metabolic activity of bone but which themselves have no function in controlling skeletal metabolism. They are traditionally categorised as markers of bone formation or bone resorption (see Table 3).

Markers of bone formation are direct or indirect products of active osteoblasts expressed during various phases of their development and reflect different aspects of osteoblast function. Type I collagen is an important component of bone matrix, and osteoblasts secrete its precursor procollagen molecule during bone formation. The extension peptides at each end of the procollagen molecule, procollagen type I N propeptide (PINP) and procollagen type I C propeptide (PICP), are cleaved by enzymes during bone matrix formation and released into the circulation. Osteocalcin (OC), one of the most abundant non-collagenous proteins in bone matrix, is also produced by osteoblasts during bone formation, and some proportion finds its way into the extracellular compartment where it can be measured. It is excreted by the kidneys and its fragments may also be measured in urine. Newly formed osteoid undergoes maturation followed by mineralisation, and during this phase, alkaline phosphatase (ALP) is secreted by osteoblasts into the extracellular fluid and can be measured in serum. However, only about half of the ALP activity in blood in healthy adults derives from bone, the other half being predominately of hepatic origin. Assays are available that detect more specifically the bone derived isoform (BALP).

The commonly used bone resorption markers are degradation products of type I collagen, but non-collagenous proteins such as the enzyme of osteoclast origin tartrate-resistant acid phosphatase 5b (TRACP) have also been investigated as resorption markers. The pyridinium cross-links, pyridinoline (PYD) and deoxypyridinoline (DPD) are formed during the maturation of bone collagen, present in significant amounts in bone and dentine, released during resorption of bone and excreted in urine in the free and peptide-bound forms without being metabolised. The peptide-bound forms of PYD and DPD include the C-terminal and N-terminal cross-linking telopeptides (CTX, NTX) of the type I collagen molecule, and these are also released into the circulation and subsequently excreted in urine.

Bone turnover markers in fracture risk prediction

Rationale

Oestrogen deficiency, associated with menopause, results in a generalised increase in bone remodelling and an imbalance between bone formation and resorption [28, 29]. This increase is maintained for several decades after the menopause [30] and is associated with accelerated bone loss [31–39]. An increased rate of bone loss from the forearm and hip is associated with an increase in the risk of vertebral fracture [40, 41], an effect independent of final BMD. Thus, it is logical to consider that high bone turnover might predict fracture.

The mechanism by which sustained high bone turnover might be associated with increased fracture risk could be related simply to the bone loss resulting in a low BMD. In addition, there are other mechanisms whereby increased bone turnover might be associated with an increased fracture risk independent of BMD [12]. Deterioration of bone architecture may contribute to skeletal fragility over and above that provided by the decrease in bone mass. For example, resorption cavities on either side of a trabeculum give rise to stress concentrators that result in the local weakening of the trabeculum that is disproportionate to the small amount of bone lost [42]. In addition, increased bone turnover increases the proportion of recently synthesised bone which is less well mineralised than mature bone [43] with fewer enzymatic post-translational modifications of bone collagen (cross-linking and β-isomerisation) [44, 45]. It is possible that these features impair the structural properties of bone.

Evidence for the utility of bone turnover markers in fracture risk prediction

Prospective studies examining the relationship between BTMs and subsequent fractures are summarised in Table 4. All but four studies [12, 46–48] showed that one or more markers of bone formation or resorption were significantly

Table 3 Bone turnover markers: nomenclature, abbreviations and description

Marker	Full name	Origin	Assay	Comments
Resorption				
u-NTX	Urinary amino-terminal cross-linking telopeptide of type I collagen	Osteoclastic hydrolysis of collagen type I	Automated Manual	Must be adjusted to levels of urinary creatinine (/Cr) Specificity: collagen type I, with highest contribution probably from bone Sources of variability: influenced by circadian rhythm
s-NTX	Serum amino-terminal cross-linking telopeptide of type I collagen	Osteoclastic hydrolysis of collagen type I, generated by cathepsin K	Automated Manual	Specificity: collagen type I, with highest contribution probably from bone; smaller response to therapy may indicate some lack of bone specificity Sources of variability: influenced by renal function and circadian rhythm
u-CTX	Urinary carboxy-terminal cross-linking telopeptide of type I collagen	Osteoclastic hydrolysis of collagen, generated by cathepsin K	Automated Manual	Must be adjusted to levels of urinary creatinine (/Cr) Specificity: collagen type I, with highest contribution probably from bone u-CTX is isomerised (β) or non-isomerised (α). Isomerised if not otherwise specified. Sources of variability: influenced by circadian rhythm
s-CTX	Serum carboxy-terminal cross-linking telopeptide of type I collagen	Osteoclastic hydrolysis of collagen, generated by cathepsin K	Automated Manual	s-CTX is always isomerised (β) Specificity: collagen type I, with highest contribution probably from bone Sources of variability: very dependent on time of day and food (must be collected after an overnight fast); influenced by renal function, liver function and circadian rhythm
s-ICTP or CTX-MMP	Carboxy-terminal cross-linking telopeptide of type I collagen	Osteoclastic hydrolysis of collagen generated by matrix metalloproteinases	Manual	Specificity: collagen type I, with highest contribution probably from bone. Results from MMP digestion of collagen and not responsive to usual treatments for osteoporosis Sources of variability: influenced by renal and liver function and circadian rhythm
u-DPD	Urinary deoxypyridinoline	Proteolytic hydrolysis of collagen, found in bone	Automated Manual	Must be adjusted to levels of urinary creatinine (/Cr) Total or free (non-peptide-bound) Specificity: highest contribution from bone, present in mature collagen only Sources of variability: independent of dietary sources, influenced by UV radiation and circadian rhythm Total or free (non-peptide-bound)
u-PYD	Urinary pyridinoline	Found in bone, cartilage, tendon, blood vessels	Automated Manual	Adjusted to levels of urinary creatinine (/Cr) Total or free (non-peptide-bound) Specificity: highest contribution from bone and cartilage, present in mature collagen only Sources of variability: independent of dietary sources; influenced by liver function, active arthritis and UV radiation, and circadian rhythm

Table 3 (continued)

Marker	Full name	Origin	Assay	Comments
s-TRACP	Serum tartrate-resistant acid phosphatase	Includes two isoforms: type 5a (platelets, erythrocytes and other sources) and type 5b (osteoclasts)	Manual	Sources of variability: influenced by haemolysis and blood clotting, and circadian rhythms Difficult to store; stable up to 2 years at −70 °C
Formation				
s-OC	Serum osteocalcin	Hydroxyapatite-binding protein exclusively synthesised by osteoblasts and odontoblasts	Automated Manual	Specificity: specific marker of osteoblast function Subject to rapid degradation in serum leads to heterogeneity of OC fragments: usually measured as intact [1–49] or N-mid [1–43] fragment, or can be undercarboxylated (ucOC) Sources of variability: influenced by renal function and circadian rhythms; large inter-laboratory variation
u-OC	Urinary osteocalcin	Hydroxyapatite-binding protein exclusively synthesised by osteoblasts and odontoblasts	Manual	Adjusted to levels of urinary creatinine (/Cr) Specificity: specific marker of osteoblast function Mid (predominant fragments) or long (only longest fragment) in urine Sources of variability: influenced by renal function and circadian rhythm
s-ALP	Serum alkaline phosphatase (total)	Ubiquitous, membrane bound tetrameric enzyme located on the outer cell surface of various tissues: liver, bone, intestine, spleen, kidney and placenta	Automated Manual	Specificity: non-specific for bone (about 50% is liver isoform in healthy individuals) Multiple assay methodologies Source of variability: very small circadian rhythm
s-BALP	Serum bone-specific alkaline phosphatase	Ubiquitous, membrane bound tetrameric enzyme located on the outer cell surface of osteoblasts	Automated Manual	Specificity: specific for bone, but with some cross-reactivity with liver isoform (up to 20%) Multiple assay methodologies Source of variability: very small circadian rhythm
s-PICP	Procollagen type I C propeptide	Precursor molecules of collagen type I synthesised by osteoblasts	Manual	Specificity: mostly derived from bone collagen type I (around 90%). Short serum half-life. Regulated by hormones (thyroid, IGF-1) Source of variability: small circadian rhythm
s-PINP	Procollagen type I N propeptide	Precursor molecules of collagen type I synthesised by osteoblasts	Automated Manual	Specificity: mostly derived from bone collagen type I Assay: may recognise trimer alone (intact) or trimer and monomer (total PINP) Source of variability: small circadian rhythm

associated with fracture risk. Several studies have reported that in women with a low BMD, the presence of increased BTMs has an additive effect on fracture risk [49–55].

An example of the contribution of u-CTX to hip fracture probability and its independence from BMD is shown in Fig. 1 [67]. Potential difficulty with such analysis is that the proportion of the population in each risk set is not defined. Thus, the small proportion of women with both low BMD and high u-CTX have an elevated risk compared to those in the lowest part of the distribution of BMD and with the

Table 4 Prospective studies of bone turnover markers to predict fracture in men and women not on treatment for osteoporosis

Study	Population and setting	Age (years)	Expression of risk	Length of follow-up	Fracture type	Outcome
[56]	France, 195 elderly institutionalised women	70–101	Higher versus lower than 2 SD above premenopausal range	18 months	Hip	Relative risk (99.9% CI), adjusted for treatment (Ca + vitamin D versus placebo), cardiovascular and neurological diseases, poor vision, treatment with psychotropic drugs s-ucOC RR=5.9 (1.5–22.7)
[49]	Sweden, population-based sample of 328 women of Scandinavian background, one city, urban, randomly selected from city population files	40–80	OR/SD change in BTM	5 years	All	Odds ratio adjusted for age and BMC by single photon absorptiometry s-OC OR=1.3 NS s-ICTP OR=1.9 p=0.043 s-PICP OR=1.8 p=0.015 OR=2.4 p=0.036 in patients aged 70–80 years old
[50]	Five French cities, volunteers from population-based listing, nested case–control study, EPIDOS cohort, 109 patients with hip fracture, 292 controls	>74	OR for 1 SD increase in BTM and 1 SD decrease in FN BMD; or Three highest quartiles versus lowest quartile; or ≥2 SD above premenopausal range	Mean 22 months	Hip	Odds ratio (95% CI) Total s-OC OR=1.0 (0.8–1.2)[a] OR=1.1 (0.7–1.9)[b] OR=1.0 (0.6–1.6)[c] s-BALP OR=0.9 (0.7–1.2)[a] OR=0.9 (0.6–1.4)[b] OR=1.1 (0.7–1.7)[c] u-NTX OR=1.1 (0.9–1.4)[a] OR=1.1 (0.7–1.9)[b] OR=1.4 (0.9–2.2)[c] u-CTX OR=1.3 (1.0–1.6)[a] OR=2.1 (1.3–3.3)[b] OR=2.2 (1.3–3.6)[c] Sens. 0.36[c] Spec. 0.81[c] u-DPD OR=1.4 (1.1–1.7)[a] OR=1.5 (0.9–2.5)[b] OR=1.9 (1.1–3.2)[c] Sens. 0.30[c] Spec. 0.81[c]
[54]	Residents of one district of Rotterdam, nested case–control study, Rotterdam cohort, 36 women with hip fracture, 163 without hip fracture	>55	RR/SD increase in BTM	Median 2.4 years	Hip	Relative risk (95% CI), adjusted for age only or for age and disability. HPLC measurement if not otherwise stated u-PYD, total RR=3.3 (1.3–8.6) RR=1.6 (0.4–5.9) if disability adjusted free RR=3.0 (1.2–7.2) RR=1.9 (0.6–5.6) if disability adjusted u-DPD, total RR=2.2 (0.8–6.0) RR=1.0 (0.3–3.8) if disability adjusted free RR=1.8 (0.8–4.1) RR=1.2 (0.5–3.2) if disability adjusted free (ELISA) RR=10.2 (1.4–74.6) RR=4.5 (0.4–46.8) if disability adjusted
[55]	EPIDOS cohort, 109 patients with hip fracture, 255 controls	> 74	Highest quartile of controls	Mean 22 months	Hip	Odds ratio (95% CI), with or without adjustment for FN BMD and gait speed Total s-OC OR=1.3 (0.7–2.1) p=0.39 s-ucOC (ELISA) OR=1.9 (1.2–3.0) p<0.008 OR=1.8 (1.1–3.0) if FN BMD adjusted OR=1.9 (1.1–3.1) if FN BMD and gait speed adjusted s-ucOC (HAP) OR=1.6 (0.9–2.7) p=0.07 % s-ucOC (HAP) OR=2.0 (1.3–3.3) p<0.004 OR=1.8 (1.1–2.9) if FN BMD adjusted OR=1.7 (1.0–2.8) if FN BMD and gait speed adjusted
[46]	700 women from the Study of Osteoporotic Fracture, randomly selected and recruited from population-based listings	>65	Lowest or highest quintile versus other quintiles	3.7 years	Hip and vertebral	Total s-OC s-BALP s-CTX For all markers, a trend was found between lowest quintiles and fracture rate but non-significant (p>0.05), even after adjustment for age and oestrogen use.
[52]	Hawaii, 512 community dwelling, postmenopausal women	43–80	OR/SD change in BTM	Mean 2.7 years	All	Odds ratio (95% CI), all adjusted for age and time of sample collection Spine s-BALP OR=1.54 (1.12–2.12) OR=1.49 (1.07–2.07) if BMD adjusted u-CTX/Cr OR=1.43 (1.04–1.98) OR=1.33 (0.94–1.88) if BMD adjusted Non-spine s-BALP OR=1.88 (1.34–2.65) OR=1.80 (1.27–2.56) if BMD adjusted u-CTX/Cr OR=1.84 (1.31–2.58) OR=1.70 (1.18–2.45) if BMD adjusted All s-BALP OR=1.53 (1.18–1.98) OR=1.45 (1.11–1.89) if BMD adjusted u-CTX/Cr OR=1.54 (1.19–1.99) OR=1.39 (1.06–1.83) if BMD adjusted
[57]	408 elderly women from the EPIDOS cohort	>75	>2 SD above the premenopausal range	3.3 years	Hip	Hazard ratio (95% CI) s-CTX HR=1.86 (1.01–3.76) For samples taken only in early afternoon u-CTX HR=1.67 (1.19–2.32) Free u-DPD HR=2.07 (1.49–2.9)

Table 4 (continued)

Study	Population and setting	Age (years)	Expression of risk	Length of follow-up	Fracture type	Outcome				
[58]	792 Finnish home-dwelling women and men	≥70	Cases versus controls with Z-score≥1 SD	5 years	All	Risk ratio (95% CI), with and without adjustment for age, sex, habit of doing heavy outdoor work, ability to go out unassisted, ability to carry a 5-kg load 100 m, body mass index, fear of falling, stroke, knee extension strength, cognitive status, visual acuity, PEF, use of psychotropic medication and use of anti-Parkinson medication				
						Total s-OC	RR=1.22 (0.68–2.17)	RR=1.09 (0.57–2.07) adjusted		
						Carboxylated s-OC	RR=1.77 (1.10–2.86)	RR=2.00 (1.20–3.36) adjusted		
						Carboxylated/ Total s-OC	RR=3.47 (2.23–5.42)	RR=5.32 (3.26–8.68) adjusted		
[59]	435 postmenopausal untreated women, from the OFELY cohort, 55 patients with osteoporotic fractures, 380 controls	50–89	Highest versus lowest quartile Levels >2 SD of premenopausal women	5 years	All	Relative risk (95% CI) adjusted to age, presence of prevalent fracture and physical activity				
						s-OC	RR=1.5 (0.8–2.7)[d]	p=0.20	RR=1.5 (0.8–2.7)[e]	p=0.20
						s-BALP	RR=2.4 (1.3–4.2)[d]	p=0.005	RR=1.9 (1.13–3.4)[e]	p=0.03
						s-PICP	RR=1.3 (0.7–2.5)[d]	p=0.40	RR=1.7 (0.7–2.5)[e]	p=0.43
						s-PINP	RR=1.3 (0.7–2.4)[d]	p=0.42	RR=1.6 (0.8–3.4)[e]	p=0.22
						u-CTX	RR=2.3 (1.3–4.1)[d]	p=0.008	RR=2.3 (1.3–4.1)[e]	p=0.008
						s-CTX	RR=2.1 (1.2–3.8)[d]	p=0.01	RR=1.9 (1.05–3.6)[e]	p=0.04
						u-NTX	RR=1.7 (0.9–3.2)[d]	p=0.09	RR=1.7 (0.9–3.2)[e]	p=0.09
						Free u-DPD	RR=1.8 (1.0–3.4)[d]	p=0.07	RR=1.8 (0.9–3.6)[e]	p=0.07
[48]	229 elderly Caucasian women from Amsterdam from homes or apartments for elderly	70–96	Highest quartile versus Q2-4	5 years (up to 7.6 years)	All	Relative risk (95% CI), adjusted for age				
						u-NTX	Hip	RR=2.6 (0.8–8.1)	p>0.05	
							Osteoporotic	RR=1.9 (0.8–4.6)	p>0.05	
							Non-vertebral	RR=2.6 (1.3–5.0)	p<0.05	
[60]	375 women recruited by age-stratified randomization from general practice populations in Sheffield	50–85 (mean 64.5)	Cases versus controls in upper quartile	5 years	All	Relative risk (95% CI)				
							Non-vertebral	Vertebral		
						u-PYD/Cr	RR=1.2 (1.2–1.3)	RR=1.3 (1.1–1.5)		
						u-NTX/Cr	Non-significant	RR=1.2 (1.0–1.5)		
						u-DPD/Cr, Free uDPD/Cr, s-TRACP, s-PICP, s-BALP, s-OC All non-significant				
[61]	408 postmenopausal untreated women, enrolled in the OFELY study, 65 with fractures, 343 in control group	50–89	Highest quartile compared to lower three quartiles	6.8 years	All	Relative risk (95% CI), adjusted for age, presence of prevalent fracture and physical activity				
						u-CTX isoforms	α-L	RR=2.0 (1.1–3.4)		
							β-L	RR=1.7 (1.0–2.9)		
							α-D	RR=1.2 (0.6–2.2)		
							β-D	RR=1.5 (0.8–2.6)		
							Total	RR=1.9 (1.1–3.2)		
						u-CTX ratio	α-L/β-L	RR=2.0 (1.2–3.5)	RR=1.7 (95% CI 0.95–2.9)[f]	
							α-L/α-D	RR=1.8 (1.0–2.7)	RR=1.6 (95% CI 0.89–2.8)[f]	
							α-L/β-D	RR=1.5 (0.9–2.7)		
[62]	603 calcium-replete postmeno pausal women, placebo arm of the intermittent cyclical tiludronate, with 1 moderate or 2 mild prevalent vertebral fractures and a BMD <−1.7 SD in two lumbar sites.	50–80	OR/3-month change in BTM level; or (log-transformed); or RR highest versus lowest quartile of baseline BTM; or RR highest versus lowest 3-month change in BTM	3 years	Vertebral	Odds ratio or relative risk (95% CI)				
						s-OC	OR=10.92 (2.22–53.78)[g]	RR=1.12 (0.62–2.04)[h]	RR=0.31 (0.15–0.65)[i]	
						u-CTX	OR=1.37 (1.00–1.87)[g]	RR=0.88 (0.53–1.53)[h]	RR=0.60 (0.34–1.09)[i]	
						s-BALP		RR=0.66 (0.37–1.17)[h]	RR=0.89 (0.51–1.54)[i]	

[63]	225 postmenopausal women, from Rochester cohort of free-living and institutional ised women 30 years +	68±13.6 mean	1 SD increase in baseline BTM	14 years	All	Hazard ratio (95% CI), age-adjusted				
						s-OC	Thoracic/lumbar	HR=0.80 (0.65–0.98)	$p<0.05$	
							Proximal femur	HR=1.16 (0.75–1.81)	$p>0.05$	
							Distal forearm	HR=1.34 (0.87–2.06)	$p>0.05$	
							Any OP fractures	HR=0.92 (0.78–1.10)	$p>0.05$	
						s-ALP	Thoracic/lumbar	HR=1.09 (0.80–1.47)	$p>0.05$	
							Proximal femur	HR=1.07 (0.80–1.42)	$p>0.05$	
							Distal forearm	HR=0.92 (0.66–1.29)	$p>0.05$	
							Any OP fractures	HR=1.06 (0.84–1.34)	$p>0.05$	
[51]	Random population enrolment of 1,040 women in Malmo.	75	Highest quartile compared to lower three quartiles	3–6.5 years	All	Odds ratio (95% CI)				
								At least 1	Hip fracture	Clinical vertebral	Multiple fractures
						s-BALP		0.89 (0.61–1.29)	0.94 (0.46–1.93)	1.43 (0.78–2.71)	1.25 (0.61–2.54)
						Total s-OC (N-mid)		1.17 (0.80–1.69)	0.65 (0.28–1.50)	1.80 (0.97–3.36)	1.35 (0.65–2.80)
						s-OC [1–49]		1.19 (0.83–1.72)	0.77 (0.35–1.71)	1.41 (0.75–2.64)	1.00 (0.58–1.70)
						Total s-OC		0.98 (0.67–1.43)	0.52 (0.21–1.25)	1.33 (0.71–2.50)	0.68 (0.30–1.58)
						s-OC		1.03 (0.71–1.50)	0.88 (0.41–1.87)	1.51 (0.81–2.81)	0.83 (0.37–1.85)
						u-DPD/Cr		0.94 (0.64–1.38)	1.02 (0.50–2.12)	1.48 (0.80–2.76)	1.21 (0.59–2.48)
						s-TRACP(5b)		1.55 (1.09–2.20)	1.54 (0.78–3.05)	2.28 (1.26–4.15)	1.95 (0.98–3.86)
						s-CTX		1.18 (0.81–1.70)	1.01 (0.48–2.11)	1.94 (1.05–3.58)	1.16 (0.55–2.45)
						u-OC(Long)/Cr		1.53 (1.07–2.18)	1.28 (0.62–2.61)	2.75 (1.52–4.96)	2.12 (1.08–4.14)
						u-OC(Mid)/Cr		1.40 (0.98–2.01)	1.79 (0.91–3.52)	2.71 (1.50–4.89)	1.48 (0.73–2.99)
								Clinical vertebral, FN BMD adjusted		Clinical vertebral, LS BMD adjusted	
						s-CTX		1.53 (0.79–2.97)		1.58 (0.83–2.98)	
						s-TRACP(5b)		2.21 (1.17–4.17)		1.78 (0.95–3.33)	
						u-OC(Long)/Cr		2.15 (1.15–4.05)		2.25 (1.21–4.18)	
						u-OC(Mid)/Cr		1.86 (0.99–3.52)		2.02 (1.08–3.77)	
[64]	Case-cohort control study of 151 elderly men, the Dubbo Study	≥70	Highest quartile of the distribution compared to lowest Uni- and multi-variate analysis, RR for 1 SD change s-ICTP +1.5 g/L s-CTX +0.15 ng/ml s-PINP +15 g/L	6.3 years	All	Relative risk (95% CI), variables used: age, weight, height, FN BMD, FN BMD/year, prevalent fracture, calcium intake, smoking habits, s-ICTP, s-CTX, s-PINP, s-creatinine and s-albumin				
						s-ICTP		RR=2.8 (1.4–5.4)[j]	RR=1.8 (1.4–2.3)[k]	$p<0.001$	
							All fractures	RR=1.4 (1.0-1.9)[j]		$p<0.05$	
							Hip	RR=1.7 (1.2-2.6)[j]		$p<0.01$	
							Non-vertebral	RR=2.1 (1.3-3.3)[j]		$p<0.005$	
							Non-hip, non-vertebral	RR=1.7 (1.1-2.4)[j]		$p<0.05$	
						s-CTX		RR=1.6 (0.8–3.3)[j]	RR=1.2 (0.98–1.6)[k]		
						s-PINP		RR=1.4 (0.8–1.6)[j]	RR=1.1 (0.9–1.4)[k]		
[53]	322 postmenopausal osteopenic women from the OFELY study on randomly selected 1,039 volunteer women aged 31–89 years	Mean 64	Highest quartile, but fail to explain the comparator	9.1 years	All	Hazard ratio (95% CI), age-adjusted. 10-year probability adjusted for mortality				
						s-BALP		HR=2.2 (1.4–3.8) Sens. 0.43 Spec. 0.75	$p<0.003$	10-year probability	28.4%
						s-OC(intact) and s-CTX were measured, but data not reported					
[47]	Prospective cohort study of 960 elderly Austrian women from nursing homes	>70	Per increment of 1 of the respective unit	2 year	Hip and non-vertebral	Relative risk (95% CI)				
						s-OC	Hip	RR=0.99 (0.97–1.00)	$p=0.21$		
							Non-vertebral	RR=0.99 (0.99–1.00)	$p=0.80$		
						s-CTX	Hip	RR=1.27 (0.45–3.60)	$p=0.57$		
							Non-vertebral	RR=1.41 (0.77–2.60)	$p=0.26$		
[12]	790 men from the MINOS study	50–85	1 SD of log-transformed BTM level	7.5 years	All	Odds ratio for s-OC(total), s-BALP, s-PINP, s-CTX, u-CTX/Cr, u-DPD(total)/Cr					
						No markers predicted incident fractures. After adjustment for age, BMI, BMD and prevalent fracture, the average odds ratio varied from 1.015 to 1.382 with $p>0.37$					

Table 4 (continued)

Study	Population and setting	Age (years)	Expression of risk	Length of follow-up	Fracture type	Outcome			
[65]	Population-based cohort of 1,044 elderly women from the Malmö OPRA study	75	Per SD change Highest tertile compared to lowest	9 years (7.4–10.9)	All and vertebral only	Hazard ratio (95% CI)			
							All	Vertebral	
						s-CTX	HR=1.13 (1.01–1.27)	HR=1.32 (1.05–1.67)	
							HR=1.29 (0.99–1.67)	HR=1.42 (0.88–2.28)	
						s-TRACP(5b)	HR=1.16 (1.04–1.29)	HR=1.22 (1.01–1.48)	
							HR=1.40 (1.09–1.81)	HR=1.43 (0.9–2.28)	
						u-OC(Mid)	HR=1.08 (0.97–1.20)	HR=1.14 (0.93–1.40)	
							HR=1.34 (1.04–1.74)	HR=1.43 (0.90–2.26)	
						No markers were able to predict hip fractures only (data not shown). All HRs were not statistically significant when adjusted to total body BMD at baseline			
[66]	1,005 men randomly selected from the MrOs study, a prospective cohort study in several US communities	>65	Highest quartile versus three lower quartile	5 years	Hip and non-spinal	Hazard ratio (CI 95%), adjusted for age and clinic			
						s-PINP	Hip	HR=2.13 (1.23–3.68)	HR=1.16 (0.57–2.36)[m]
							Non-spine	HR=1.57 (1.21–2.05)	HR=1.31 (0.98–1.74)[m]
						s-CTX	Hip	HR=1.76 (1.04–2.98)	HR=1.04 (0.55–1.97)[m]
							Non-spine	HR=1.29 (0.99–1.69)	HR=1.07 (0.80–1.42)[m]
						s-TRACP(5b)	Hip	HR=0.92 (0.50–1.71)	HR=0.66 (0.32–1.39)[m]
							Non-spine	HR=0.17 (0.88–1.55)	HR=1.05 (0.77–1.42)[m]

HPLC high-performance liquid chromatography, *ELISA* enzyme-linked immunosorbent assay, *PEF* peak expiratory flow, *HAP* hydroxyapatite binding assay, *BMI* body mass index, *BMD* bone mineral density (*FN* femoral neck, *LS* lumbar spine), *CI* confidence interval, *SD* standard deviation, *Sens.* Sensitivity, *Spec.* Specificity, *NS* non-significant, *OP* osteoporosis

[a]OR for 1 SD increase in BTM and 1 SD decrease in FN BMD

[b]Three highest quartiles versus lowest quartile

[c]≥2 SD above premenopausal range

[d]Highest versus lowest quartile

[e]Levels >2 SD of premenopausal women

[f]RR adjusted for bone ALP and FN BMD also

[g]OR/3-month change in BTM level (log-transformed)

[h]RR Highest versus lowest quartile of baseline BTM

[i]RR Highest versus lowest 3-month change in BTM

[j]Highest quartile of the distribution compared to lowest

[k]Uni-variate analysis, RR for 1 SD change

[l]Multi-variate analysis, RR for 1 SD change

[m]Adjusted for also BMI, race, diabetes, grip strength and baseline total hip BMD

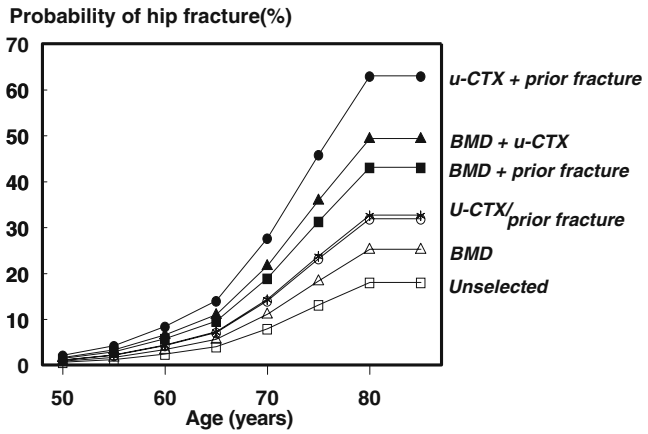

Fig. 1 The impact of u-CTX, bone mineral density (*BMD*) and prior fracture on the 10-year hip fracture probability based on the EPIDOS data applied to women from Sweden [67]. BMD refers to a T-score of less than or equal to −2.5 SD at the femoral neck and u-CTX to a urinary value that exceeds the upper limit of normal for premenopausal women

highest u-CTX alone. ROC analysis methods may be preferable to assess the additive benefit of multiple tests.

Men have been less extensively studied than women, but several studies suggest that one or more BTM may be of value in fracture risk prediction. In a study of elderly men in northern Finland, a decrease in carboxylated s-OC/total s-OC ratio was associated with increased risk of subsequent fractures [58]. In the Dubbo Osteoporosis Study of elderly men in Australia, an increased serum carboxyterminal crosslinking telopeptide of type I collagen (s-ICTP) was associated with an increased risk of osteoporotic fractures independent of BMD [64]. In the US MrOS study, hip and non-spine fractures were associated with increased s-PINP and s-CTX, an association no longer evident after adjustment for hip BMD [66]. In contrast, a range of markers of formation and resorption were of no predictive value in men from the MINOS study [12].

Limitations in fracture risk prediction

It would appear that BTMs, particularly those of bone resorption, have some utility in predicting fracture outcomes. It is a challenge, however, to draw clear conclusions from these 22 studies detailed for several reasons:

1. Table 4 includes 17 different BTMs. In a given study, there have been up to ten different BTMs measured [51]. The large number of predictions published raises the possibility of false positive results.
2. There is heterogeneity in the fracture outcomes reported. There have been up to four different fracture classifications, such as spine, hip, non-spine and all fractures [51].
3. In some studies, the statistical approach was multiple; for example, bone turnover was considered as odds ratio per standard deviation increase in BTM, a BTM lying within the top three quartiles (compared to the lowest quartile) or value more than two standard deviations above the premenopausal reference interval [50]. This is further discussed below.
4. For any given analyte, there is some inconsistency in the predictive value of specific markers. For example, s-OC is variously a strong [56, 62], moderate [55, 63], borderline [49, 50] or non-significant [47, 51, 58–60, 68] predictor of fracture risk.
5. The association with bone formation markers and fracture risk was usually, though not invariably, not statistically significant; this included OC, BALP, PICP and PINP. Indeed, the one study that showed a significant association with PICP indicated that fractures were associated with a lower PICP [49].
6. The association of bone resorption markers and fracture risk appeared more consistent than that with bone formation markers, particularly for urinary free DPD and u-CTX. However, the closely chemically related total DPD [54, 60] and u-NTX were not usually associated with fracture risk.
7. The lack of consistency also related to the analytic method used. Thus, although undercarboxylated OC was related to fracture risk in at least two studies, the association was significant when the assay was an immunoassay, but not when there was a hydroxyapatite binding step [55]. The assay for free DPD was significant when performed by immunoassay but not when performed by high-performance liquid chromatography [54].
8. The time of day is critical to the level of some BTMs (Table 1). For example, levels of CTX are much lower in the afternoon than in the morning and related to the ingestion of food, so that fasting samples are normally recommended. Surprisingly, it was the afternoon level of CTX that was more closely related to fracture risk than the morning sample in the one study in which this was examined [57].
9. BTMs would be particularly helpful if their association with fracture risk were independent of BMD. There is a negative correlation between BMD and BTMs, which becomes stronger with advancing age [50, 69–71]. The prediction of BTMs for fracture was independent of BMD in some studies [49, 51, 55, 64] but not in all studies [61, 66].

Expression of risk

As noted above, there has been inconsistency in the manner by which risk is expressed. Examples include comparison of the lowest with the highest quartile of bone marker, comparison of the lowest with the other quartile (or quintile) of bone marker, values above or

below an arbitrary threshold value or as a gradient of risk (GR; in this context meaning the increase in fracture risk per SD increase in marker value). These various approaches hamper assessment of the comparative value of the markers between studies. Interpretation is difficult even within studies. For example, one study [50] reported that u-CTX predicted hip fracture in elderly women with a gradient of risk of 1.3 (1.0–1.6 per SD increase) that might be described as modest. When the same data were presented comparing the highest quartiles with the lowest quartile, the odds ratio was 2.1 (1.3–3.3) which sounds all the more impressive. In another study, hazard ratios (HR) expressed as a gradient of risk failed to show predictive value of s-CTX for fracture (HR/SD=1.12; 95% confidence interval (CI)=0.99–1.26) [65]. When the hazard ratio was the comparison of the upper to the lower tertile, s-CTX was a 'significant' predictor of fracture (HR=1.40; 95% CI=1.05–1.87).

Nearly all these methods of expressing risk have been uncritically used. The starting point is to ascertain the distribution of the analyte. For some, this may follow a Gaussian distribution whereas others will be skewed. Logarithmic transformation has been used in a minority of studies, presumably to normalise the distribution of the measurement [12, 62, 65]. The comparison of the upper quartiles of two different distributions will be akin to comparing apples with oranges.

It is instructive to examine the relationships between gradients of risk and hazard ratios derived from the comparison of quartiles. If it is assumed that a biochemical marker X has a normal distribution (before or after transformation) and that the increase of fracture risk (hazard function) per unit of X is constant over the whole range of X, then the performance characteristics of the analyte can be described as a gradient of risk, e.g. the increase in fracture risk/SD difference in analyte. With these assumptions, hazard ratios can be computed where quartiles are compared, e.g. Q_4/Q_1 or combinations are compared, e.g. $Q_4/Q_{1–3}$ (Fig. 2).

Q_i is the mean of the hazard function in the corresponding quartile. $Q_{1–3}$ is the mean for the range corresponding to Q_3, Q_2 and Q_1. $Q_{1–4}$ is the mean of the hazard function. Let GR denote the gradient of risk per 1 standard deviation. The mean of the hazard function in the interval a to b is equal to a constant time.

$$\int_a^b \exp(\log(\mathrm{GR}) \times x) \times \frac{1}{\sqrt{2\times\pi}} \times \exp(-x^2/2)\mathrm{d}x/[\Phi(b)-\Phi(a)] =$$
$$\int_a^b \frac{1}{\sqrt{2\times\pi}} \exp\left(-\frac{1}{2}\left(x^2 - 2 \times \log(\mathrm{GR}) \times x + (\log(\mathrm{GR}))^2\right) + \frac{1}{2} \times (\log(\mathrm{GR}))^2\right)\mathrm{d}x/[\Phi(b)-\Phi(a)] =$$
$$\exp\left(\frac{1}{2} \times (\log(\mathrm{GR}))^2\right) \times [\Phi(b-\log(\mathrm{GR})) - \Phi(a-\log(\mathrm{GR}))]/[\Phi(b)-\Phi(a)]$$

The upper quartile limit is −0.6745 SD. By use of the relationship above, the hazards for Q_4, $Q_{1–3}$ etc. can be calculated (Anders Oden, personal communication).

Table 5 shows the hazard ratios when comparing quartiles according to the increase in fracture risk/SD difference in analyte (gradient of risk). It is evident that comparisons of quartiles may give results that are difficult to interpret. For example, for an analyte with a gradient of risk of 2.0, hazard ratios associated with the highest quartile vary from 2.0 to 5.9 depending on the denominator used.

The comparison of adjacent quartiles may give results that are difficult to interpret. If, for example, a variable has a normal distribution and a continuous gradient of risk of say 2.0 per standard deviation (Table 5), then the hazard ratio between Q_4 and Q_3 will be 2.0 and the hazard ratio between Q_3 and Q_2 will be 1.6. The difference in HR arises because Q_1 captures a greater range of the index variable (−5 to −0.7 SD) whereas Q_2 and Q_3 cover a smaller interval (0.7 SD). Thus, differences in hazard ratios should not be interpreted as evidence for non-linearity of risk and vice versa.

In the context of groups, the appropriate risk is that of the group (e.g. above a specified limit) compared to the risk of the general population (e.g. $Q_4/Q_{1–4}$). In the context of fracture risk assessment for an individual, then the most appropriate relative risk (assuming a normal distribution) is the risk of the individual compared with the risk in the normal population.

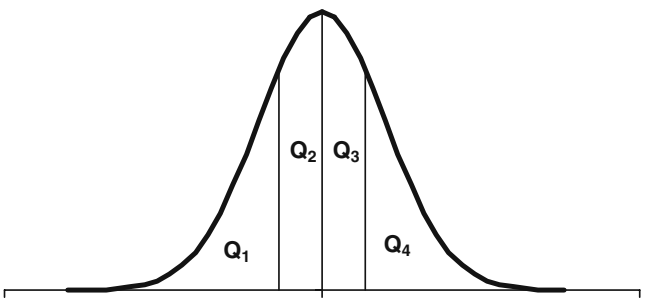

Fig. 2 Normal distribution of a hypothetical analyte according to quartile of the distribution

Table 5 Hazard ratios comparing quartiles according to the increase in fracture risk/SD difference in analyte (gradient of risk)

Gradient of risk	Q_4/Q_{1-3}	Q_4/Q_{1-4}	Q_4/median	Q_4/Q_3	Q_4/Q_{1-2}	Q_4/Q_1	Q_3/Q_2
1.0	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1.5	1.950	1.576	1.711	1.495	2.300	2.812	1.301
2.0	3.090	2.030	2.581	2.042	4.157	5.921	1.568
2.5	4.416	2.382	3.625	2.650	6.625	10.667	1.813
3.0	5.934	2.657	4.858	3.324	9.770	17.430	2.042

Future developments

There have been recent developments in fracture risk assessment, particularly the recognition that the combination of information from independent risk factors for fracture improves the ability to characterise risk. Risk factors for fracture that contribute independently of BMD include age, sex, a prior fragility fracture and a range of clinical risk factors. More recently, the independent contribution of different risk factors for fracture has been quantified [72] permitting the calculation of absolute risk with the FRAX® tool (http://www.shef.ac.uk/FRAX). Since the availability of FRAX, treatment decisions in the management of osteoporosis are increasingly being based on the assessment of a patient's probability of sustaining a fragility fracture [8, 21, 73–77].

Risk ratios can be converted to fracture probabilities with knowledge of the fracture and death hazards and the prevalence of the risk factor of the country concerned. Table 6 shows the conversion for the UK for an analyte where a value above a certain threshold is associated with a 2.5-fold increase in risk of fracture. Assume, for example, that the prevalence of a high marker value is 25% (s-CTX in women aged 80 years is approximately 25%), then the 10-year hip fracture probability is 20% when applied to a population from the UK. A further example in a Swedish setting is provided in the Epidemiology of Osteoporosis (EPIDOS) study for s-CTX (see Fig. 1).

BTMs are currently not included in the FRAX algorithms because of the scarcity of population-based prospective studies with any single analyte. The applicability of the research data base in an international setting is also insecure; for example, more than one third of studies are from France and none from Asia. The remedy is to enlarge the experience of the value of BTMs for fracture risk assessment in population-based studies around the world. In so doing, the incorporation of reference analytes using standardised methodology would permit the synthesis of large data bases suitable for meta-analyses to determine the quantum of their predictive value for different fracture outcomes. In addition to the estimate of relative risk, research questions include the distribution of the analytes and subsequent performance characteristics and the dependence of BTMs on the other clinical risk factors used in FRAX. A further consideration is whether the predictive value is constant with time, since the limited data available raise the possibility that the performance characteristics of BTMs may attenuate with time (Fig. 3) [58, 65] as observed with some other risk factors.

Table 6 Ten year probability (percent) of hip fracture in women from the UK according to age, risk ratio and proportion of population with the risk factor

Age (years)	Proportion of population having the condition									
	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
50	1.2	1.1	1.1	1.0	0.9	0.9	0.9	0.8	0.8	0.7
55	1.9	1.8	1.7	1.6	1.5	1.4	1.4	1.3	1.2	1.2
60	4.1	3.9	3.6	3.4	3.3	3.1	2.9	2.8	2.7	2.6
65	8.3	7.8	7.3	6.9	6.6	6.2	5.9	5.7	5.4	5.2
70	13	13	12	11	11	10	9.7	9.3	8.9	8.5
75	20	19	18	17	16	15	15	14	14	13
80	24	23	22	21	20	19	18	18	17	16
85	23	22	21	20	19	18	17	16	16	15

Risk ratio=2.50 (JA Kanis, A Oden, H Johansson, EV McCloskey, previously unpublished)

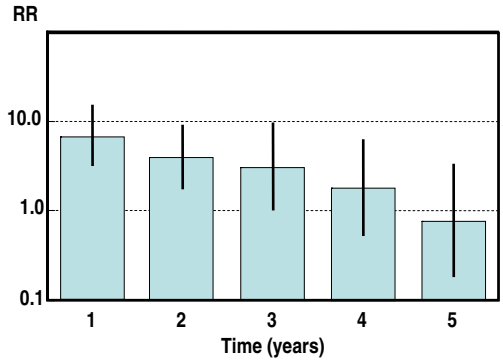

Fig. 3 Relative risk of fracture with 95% confidence intervals for the ratio of carboxylated to total serum osteocalcin in elderly men and women according to duration of follow-up [58]

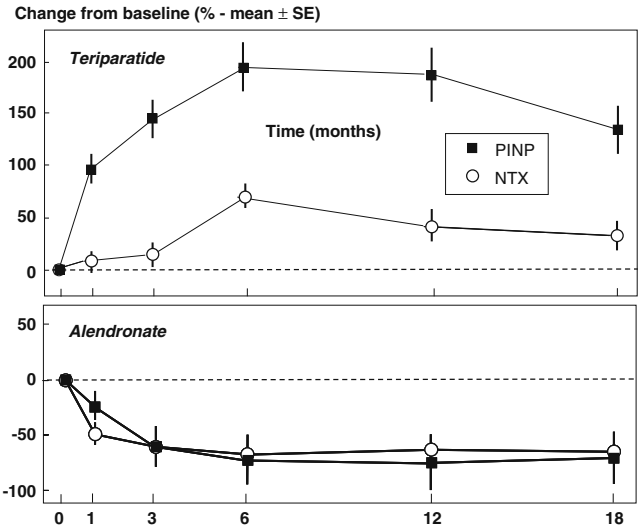

Fig. 4 Changes (% ± SEM) in markers of bone resorption (*NTX*) and bone formation (*PINP*) following treatment with an anti-resorptive therapy (alendronate) and an anabolic therapy (teriparatide), redrawn from [79]

Bone turnover markers in monitoring of osteoporosis treatment

Rationale

BTMs may show large and rapid responses to the treatments used for osteoporosis, and their measurement has proved useful during drug development. Their response to treatment may allow the best choice of dose and dose frequency. They may also help with proof of principle and help establish mechanism of action. The decrease in marker values, particularly the indices of bone resorption, occurs within days or weeks of starting treatment with anti-resorptive agents. In contrast, the change in BMD occurs over months or years so that BTMs may give earlier information on the response to treatment than BMD. Moreover, the decrement in marker values is large in the case of bisphosphonates (e.g. by 50% or more), whereas the increment in BMD is modest (e.g. 5%). The responsiveness of the markers to intervention provides a rationale for their use to monitor treatment in a clinical setting.

Patterns of response

The direction of the response and its magnitude and time course differ by treatment and by BTM. The nature of the BTM response is determined by the mechanism of action of the drug. Thus, treatment of postmenopausal osteoporosis with an anti-resorptive treatment, such as the bisphosphonate alendronate, results in an early decrease in bone resorption markers followed by a decrease in bone formation markers after a delay of about 4 weeks (Fig. 4). Bisphosphonates reduce the rate of bone remodelling, and as remodelling begins with bone resorption to be followed about 4 weeks later by bone formation at the same location ('coupling'), the action of the drug is first evident on bone resorption. These changes in BTMs following anti-resorptive therapy are followed by an increase in BMD, assumed to be related to the decrease in bone turnover and the consequences therefrom [78].

This pattern of response contrasts with treatment with an anabolic agent such as teriparatide, which results initially in an increase in bone formation and later with an increase in bone resorption (see Fig. 4) [79]. The early increase in bone formation is not due to changes in bone remodelling rate but reflects a direct stimulation of bone formation.

The dose of the drug is another major determinant of the BTM response. It is usual during drug development to evaluate the rate of onset, the magnitude of response and possible offset using BTMs, as illustrated by the example of denosumab (Fig. 5) [80]. The onset of action on markers of bone resorption, as illustrated by s-CTX, is rapid (within hours). After several months, there is a subsequent resolution of effect (particularly at the lower doses) until the next dose is administered at 6 months. The magnitude of response of BTMs usually relates to that of BMD (greater reductions in BTMs are associated with greater increases in BMD), but the changes usually occur earlier and so allow more rapid evaluation of treatment response.

The route of administration of the drug is another determinant of the BTM response, probably related to the total dose administered. As noted above, denosumab therapy results in a rapid decrease in bone resorption (see Fig. 5) [80]. More rapid decreases in bone resorption are seen with intravenous alendronate than with the oral formulation [81]. Zoledronic acid is a bisphosphonate that is administered intravenously as an annual dose of 5 mg and also reduces bone resorption more rapidly than alendronate by mouth (Fig. 6) [82].

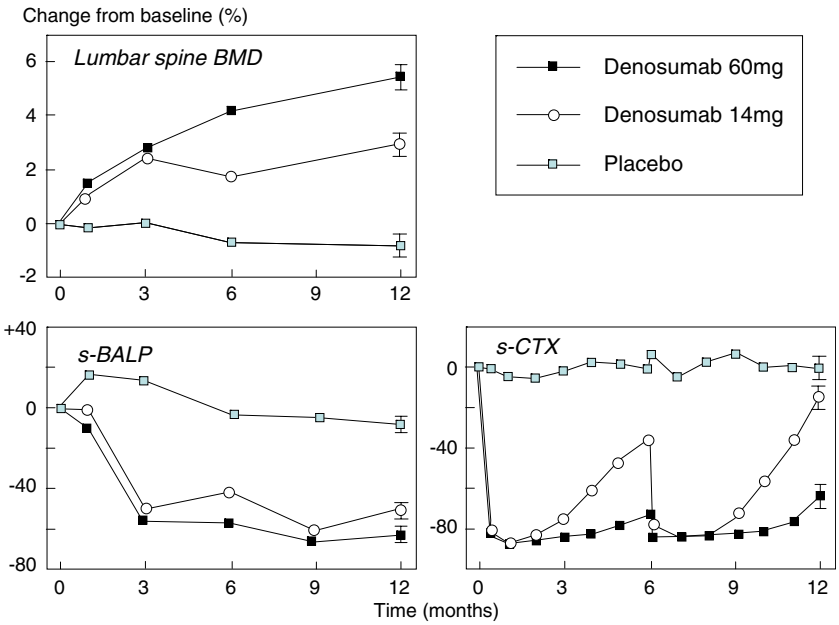

Fig. 5 Effect of denosumab 60 mg and 14 mg and placebo given subcutaneously every 6 months on lumbar spine BMD and a marker of bone resorption (*s-CTX*) and bone formation (*s-BALP*). Denosumab showed a dose-dependent increase in BMD and decrease in BTMs. The dose of 60 mg subcutaneously every 6 months was chosen as the licensed dose [80]

The various drugs licensed for the treatment of osteoporosis have a differing spectrum of effects on BTMs (Table 7). Amongst the anti-resorptive agents, some have a modest effect (such as nasal calcitonin), whereas others have a marked effect (denosumab, zoledronic acid, alendronate).

Not all drugs have the same classical anti-resorptive or anabolic effect (see Fig. 4). Strontium ranelate treatment results in a small decrease in bone resorption and an increase in bone formation [84]. It may be a weak anti-resorptive drug with anabolic properties, or it may have its most important effects through mechanisms that do not involve remodelling (such as changes to crystal properties) [105]. Odanacatib is a cathepsin K inhibitor that is in phase III development which inhibits bone resorption as judged by s-CTX [106] but has no clear effect on TRACP. This may reflect its mode of action to inhibit the degradation of type I collagen without having any effect on the osteoclast viability (TRACP may reflect osteoclast number rather than their activity).

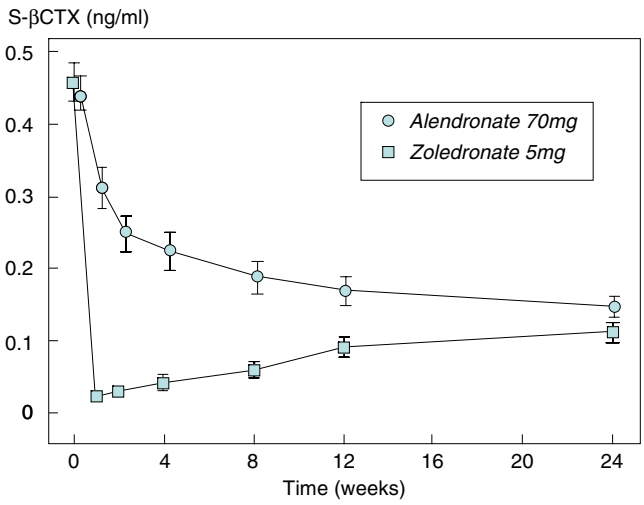

Fig. 6 The time course of s-CTX (mean ± SEM) following treatment with the bisphosphonate alendronate given weekly by mouth and zoledronic acid (zoledronate) given as a single intravenous dose, redrawn from [82]

Not all markers respond by the same amount for a given degree of bone resorption. Amongst the bone resorption markers, s-CTX tends to change more than u-NTX which tends to change more than TRACP. Amongst the bone formation markers, s-PINP tends to change more than BALP (see Table 7). Even closely related markers show different responses; for example, the response of free DPD to alendronate is modest or not present, but the total DPD changes as much as NTX [107]. It is important to recognise that different bone active treatments have different mechanisms of action at the cellular level, and BTMs should be chosen to capture the multiple effects of these agents.

Monitoring osteoporosis treatment

The use of BTMs for the monitoring of treatment requires a baseline assessment with a repeat measurement at some defined point during treatment. In order to do this effectively, it is important to appreciate the expected level of change (see Table 7). Thus, for the more potent drugs, it is possible to monitor treatment effect in the individual. The ability to detect change between the two values with confidence is also related to the imprecision of the measurement as well as biological (intra-individual) variability which may be influenced by factors such as time of day, fasting, adherence to instructions etc. Accuracy is less relevant in this context. Reproducibility is usually expressed as a coefficient of variation (CV).

Table 7 Percentage difference in BTM steady state response to treatment at licensed dose compared to placebo

Treatment	Author	Dose	PINP	OC	BALP	s-CTX	u-NTX	TRACP
Calcitonin	Chesnut [83]	200 IU/day, intranasal				−10		
Strontium ranelate	Meunier [84]	2 g/day			+8	−12		
	Bruyère [85]						−11	
Raloxifene	Naylor [86]	60 mg/day	−34				−25	
	Chesnut [83]			−21		−21		
	Meunier [84]			−20		−28		
	Ettinger [87]			−18				
HRT, oral CEE	Prestwood [88]	0.625 mg/day		−30[a]	−45[a]		−50[a]	
HRT, oestradiol implant	Pereda [89]	25 mg/6 months	−35	−15	−15		−40	
Risedronate	Harris [90]	5 mg/day			−23			
	Rosen [91]	35 mg/week	−48[a]		−28[a]	−55[a]	−40[a]	
Alendronate	Naylor [86]	10 mg/day		−28	−31		−49	
	Hannon [92]	10 mg/day				−71		−27
	Rosen [91]	70 mg/week	−64[a]		−41[a]	−74[a]	−53[a]	
	Emkey [93]	70 mg/week	−68[a]			−81[a]		
	Arlot [79]	70 mg/week	−70[a]				−70[a]	
Ibandronate	Delmas [94]	2.5 mg/day		−34			−31	
	Delmas [95]	2.5 mg/day				−63[a]		
	Miller [96]	150 mg/month				−76[a]		
	Emkey [93]	150 mg/month				−76[a]		
	Delmas [95]	3 mg/3 months i.v.				−58[a]		
Zoledronate	Black [97]	5 mg/year i.v.	−59		−30	−58		
Denosumab	Cummings [98]	60 mg/6 months, s.c.	−50			−72		
	Bone [99]	60 mg/6 months, s.c.	−60			75		−38
	Lewiecki [100]	60 mg/6 months, s.c.			−60	−70	−60	
Teriparatide	Glover [101]	20 μg/day	+111[a,b]	+76[a,b]	+18[a,b]	+5[a,b]	+8[a,b]	+3[a,b]
	Arlot [79]	20 μg/day	+135[a]				+32[a]	
PTH 1–84	Greenspan [102]	100 μg/day			+90		+140	
	Black [103]	100 μg/day	+90[b]		+20[b]	+10[b]		
	Black [104]	100 μg/day	+150[a]			+100[a]		

CEE conjugated equine oestrogen, *PTH* parathyroid hormone, *i.v.* intravenous, *s.c.* subcutaneous, *HRT* hormone replacement therapy

[a]Percent change from baseline (not compared to placebo)

[b]Percent change at 1 month

When BTMs are measured in the untreated state on more than one occasion, the results can be used to calculate the variability within a subject and derive the total intra-individual CV. Intra-individual CVs are shown in Table 8 for some of the analytes. In order to be confident ($p<0.05$) that a change in marker value has occurred, then (assuming a normal distribution) the change in measured value must exceed $\sqrt{2}\times1.96\times CV=2.77\times CV$ which is termed the least significant change (LSC). For example, the LSC for CTX might be $9.6\times2.77=27\%$. In a woman with a baseline value of 0.50 μg/l for CTX, the LSC would be ±0.13 μg/l, and so a significant decrease would be a value of 0.37 μg/l or below and a significant increase would be a value of 0.63 μg/l or above.

One method of improving confidence (i.e. improve the LSC) is to undertake several baseline estimates and to use the mean value. The confidence is inversely proportional to the square root of the number of observations. Thus, confidence is increased two-fold when four baseline measurements are made.

When monitoring treatment in clinical practice, a one-sided rather than two-sided probability of 0.05 is appropriate since the direction of change is known and the LSC would be $\sqrt{2}\times1.65\times CV=2.33\times CV$. In addition, some clinicians consider that an 80% probability ($p<0.2$) is adequate. In this case, the LSC with a one-tailed test is ($\sqrt{2}\times0.84$) 1.19 times the intra-individual variation ($1.19\times$ CV) [13].

Table 8 Currently available commercial assays and their clinical performance for s-CTX and s-PINP

	Commercial assay	Manufacturer	Automated	Assay analytical range (μg/L)	Premenopausal 95% reference interval	Analytical CV (%)	Intra-individual CV (%)
s-CTX	β-CrossLaps ECLIA	Roche Diagnostics, Indianapolis, IN, USA	Yes	0.01–6 [108]	0.11–0.63 [108]	1.3–4.3 [108]	9.4 [112]
					0.10–0.62 [109]	3.7–5.7 [109]	<4.1 [113]
					0.09–0.66 [110]	<5.7 [112, 113]	
					0.07–0.61 [111]		
s-CTX	β-CrossLaps ELISA	IDS, Tyne and Wear, UK	No	0.02–3.38	0.20–0.90 [114]	2.2–5.5 [114]	10.0 [114]
s-PINP	Total PINP ECLIA	Roche Diagnostics, Indianapolis, IN, USA	Yes	5–1,200 [108, 113]	16.3–78.2 [108]	2.7–4.4 [109, 113]	7.2 [113]
					16.2–60.9 [109]		
					14.6–63.5 [111]		
s-PINP	Intact PINP CLIA	IDS, Tyne and Wear, UK	Yes	2–230		<6	
s-PINP	Intact PINP RIA	UniQ PINP RIA Orion Diagnostica, Epsoo, Finland	No	5–250	16–83 [110]	4.1–7.6 [113]	3.7–5 [113]
					17.4–61.6 [114]	2.2 [115]	7.4 [115]
						2.9 [114]	9.1 [114]

CV coefficient of variation, *ECLIA* electrochemiluminescence immunoassay, *CLIA* chemiluminescence immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RIA* radioimmunoassay

Serum CTX and s-PINP show responsiveness to treatment and low within-subject variability. Thus, their measurement usually enables the identification of most responders to treatment using the LSC approach [114, 116].

Evidence for the utility of bone turnover markers in monitoring osteoporosis treatment

Changes in BTMs with treatment are associated with changes in BMD, both for anti-resorptive therapy (see Fig. 5) [117] and for anabolic therapy [118]. However, the changes in BMD with therapy are not closely related to the fracture risk reduction, particularly with anti-resorptive therapy. For example, the change in spine BMD over 3 years explained only 11% of the reduction in spine fracture risk with alendronate [119], 18% for risedronate [120] and close to zero for raloxifene [121]. It has been proposed that the increase in bone strength following anti-resorptive treatment may be partly explained by a reduction in trabecular perforations (i.e. improved bone microarchitecture) that might be captured in the measurement of BTMs, but not by BMD [27, 122].

Several studies have described the relationship between the reduction in BTMs following anti-resorptive therapy and the reduction in vertebral and non-vertebral fracture risk (Table 9). These studies showed in general that the larger the decrease in BTM, the larger the reduction in fracture risk. The percent of treatment effect explained was only calculated for two of these studies, the Vertebral Efficacy with Risedronate Therapy (VERT) and the Multiple Outcomes of Raloxifene Evaluation (MORE) trials. In the VERT study, the change in u-CTX and u-NTX at 3 to 6 months explained between 54% and 77% of the fracture risk reduction with risedronate, depending on the marker, the method of analysis and the fracture type [123]. In the MORE study, the change in PINP and OC explained 28% and 34%, respectively, of the vertebral fracture risk reduction with raloxifene [124, 125].

The alternative way of examining these relationships is to calculate the relative hazard (or odds ratio) for each standard deviation decrease in each BTM on treatment. This analysis has been reported for the Fracture Intervention Trial (FIT; alendronate), Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly (HORIZON; zoledronic acid) and MORE (raloxifene) trials and was of similar magnitude for each marker and each fracture type, although not all of these reached statistical significance (see Table 9). It should be noted that these analyses were also undertaken for teriparatide, but there was no association between BTM changes and fracture risk reduction [118]. However, the total number of fractures in this study was only 74, much fewer than FIT (897), VERT

Table 9 The performance of BTMs in monitoring treatment

Treatment	Trial	Author	Sample size[a]	BTM	Measurement of BTM (months)	% change in BTM	Follow-up for fracture (years)	Fracture endpoint	Outcome (95% CI)
Alendronate	FIT	Bauer 2004 [126]	6,151 (336)	s-BALP	12	−31.1 SD 22.1	Mean 3.6	Vertebral	OR 0.74 (0.63–0.87)
			(515)	s-BALP	12	−31.1 SD 22.1	Mean 3.6	Non-vertebral	RH 0.89 (0.78–1.00)
			(46)	s-BALP	12	−31.1 SD 22.1	Mean 3.6	Hip	RH 0.61 (0.46–0.80)
			6,087 (336)	s-PINP	12	−50.9 SD 30.7	Mean 3.6	Vertebral	OR 0.77 (0.66–0.90)
			(515)	s-PINP	12	−50.9 SD 30.7	Mean 3.6	Non-vertebral	RH 0.90 (0.80–1.03)
			(46)	s-PINP	12	−50.9 SD 31.1	Mean 3.6	Hip	RH 0.78 (0.51–1.19)
			6,142 (336)	s-CTX	12	−59.2 SD 31.1	Mean 3.6	Vertebral	OR 0.83 (0.73–0.95)
			(515)	s-CTX	12	−59.2 SD 31.1	Mean 3.6	Non-vertebral	RH 0.94 (0.84–1.06)
			(46)	s-CTX	12	−59.2 SD 31.1	Mean 3.6	Hip	RH 0.89 (0.61–1.31)
			1114	s-CTX[c]	12	−53.3 SD 35.5	Mean 3.6	Vertebral	OR 0.77 (0.58–1.03)
				s-CTX[c]	12	−53.3 SD 35.5	Mean 3.6	Non-vertebral	RH 1.02 (0.75–1.37)
Risedronate	VERT	Eastell 2003 [123]	693 (42)	u-CTX	3–6	−60	1	Vertebral	TEE 55 (33–92)[f]
			(42)	u-CTX	3–6	−60	1	Vertebral	TEE 34 (0–100)[g]
			(100)	u-CTX	3–6	−60	3	Vertebral	TEE 67 (35–100)[f]
			(100)	u-CTX	3–6	−60	3	Vertebral	TEE 62 (0–100)[g]
			(60)	u-CTX	3–6	−60	3	Non-vertebral	TEE 77[f]
			(42)	u-NTX	3–6	−51	1	Vertebral	TEE 49 (29–84)[f]
			(42)	u-NTX	3–6	−51	1	Vertebral	TEE 29 (0–100)[g]
			(100)	u-NTX	3–6	−51	3	Vertebral	TEE 66 (24–100)[f]
			(100)	u-NTX	3–6	−51	3	Vertebral	TEE 59 (0–100)[g]
			(60)	u-NTX	3–6	−51	3	Non-vertebral	TEE 54[f]
Zoledronic acid	HORIZON	Delmas 2009 [127]	1,270 (130)	s-PINP	36	−56	3	Any clinical	RR 0.62 (0.43–0.88)[h]
			(114)	s-PINP	36	−56	3	Non-vertebral	RR 0.67 (0.46–0.98)[h]
			(20)	s-PINP	36	−56	3	Hip	RR 0.43 (0.17–1.13)[h]
			(21)	s-PINP	36	−56	3	Vertebral	OR 0.32 (0.11–0.86)[h]
			(40)	s-PINP	36	−56	3	Vertebral[d,e]	OR 0.17 (0.07–0.41)[h]
Raloxifene	MORE	Bjarnason 2001 [128]	2,312 (150)	u-CTX	6		3	Vertebral	OR 0.91 (0.73–1.13)
				u-CTX	12	−27.8 SEM 2.3	3	Vertebral	OR 0.81 (0.64–1.03)
			2,403 (155)	s-BALP	6		3	Vertebral	OR 0.63 (0.5–0.8)
				s-BALP	12	−29 SEM 1.2	3	Vertebral	OR 0.75 (0.62–0.92)
			2,413 (161)	s-OC	6		3	Vertebral	OR 0.76 (0.61–0.96)
				s-OC	12	−28.3 SEM 0.7	3	Vertebral	OR 0.69 (0.54–0.88)
Raloxifene	MORE	Sarkar 2004 [125]	2,503 (–[b])	s-OC	24		3	Vertebral	TEE 34 (−0.7–61)[g]
Raloxifene	MORE	Reginster 2004 [124]	967 (–[b])	u-CTX	12	−46.5	3	Vertebral	Slope 0.0027 (−0.0014–0.0068)
				s-BALP	12	−34.6	3	Vertebral	Slope 0.0056 (0.0003–0.0109)
				s-OC	12	−31.8	3	Vertebral	Slope 0.0068 (0.0005–0.0131)
				s-PINP	12	−40.8	3	Vertebral	Slope 0.0085 (0.0021–0.0150)
				s-PINP	12	−40.8	3	Vertebral	TEE 27.5 (3–51)[g]

Strontium ranelate	SOTI/TROPOS	Bruyere 2010 [85]	1,737 (228) (–[b])	s-BALP	3		9.6	3	Vertebral	ROC 0.51 (0.47–0.56)
					3				Non-vertebral	ROC 0.51 (0.47–0.57)
			(228) (–[b])	s-PICP	3		9.9	3	Vertebral	ROC 0.52 (0.48–0.57)
					3				Non-vertebral	ROC 0.47 (0.43–0.52)
			(228) (–[b])	s-CTX	3		–5.9	3	Vertebral	ROC 0.48 (0.43–0.53)
					3				Non-vertebral	ROC 0.46 (0.42–0.51)
			(228) (–[b])	u-NTX	3		11.0	3	Vertebral	ROC 0.52 (0.47–0.57)
					3				Non-vertebral	ROC 0.47 (0.42–0.51)

OR odds ratio per 1 SD decrease in BTM, *RH* relative hazard per 1 SD decrease, *TEE* treatment effect explained (percent), *ROC* area under the ROC curve, *Slope* slope from log regression analyses of vertebral fracture risk and percentage change in BTM

[a]Sample size for BTM measurements (number of patients with fractures)

[b]Number of fractures not given

[c]s-CTX fasting at baseline

[d]Stratum 1 only (no osteoporosis medication at randomisation)

[e]Morphometric vertebral fractures (logistic model analysis)

[f]Method of Li et al. [129]

[g]Method of Freedman et al. [130]

[h]Relative risk in those with BTMs above the premenopausal range (57.7%) compared to those within the reference interval (42.3%)

(160) or HORIZON (170)—the number was not given for the raloxifene study.

These analyses also permitted an evaluation of the relationship between the level of BTM on treatment and fracture risk (for HORIZON and VERT). In the VERT study [131], the fracture risk was at its lowest when the u-CTX level was below a critical point that was equivalent to the mean value for premenopausal women. This introduced the notion that one of the goals of treatment might be to return BTMs to levels in the lower half of the reference interval for premenopausal women.

Strengths and limitations

There are a number of strengths to these analyses. It appears that the percent of treatment effect explained is greater for BTMs than for BMD. This was specifically examined in the FIT trial [126]. Furthermore, the effect of change in BTMs is independent of change in BMD [126]. These important observations led to the flowering of a research effort in bone quality to try to identify treatment benefits other than BMD [122].

The studies provide support for treatment monitoring using percentage change. Thus, in the FIT study, a decrease in serum PINP of more than 30% was associated with a vertebral fracture risk that was 55% less than those with a decrease of less than 30%. This information can be used alongside the LSC approach described above to identify treatment targets. Another treatment target that these studies have identified is to use anti-resorptive treatments to reduce BTMs into the lower half of the reference interval for premenopausal women.

There are limitations to these studies, too. Only five of the many clinical trials of recent years have been analysed. Furthermore, only a subset of patients had the BTM measured in these trials, so that the number of fractures considered was small; only the FIT study was of large size [132]. It is possible that the Fracture Prevention Trial was negative because the BTM sub-study was too small [118].

The BTMs were not always collected in the correct way—the s-CTX samples from FIT were mostly non-fasting [126] and the u-CTX samples from MORE were on first morning void (not the usual second morning void) [125]. In the FIT study, the fractures were only counted if they occurred after the BTM sample was taken [133].

There are a number of statistical pitfalls in these analyses. Changes in BTMs have a skewed distribution and data were not consistently normalised. There are two methods that allow calculation of percent of treatment effect explained, the Freedman and the Li methods [123]. The Li method is based on survival analysis and so is the more robust; however, these approaches assume that the

BTM subset had a reduction in fracture risk, and this is not always reported. Additional limitations of the percent effect explained include wide and often uninterpretable confidence intervals and disagreement about the theoretical underpinnings of the concept.

Future developments

In summary, the available studies relating BTM changes to fracture risk reduction with osteoporosis treatments are promising. Further studies are needed that take care of sample handling, ensure that BTMs are measured in all available patients and use the appropriate statistical methods, including an assessment of whether the final BTM level is a guide to fracture risk.

Other uses for BTMs

Prediction of rate of bone loss

Much of the early work on the clinical utility of BTMs focused on the prediction of bone loss in women at the time of the menopause. It was considered that a low BMD along with a high rate of bone loss might help identify those who would benefit most from hormone replacement therapy (HRT) [134]. This approach had assumed that there would be a subpopulation of 'fast losers' but bone loss is normally distributed. Also, the use of HRT for the prevention of osteoporosis is an approach no longer widely adopted.

BTMs, together with demographic variables, predict 30–40% of the variance in bone loss in untreated postmenopausal women [135]. There are consistent associations between BTMs and bone loss at the distal forearm and the calcaneus, a modest relationship with bone loss at the hip and only a weak relationship with bone loss at the spine; the latter may be related to BMD measurement artifact due to the high prevalence of spinal osteoarthritis in the elderly [135]. Increased BTMs in early menopause have an 80% sensitivity for detecting fast bone losers (bone loss >3%/year) at the forearm in the next 2–12 years, but they have not been shown to be sufficiently predictive of bone loss at the hip or spine in individual patients [135]. Some physicians might use a high BTM to indicate that rapid bone loss is quite likely and so review the patient earlier for BMD monitoring, but BTM thresholds for intervention to prevent bone loss in menopausal and elderly subjects have not been defined.

Identification of secondary osteoporosis

The clinical approach to the patient at risk of fractures should always include a consideration of secondary osteoporosis. The level of BMD has proven less useful in selecting individuals for further workup for secondary osteoporosis [136]. It is possible that BTMs could be used for this purpose. High levels of BTMs are found in metabolic bone disease (such as osteomalacia and Paget's disease), various endocrine disorders (thyrotoxicosis, primary hyperparathyroidism) and in malignant bone disease (e.g. multiple myeloma). Low BTMs may be found in glucocorticoid-induced osteoporosis. Although experienced clinicians use BTMs as a signal to investigate further for secondary osteoporosis, there is no systematic study of this topic available.

Prediction of response to therapy

BTMs might be used to target interventions. For example, a high level of bone resorption in an untreated patient might indicate that a good response to anti-resorptive therapy is likely whereas a low bone formation might indicate a favourable response to anabolic therapy.

The effect of anti-resorptive therapy on BMD may be greater in women with higher BTMs at baseline. For example, the increase in spine BMD in response to HRT is greater in those with higher u-NTX at baseline [31]. However, it is more important to know whether high BTMs predict better fracture risk reduction with treatments. This has been examined in response to alendronate and risedronate. In the FIT, the non-spine fracture efficacy of alendronate was greater in women with higher s-PINP before treatment, suggesting that bisphosphonate treatment may be most effective in women with elevated bone turnover [132]. This was one positive finding against five negative findings (no association of non-vertebral fracture risk reduction with BALP or s-CTX or of vertebral fracture risk with any marker), and so care must be taken in its interpretation. In the study of risedronate [137], a higher baseline u-DPD (total) was associated with a greater gain in spine BMD in the first year, but it was not related to greater fracture risk reduction.

Contrary perhaps to expectation, the effect of anabolic therapy on BMD was greater in women with higher BTMs at baseline. Baseline BTMs were correlated with change in spine BMD in response to teriparatide 20 μg/day (with r values of around 0.4) [118]. However, the baseline BTM did not predict the relative fracture risk reduction with teriparatide [138]. Similarly with strontium ranelate, higher baseline BALP and CTX were associated with greater increases in spine BMD, but no greater reduction in vertebral fracture relative risk [139]. There has been no study in which subjects were stratified according to BTMs and then randomised to treatment or placebo; results from such studies would be of interest [67].

Improving adherence

Adherence to treatment is poor in all chronic diseases; osteoporosis is no exception [140]. There is some evidence that the response to treatment is suboptimal in those who adhere poorly [141, 142].

It is a widespread clinical experience that patients are encouraged to adhere to medication when information about the beneficial effects of the drug is fed back to them. It has been hard to test this in the clinical trial setting because patients who enter clinical trials usually adhere to therapy.

Two studies have examined the effect of monitoring treatment on adherence [143, 144]. In a randomised open study of women treated with raloxifene [143], one group was unmonitored and two other groups monitored, – one by feedback from regular visits to a nurse and the other with the addition of feedback about the results of measurement of u-NTX. When the results of the two monitored groups were examined, adherence to treatment was significantly improved compared to the unmonitored group. However, when the monitored groups were split, there was no significant difference in adherence between those who were interviewed alone and those who had feedback on marker values. This suggests that it was the contact with the nurse that was the determinant of adherence, rather than the feedback on BTMs.

A second study, the Improving Measurements of Persistence on Actonel Treatment study [144], was a controlled trial of risedronate where one group received feedback from measurements of u-NTX at 10 and 22 weeks of a year-long treatment. No difference in persistence was seen between the two groups at 1 year, though persistence was high (approximately 80%). In women who had a good response to treatment as judged by a fall in u-NTX of 30% or more, persistence was improved, but in those who responded poorly in terms of a 30% or greater increase in u-NTX, persistence was worse.

Both studies found that patients who received a positive message (they were responding) also had better compliance. Unfortunately, the converse was also true that patients who received a negative message had worse compliance than the rest of the group.

Research priorities and the need for bone turnover marker reference standards

The review has identified several challenges in the clinical utility of BTMs. Some of these challenges could be met by the adoption of reference analytes and reference standards that should be included in all studies, ideally one bone formation and one bone resorption marker. This would not preclude the use of other BTMs in these studies but provide internal references and the ability to pool studies more easily. Specifically, this would permit:

1. Meta-analyses of prediction studies; whether BTMs predict fractures, and if so, to examine the nature of that relationship.
2. Appropriately powered cohort studies with samples collected and stored under optimal conditions so that BTMs can be considered alongside other risk factors for fracture in the FRAX® algorithm.
3. Inclusion in clinical trials, measurements of the reference BTMs in all subjects to allow careful study of the relationship between change in BTM and fracture risk reduction and also examine the relationship between the baseline BTM and fracture risk reduction and the relationship between BTM on treatment and fracture risk.

Furthermore, appropriate characterisation of the reference range for different analytes and efficient generation of normative values remain a priority. These should be supplemented by regular and consistent external control.

Criteria for the selection of reference bone turnover marker standards

1. The reference BTMs should be adequately characterised and clearly defined.
2. The reference BTMs should be bone specific and should ideally perform well both in fracture risk prediction as well as in monitoring treatments used or trialled for osteoporosis treatment amongst women and men.
3. The reference analyte assay should be widely available and the intellectual property covering its use should preferably not be the monopoly of a single owner.
4. The reference BTMs should have biological and physicochemical characteristics that make them suitable candidates for practical laboratory use in terms of biological and analytical variability, sample handling, stability, ease of analysis etc.
5. The reference BTMs should be measurable by methodology (ideally automated) that is widely available in routine clinical laboratories.
6. Whilst the medium of measurement could be either blood or urine, the ideal medium is blood as intra-individual variation is significantly greater for urine than for blood since urine measurements have to be corrected for creatinine, which introduces another source of variation. On the other hand, the use of a urine sample avoids the invasive venepuncture associated with a blood sample and may be preferred by patients.

Recommendations for reference bone turnover markers

Serum CTX

We have chosen s-CTX as the reference standard for bone resorption. The rationale for this is given in relation to the above criteria:

1. The standard in the assay is well characterised and is an eight-amino acid peptide, and this allows the development of clearly defined reference standard. An immunoassay has been available for some time. Of course, the peptides in the serum that cross-react with such antibodies have a wide range of molecular weights [145].
2. None of the degradation products of type I collagen is specific to bone, and CTX is no exception. However, it is likely that most CTX is derived from osteoclastic bone resorption given that treatments that reduce bone turnover such as denosumab reduce such markers to very low levels in most individuals (see Fig. 5). It has been evaluated both for fracture prediction and monitoring osteoporosis therapies (see Tables 4 and 9).
3. The marker is widely available as an ELISA kit or on automated immunoassay analysers (Roche, IDS). However, the intellectual property is with a single owner.
4. The biological and analytical variability of s-CTX have been well documented (see Table 8) as are the requirements of sample handling and stability.
5. The assay has been automated and is widely available. When the automated platform is not available, the ELISA would be available.
6. The assay is available for serum or plasma (EDTA preferred).

Serum PINP

We have chosen s-PINP as the reference standard for bone formation. The rationale for this is given in relation to the above criteria:

1. PINP reflects the synthesis of the most abundant protein of bone tissue. The standard in the assay is less well characterised than for CTX. The molecular weight is much larger at 35,000 Da. Immunoassay has been available for some time. These may recognise the trimeric (intact) molecule or both the monomer and trimer (total).
2. Neither of the formation products of type I collagen is specific to bone, and PINP is no exception. However, it is believed that most PINP is produced during bone formation. It has been evaluated already for fracture prediction and monitoring osteoporosis therapies (see Tables 4 and 9).
3. The marker is widely available as an RIA (Orion), or on automated immunoassay analysers (Roche, IDS). There are two types of assay and so the intellectual property is not just held by one source.
4. The biological and analytical variability of s-PINP have been well documented (see Table 8) as is knowledge of its sample handling and stability.
5. The assay has been automated and is widely available. When the automated platform is not available, the RIA or ELISA would be available.
6. The assay is available for serum or plasma.

Standardisation

Over and above the identification of the reference BTMs, an important further step is to standardise the measurement of each marker with the aim of obtaining comparable values for each marker irrespective of the laboratory in which the measurement is made or the method utilised [146]. The use of internationally agreed decision limits and target values for these markers requires that measurements are universally comparable. Standardisation and the establishment of a reference system [147] for the BTMs is the route to achieve this. IFCC has an extensive experience with this process [148–152].

Standardisation of measurement requires the development of a reference measurement system for each BTM, the components of which are as follows [153]: Where possible, the marker (the measurand) should be clearly defined by molecular structure and weight. The pure form of the primary standard material is produced, and a certified value is assigned to that by first principles, e.g. amino acid sequence analysis, and is used to calibrate a primary reference measurement procedure. The National Institute of Standards and Technology and the Institute for Reference Materials and Measurements are usually the repositories of the reference standard material (http://irmm.jrc.europa.eu; http://ts.nist.gov//measurementservices/referencematerials/index.cfm). This primary reference standard material is then used to value assign secondary reference materials using a higher-order reference method such as liquid chromatography–mass spectrometry. Adequate amounts of the secondary reference material are produced and distributed to manufacturers of commercial assays of the analyte in order to calibrate and audit their reference measurement procedure. The manufacturers' measurement procedures are then used to produce calibrators for their routine commercial assays, which are used in the clinical laboratories. In this way, all routine measurements can be traced to the primary reference material [154, 155]. The

cooperation of in vitro diagnostics companies is of course critical to this process.

It has to be borne in mind that issues of commutability of reference materials (that is the ability of a reference or calibrator material to behave similarly to human samples such as serum or urine in assay systems) as well as the imprecision and specificity of particular commercial assays will also have a significant impact on the practical outcome of the implementation of the reference system [146]. However a considerable body of knowledge has been developed on these issues in recent years [156].

Harmonisation

The steps towards attaining international standardisation of assays can be slow and laborious. It is possible that a strategy of harmonisation of assays could be adopted as a short-term interim solution. This strategy would involve comparison studies between different routine clinical assays, plus a higher-order assay, by distributing a panel of human samples for measurement by each of the available assays. The results produced by different assays are analysed by Deming regression and compared to the overall mean for all assays to identify the bias for each routine assay. These data can then be used to correct the bias of various systems in order to obtain a consensus mean and harmonisation of results [157]. The use of correction factors will minimise the systematic differences between results produced by different assays.

Conclusion

Whilst recognising that there is no perfect BTM (or gold standard), the adoption of reference analytes would assist in the accumulation of trial data on BTMs in order to expedite their incorporation into clinical practice. Following the identification of these reference analytes, standardisation of their measurement is envisaged as the next step to be undertaken by this working group in ensuring consistency and comparability of data. IOF and IFCC consider that reference standards for bone formation and resorption markers should be established and that assays based on these standards be used consistently in future clinical trials and observational studies. In conclusion, this review supports the role of BTMs in the management of patients with osteoporosis. The adoption of international reference standards will markedly enhance laboratory consistency and facilitate their inclusion in routine clinical practice.

Acknowledgements This review was undertaken through grants from the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine. It is endorsed by the Committee of Scientific Advisors of the IOF and the Education and Management Division of IFCC. None of the authors were compensated for their contribution.

Conflicts of interest JAK, CC, OB, AJF, MMcC and MS have no competing interests with regard to markers of bone turnover. AG, SDV, TT and DAW declare no competing interests. HAM has participated in speakers bureaux for Roche Diagnostics Australia and Abbott Diagnostics with the provision of travel expenses. RE has received research funding and/or consultancy funding from Inverness Medical, Unipath, Roche Diagnostics and IDS.

References

1. Consensus Development Conference (1993) Diagnosis, prophylaxis and treatment of osteoporosis. Am J Med 94:646–650
2. Kanis JA, Johnell O (2005) Requirements for DXA for the management of osteoporosis in Europe. Osteoporos Int 16:229–238
3. Burge R, Dawson-Hughes B, Solomon D, Wong J, King A, Tosteson A (2007) Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. J Bone Miner Res 22:465–475
4. Kanis JA, McCloskey EV, Johansson H, Oden A, Melton LJ, Khaltaev N (2008) A reference standard for the description of osteoporosis. Bone 42:467–475
5. Kanis JA, Melton LJ, Christiansen C, Johnston C, Khaltaev N (1994) The diagnosis of osteoporosis. J Bone Miner Res 9:1137–1141
6. Delmas PD (2002) Treatment of postmenopausal osteoporosis. Lancet 359:2018–2026
7. MacLean C, Newberry S, Maglione M, McMahon M, Ranganath V, Suttorp M, Mojica W, Timmer M, Alexander A et al (2008) Systematic review: comparative effectiveness of treatments to prevent fractures in men and women with low bone density or osteoporosis. Ann Intern Med 148:197–213
8. Kanis JA, Burlet N, Cooper C, Delmas PD, Reginster J-Y, Borgstrom F, Rizzoli R, on behalf of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) (2008) European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int 19:399–428
9. Bergmann P, Body JJ, Boonen S, Boutsen Y, Devogelaer JP, Goemaere S, Kaufman JM, Reginster JY, Gangji V (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
10. Vesper H, Cosman F, Endres DB, Garnero P, Hoyle NR, Kleerekoper M, Mallinak NJ (2004) Application of biochemical markers of bone turnover in the assessment and monitoring of bone diseases—approved guidelines. NCCLS document C48-A. ISBN 1-56238-539-9
11. Brown JP, Albert C, Nassar BA, Adachi JD, Cole D, Davison KS, Dooley KC, Don-Wauchope A, Douville P et al (2009) Bone turnover markers in the management of postmenopausal osteoporosis. Clin Biochem 42:929–942
12. Szulc P, Delmas P (2008) Biochemical markers of bone turnover: potential use in the investigation and management of postmenopausal osteoporosis. Osteoporos Int 19:1683–1704
13. Vasikaran SD (2008) Utility of biochemical markers of bone turnover and bone mineral density in management of osteoporosis. Crit Rev Clin Lab Sci 45:221–258

14. Hannon R, Eastell R (2000) Preanalytical variability of biochemical markers of bone turnover. Osteoporos Int 11:S30–S44
15. Seibel MJ, Lang M, Geilenkeuser WJ (2001) Interlaboratory variation of biochemical markers of bone turnover. Clin Chem 47:1443–1450
16. Eisman J, Ebeling P, Ewald D, Flicker L, Holborow B, Nash P, Sambrook P, Seibel M, Stenmark J, Winzenberg T, Herjandono J (2010) Clinical guideline for the prevention and treatment of osteoporosis in postmenopausal women and older men. The Royal Australian College of General Practitioners. www.racgp.org.au. Accessed March 2010
17. Sociedad Iberoamericana de Osteología y Metabolismo Mineral (SIBOMM) (2009) Ibero-American consensus on osteoporosis (Osteoporosis: Prevención, Diagnóstico y Tratamiento). www.aaomm.org.ar. Accessed March 2010
18. Lorenc R, Głuszko P, Karczmarewicz E, Księżopolska-Orłowska K, Misiorowski W, Franek E, Horst-Sikorska W, Kaleta M, Męczekalski B et al (2007) Recommendations on the diagnosis and treatment of osteoporosis. Reducing the incidence of fractures through effective prevention and treatment. Terapia 9:11–39
19. Singapore Ministry of Health (2008) Clinical practice guidelines for osteoporosis. Ministry of Health, Singapore. www.moh.gov.sg/cpg. Accessed March 2010
20. National Osteoporosis Guideline Group (2008) Osteoporosis—clinical guideline for prevention and treatment—executive summary. www.shef.ac.uk/NOGG. Accessed March 2010
21. Dawson-Hughes B, Lindsay R, Khosla S, Melton LJ, Tosteson AN, Favus MJ, Baim S (2008) Clinician's guide to prevention and treatment of osteoporosis. National Osteoporosis Foundation. www.nof.org. Accessed March 2010
22. Nelson HD, Morris CD, Kraemer DF, Mahon S, Carney N, Nygren PM, Helfand M (2001) Osteoporosis in postmenopausal women: diagnosis and monitoring. Evidence report/technology assessment no. 28. Agency for Healthcare Research and Quality, Rockville
23. Civitelli R, Armamento-Villareal R, Napoli N (2009) Bone turnover markers: understanding their value in clinical trials and clinical practice. Osteoporos Int 20:843–851
24. Leeming D, Alexandersen P, Karsdal M, Qvist P, Schaller S, Tankó L (2006) An update on biomarkers of bone turnover and their utility in biomedical research and clinical practice. Eur J Clin Pharmacol 62:781–792
25. Wickham C, Walsh K, Cooper C, Barker D, Margetts B, Morris J, Bruce S (1989) Dietary calcium, physical activity, and risk of hip fracture: a prospective study. Br Med J Clin Res Ed 299:889–892
26. Huopio J, Kröger H, Honkanen R, Saarikoski S, Alhava E (2000) Risk factors for perimenopausal fractures: a prospective study. Osteoporos Int 11:219–227
27. Hochberg MC, Greenspan S, Wasnich RD, Miller P, Thompson DE, Ross PD (2002) Changes in bone density and turnover explain the reductions in incidence of nonvertebral fractures that occur during treatment with antiresorptive agents. J Clin Endocrinol Metab 87:1586–1592
28. Darby AJ, Meunier PJ (1981) Mean wall thickness and formation periods of trabecular bone packets in idiopathic osteoporosis. Calcif Tissue Int 33:199–204
29. Jilka RL (2003) Biology of the basic multicellular unit and the pathophysiology of osteoporosis. Med Pediatr Oncol 41:182–185
30. Garnero P, Sornay-Rendu E, Capuy MC, Delmas PD (1996) Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. J Bone Miner Res 11:337–349
31. Chesnut CH, Bell NH, Clark GS, Drinkwater BL, English SC, Johnson CCJ, Notelovitz M, Rosen C, Cain DF, Flessland KA, Mallinak NJ (1997) Hormone replacement therapy in postmenopausal women: urinary N-telopeptide of type I collagen monitors therapeutic effect and predicts response of bone mineral density. Am J Med 102:29–37
32. Peichl P, Griesmacher A, Pointinger P, Marteau R, Hartl W, Gruber W, Bröll H (1998) Association between female sex hormones and biochemical markers of bone turnover in peri- and postmenopausal women. Calcif Tissue Int 62:388–394
33. Christiansen C, Riis BJ, Rodbro P (1987) Prediction of rapid bone loss in postmenopausal women. Lancet 1:1105–1108
34. Christiansen C, Riis BJ, Rodbro P (1990) Screening procedures for women at risk of developing postmenopausal osteoporosis. Osteoporos Int 1:35–40
35. Cosman F, Nieves J, Wilkinson C, Schnering D, Shen V, Lindsay R (1996) Bone density change and biochemical indices of skeletal turnover. Calcif Tissue Int 58:236–243
36. Mole PA, Walkinshaw MH, Robins SP, Paterson CR (1992) Can urinary pyridinium crosslinks and urinary oestrogens predict bone mass and rate of bone loss after the menopause? Eur J Clin Investig 22:767–771
37. Reeve J, Pearson J, Mitchell A, Green J, Nicholls A, Justice J, Hudson E, Klenerman L (1995) Evolution of spinal bone loss and biochemical markers of bone remodelling after menopause in normal women. Calcif Tissue Int 57:105–110
38. Rosen CJ, Chesnut CH, Mallinak NJ (1997) The predictive value of biochemical markers of bone turnover for bone mineral density in early postmenopausal women treated with hormone replacement therapy or calcium supplementation. J Clin Endocrinol Metab 82:1904–1910
39. Ross PD, Knowlton W (1998) Rapid bone loss is associated with increased levels of biochemical markers. J Bone Miner Res 13:297–302
40. Riis BJ, Hansen MA, Jensen AM, Overgaard K, Christiansen C (1996) Low bone mass and fast rate of bone loss at menopause: equal risk factors for future fracture: a 15-year follow-up study. Bone 19:9–12
41. Finigan J, Greenfield D, Blumsohn A, Hannon R, Peel N, Jiang G, Eastell R (2008) Risk factors for vertebral and nonvertebral fracture over 10 years: a population-based study in women. J Bone Miner Res 23:75–85
42. Dempster DW (2000) The contribution of trabecular architecture to cancellous bone quality. J Bone Miner Res 15:20–23
43. Follet H, Boivin G, Rumelhart C, Meunier PJ (2004) The degree of mineralization is a determinant of bone strength: a study on human calcanei. Bone 34:783–789
44. Banse X, Sims TJ, Bailey AJ (2002) Mechanical properties of adult vertebral cancellous bone: correlation with collagen intermolecular cross-links. J Bone Miner Res 17:1621–1628
45. Viguet-Carin S, Roux JP, Arlot ME, Merabet Z, Leeming DJ, Byrjalsen I, Delmas PD, Bouxsein ML (2006) Contribution of the advanced glycation end product pentosidine and of maturation of type I collagen to compressive biomechanical properties of human lumbar vertebrae. Bone 39:1073–1079
46. Bauer DC (2001) Biochemical markers of bone turnover: the Study of Osteoporotic Fracture. In: Eastell R, Baumann M, Hoyle N, Wieczorek L (eds) Bone markers—biochemical and clinical perspectives. Martin Dunitz, London, pp 219–223
47. Dobnig H, Piswanger-Solkner JC, Obermayer-Pietsch B, Tiran A, Strele A, Maier E, Maritschnegg P, Riedmuller G, Brueck C, Fahrleitner-Pammer A (2007) Hip and nonvertebral fracture prediction in nursing home patients: role of bone ultrasound and bone marker measurements. J Clin Endocrinol Metab 92:1678–1686
48. Tromp AM, Ooms ME, Popp-Snijders C, Roos JC, Lips P (2000) Predictors of fractures in elderly women. Osteoporos Int 11:134–140
49. Akesson K, Ljunghall S, Jonsson B, Sernbo I, Johnell O, Gärdsell P, Obrant KJ (1995) Assessment of biochemical markers of bone metabolism in relation to the occurrence of fracture: a retrospective and prospective population-based study of women. J Bone Miner Res 10:1823–1829

50. Garnero P, Hausherr E, Chapuy M-C, Marcelli C, Grandjean H, Muller C, Cormier C, Breart G, Meunier PJ, Delmas PD (1996) Markers of bone resorption predict hip fracture in elderly women: the EPIDOS prospective study. J Bone Miner Res 11:1531–1538
51. Gerdhem P, Ivaska KK, Alatalo SL, Halleen JM, Hellman J, Isaksson A, Pettersson K, Vaananen HK, Akesson K, Obrant KJ (2004) Biochemical markers of bone metabolism and prediction of fracture in elderly women. J Bone Miner Res 19:386–393
52. Ross PD, Kress BC, Parson RE, Wasnich RD, Armour KA, Mizrahi IA (2000) Serum bone alkaline phosphatase and calcaneus bone density predict fractures: a prospective study. Osteoporos Int 11:76–82
53. Sornay-Rendu E, Munoz F, Garnero P, Duboeuf F, Delmas PD (2005) Identification of osteopenic women at high risk of fracture: the OFELY study. J Bone Miner Res 20:1813–1819
54. van Daele PL, Seibel MJ, Burger H, Hofman A, Grobbee DE, van Leeuwen JP, Birkenhager JC, Pols HA (1996) Case–control analysis of bone resorption markers, disability, and hip fracture risk: the Rotterdam study. BMJ 312:482–483
55. Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delmas PD (1997) Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS study. J Clin Endocrinol Metab 82:719–724
56. Szulc P, Chapuy MC, Meunier PJ, Delmas PD (1993) Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest 91:1769–1774
57. Chapurlat RD, Garnero P, Bréart G, Meunier PJ, Delmas PD (2000) Serum type I collagen breakdown product (serum CTX) predicts hip fracture risk in elderly women: the EPIDOS study. Bone 27:283–286
58. Luukinen H, Kakonen S-M, Pettersson K, Koski K, Laippala P, Lovgren T, Kivela S-L, Vaananen HK (2000) Strong prediction of fractures among older adults by the ratio of carboxylated to total serum osteocalcin. J Bone Miner Res 15:2473–2478
59. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD (2000) Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. J Bone Miner Res 15:1526–1536
60. Greenfield DM, Hannon RA, Eastell R (2001) The association between bone turnover and fracture risk (Sheffield Osteoporosis study). In: Eastell R, Baumann M, Hoyle N, Wieczorek L (eds) Bone markers—biochemical and clinical perspectives. Martin Dunitz, London, pp 225–236
61. Garnero P, Cloos P, Sornay-Rendu E, Qvist PER, Delmas PD (2002) Type I collagen racemization and isomerization and the risk of fracture in postmenopausal women: the OFELY prospective study. J Bone Miner Res 17:826–833
62. Bruyère O, Collette J, Delmas PD, Rouillon A, Roux C, Seidel L, Richy F, Reginster J-Y (2003) Interest of biochemical markers of bone turnover for long-term prediction of new vertebral fracture in postmenopausal women. Maturitas 44:259–265
63. Melton LJ III, Crowson CS, O'Fallon WM, Wahner HW, Riggs BL (2003) Relative contributions of bone density, bone turnover, and clinical risk factors to long-term fracture prediction. J Bone Miner Res 18:312–318
64. Meier C, Nguyen TV, Center JR, Seibel MJ, Eisman JA (2005) Bone resorption and osteoporotic fractures in elderly men: the dubbo osteoporosis epidemiology study. J Bone Miner Res 20:579–587
65. Ivaska KK, Gerdhem P, Vaananen HK, Akesson K, Obrant KJ (2010) Bone turnover markers and prediction of fracture: a prospective follow-up study of 1040 elderly women for a mean of 9 years. J Bone Miner Res 25:393–403
66. Bauer DC, Garnero P, Harrison SL, Cauley JA, Eastell R, Ensrud KE, Orwoll E, Osteoporotic Fractures in Men (MrOS) Research Group (2009) Biochemical markers of bone turnover, hip bone loss, and fracture in older men: the MrOS study. J Bone Miner Res 24:2032–2038
67. Johnell O, Oden A, De Laet C, Garnero P, Delmas PD, Kanis JA (2002) Biochemical indices of bone turnover and the assessment of fracture probability. Osteoporos Int 13:523–526
68. Bauer DC, Sklarin PM, Stone KL, Black DM, Nevitt MC, Ensrud KE, Arnaud CD, Genant HK, Garnero P, Delmas PD, Lawaetz H, Cummings SR (1999) Biochemical markers of bone turnover and prediction of hip bone loss in older women: the study of osteoporotic fractures. J Bone Miner Res 14:1404–1410
69. Khosla S, Melton LJ, Atkinson EJ, O'Fallon WM, Klee GG, Riggs BL (1998) Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. J Clin Endocrinol Metab 83:2266–2274
70. Marcus R, Holloway L, Wells B, Greendale G, James MK, Wasilauskas C, Kelaghan J (1999) The relationship of biochemical markers of bone turnover to bone density changes in postmenopausal women: results from the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial. J Bone Miner Res 14:1583–1595
71. Stepan JJ, Pospichal J, Presl J, Pacovsky V (1987) Bone loss and biochemical indices of bone remodeling in surgically induced postmenopausal women. Bone 8:279–284
72. Kanis JA, on behalf of the World Health Organization Scientific Group (2008) Assessment of osteoporosis at the primary health-care level. Technical report, Sheffield, UK. WHO Collaborating Center, University of Sheffield. Available at http://www.shef.ac.uk/FRAX/index.htm. Accessed July 2009
73. Berry SD, Kiel DP, Donaldson MG, Cummings SR, Kanis JA, Johansson H, Samelson EJ (2010) Application of the National Osteoporosis Foundation Guidelines to postmenopausal women and men: the Framingham Osteoporosis Study. Osteoporos Int 21:53–60
74. Compston J, Cooper A, Cooper C, Francis R, Kanis JA, Marsh D, McCloskey EV, Reid DM, Selby P, Wilkins M, for the National Osteoporosis Guideline Group (2009) Guidelines for the diagnosis and management of osteoporosis in postmenopausal women and men from the age of 50 years in the UK. Maturitas 62:105–108
75. Fujiwara S, Nakamura T, Orimo H, Hosoi T, Gorai I, Oden A, Johansson H, Kanis JA (2008) Development and application of a Japanese model of the WHO fracture risk assessment tool (FRAX (TM)). Osteoporos Int 19:429–435
76. Kanis JA, Black D, Cooper C, Dargent P, Dawson-Hughes B, De Laet C, Delmas PD, Eisman J, Johnell O, on behalf of the International Osteoporosis Foundation and the National Osteoporosis Foundation, USA et al (2002) A new approach to the development of assessment guidelines for osteoporosis. Osteoporos Int 13:527–536
77. Lippuner K, Johansson H, Kanis JA, Rizzoli R (2010) FRAX (R) assessment of osteoporotic fracture probability in Switzerland. Osteoporos Int 21:381–389
78. Riggs BL, Parfitt AM (2005) Drugs used to treat osteoporosis: the critical need for a uniform nomenclature based on their action on bone remodeling. J Bone Miner Res 20:177–184
79. Arlot M, Meunier PJ, Boivin G, Haddock L, Tamayo J, Correa-Rotter R, Jasqui S, Donley DW, Dalsky GP, Martin JS, Eriksen EF (2005) Differential effects of teriparatide and alendronate on bone remodeling in postmenopausal women assessed by histomorphometric parameters. J Bone Miner Res 20:1244–1253
80. McClung M, Lewiecki E, Cohen S, Bolognese M, Woodson G, Moffett A, Peacock M, Miller P, Lederman S et al (2006) Denosumab in postmenopausal women with low bone mineral density. N Engl J Med 354:821–831
81. Vasikaran SD, Khan S, McCloskey EV, Kanis JA (1995) Sustained response to intravenous alendronate in postmenopausal osteoporosis. Bone Miner 17:517–520

82. Saag K, Lindsay R, Kriegman A, Beamer E, Zhou W (2007) A single zoledronic acid infusion reduces bone resorption markers more rapidly than weekly oral alendronate in postmenopausal women with low bone mineral density. Bone 40:1238–1243
83. Chesnut CH, Silverman S, Andriano K, Genant H, Gimona A, Harris S, Kiel D, LeBoff M, Maricic M et al (2000) A randomized trial of nasal spray salmon calcitonin in postmenopausal women with established osteoporosis: the prevent recurrence of osteoporotic fractures study. Am J Med 109:267–276
84. Meunier PJ, Roux C, Seeman E, Ortolani S, Badurski JE, Spector TD, Cannata J, Balogh A, Lemmel E-M et al (2004) The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. N Engl J Med 350:459–468
85. Bruyère O, Collette J, Rizzoli R, Decock C, Ortolani S, Cormier C, Detilleux J, Reginster J-Y (2010) Relationship between 3-month changes in biochemical markers of bone remodelling and changes in bone mineral density and fracture incidence in patients treated with strontium ranelate for 3 years. Osteoporos Int 21:1031–1036
86. Naylor K, Clowes J, Finigan J, Paggiosi M, Peel N, Eastell R (2010) The effect of cessation of raloxifene treatment on bone turnover in postmenopausal women. Bone 46:592–597
87. Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR et al (1999) Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. JAMA 282:637–645
88. Prestwood K, Gunness M, Muchmore D, Lu Y, Wong M, Raisz L (2000) A comparison of the effects of raloxifene and estrogen on bone in postmenopausal women. J Clin Endocrinol Metab 85:2197–2202
89. Pereda C, Hannon R, Naylor K, Eastell R (2002) The impact of subcutaneous oestradiol implants on biochemical markers of bone turnover and bone mineral density in postmenopausal women. Br J Obstet Gynaecol 109:812–820
90. Harris ST, Watts NB, Genant HK, McKeever CD, Hangartner T, Keller M, Chesnut Iii CH, Brown J, Eriksen EF et al (1999) Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis: a randomized controlled trial. JAMA 282:1344–1352
91. Rosen C, Hochberg M, Bonnick S, McClung M, Miller P, Broy S, Kagan R, Chen E, Petruschke R, Thompson D, de Papp A (2005) Treatment with once-weekly alendronate 70 mg compared with once-weekly risedronate 35 mg in women with postmenopausal osteoporosis: a randomized double-blind study. J Bone Miner Res 20:141–151
92. Hannon R, Clowes J, Eagleton A, Al H, Eastell R, Blumsohn A (2004) Clinical performance of immunoreactive tartrate-resistant acid phosphatase isoform 5b as a marker of bone resorption. Bone 34:187–194
93. Emkey R, Delmas P, Bolognese M, Borges J, Cosman F, Ragi-Eis S, Recknor C, Zerbini C, Neate C, Sedarati F, Epstein S (2009) Efficacy and tolerability of once-monthly oral ibandronate (150 mg) and once-weekly oral alendronate (70 mg): additional results from the Monthly Oral Therapy With Ibandronate For Osteoporosis Intervention (MOTION) study. Clin Ther 31:751–761
94. Delmas PD, Recker RR, Chesnut CH, Skag A, Stakkestad JA, Emkey R, Gilbride J, Schimmer RC, Christiansen C (2004) Daily and intermittent oral ibandronate normalize bone turnover and provide significant reduction in vertebral fracture risk: results from the BONE study. Osteoporos Int 15:792–798
95. Delmas P, Adami S, Strugala C, Stakkestad J, Reginster J, Felsenberg D, Christiansen C, Civitelli R, Drezner M et al (2006) Intravenous ibandronate injections in postmenopausal women with osteoporosis: one-year results from the dosing intravenous administration study. Arthritis Rheum 54:1838–1846
96. Miller P, McClung M, Macovei L, Stakkestad J, Luckey M, Bonvoisin B, Reginster J, Recker R, Hughes C et al (2005) Monthly oral ibandronate therapy in postmenopausal osteoporosis: 1-year results from the MOBILE study. J Bone Miner Res 20:1315–1322
97. Black DM, Delmas PD, Eastell R, Reid IR, Boonen S, Cauley JA, Cosman F, Lakatos P, Leung PC et al (2007) Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. N Engl J Med 356:1809–1822
98. Cummings SR, San MJ, McClung MR, Siris ES, Eastell R, Reid IR, Delmas PD, Zoog HB, Austin M et al (2009) Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med 361:756–765
99. Bone HG, Bolognese MA, Yuen CK, Kendler DL, Wang H, Liu Y, San Martin J (2008) Effects of denosumab on bone mineral density and bone turnover in postmenopausal women. J Clin Endocrinol Metab 93:2149–2157
100. Lewiecki E, Miller P, McClung M, Cohen S, Bolognese M, Liu Y, Wang A, Siddhanti S, Fitzpatrick L (2007) Two-year treatment with denosumab (AMG 162) in a randomized phase 2 study of postmenopausal women with low BMD. J Bone Miner Res 22:1832–1841
101. Glover S, Eastell R, McCloskey E, Rogers A, Garnero P, Lowery J, Belleli R, Wright T, John M (2009) Rapid and robust response of biochemical markers of bone formation to teriparatide therapy. Bone Miner 45:1053–1058
102. Greenspan S, Bone H, Ettinger M, Hanley D, Lindsay R, Zanchetta J, Blosch C, Mathisen A, Morris S, Marriott T (2007) Effect of recombinant human parathyroid hormone (1–84) on vertebral fracture and bone mineral density in postmenopausal women with osteoporosis: a randomized trial. Ann Intern Med 146:326–339
103. Black D, Bouxsein M, Palermo L, McGowan J, Newitt D, Rosen E, Majumdar S, Rosen C (2008) Randomized trial of once-weekly parathyroid hormone (1–84) on bone mineral density and remodeling. J Clin Endocrinol Metab 93:2166–2172
104. Black D, Bilezikian J, Ensrud K, Greenspan S, Palermo L, Hue T, Lang T, McGowan J, Rosen C (2005) One year of alendronate after one year of parathyroid hormone (1–84) for osteoporosis. N Engl J Med 353:555–565
105. Blake G, Compston J, Fogelman I (2009) Could strontium ranelate have a synergistic role in the treatment of osteoporosis? J Bone Miner Res 24:1354–1357
106. Bone H, McClung M, Roux C, Recker R, Eisman J, Verbruggen N, Hustad C, DaSilva C, Santora A, Ince B (2010) Odanacatib, a cathepsin-K inhibitor for osteoporosis: a two-year study in postmenopausal women with low bone density. J Bone Miner Res 25:937–947
107. Garnero P, Shih W, Gineyts E, Karpf D, Delmas P (1994) Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. J Clin Endocrinol Metab 79:1693–1700
108. Glover SJ, Gall M, Schoenborn-Kellenberger O, Wagener M, Garnero P, Boonen S, Cauley JA, Black DM, Delmas PD, Eastell R (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
109. Glover SJ, Gamero P, Naylor K, Rogers A, Eastell R (2008) Establishing a reference range for bone turnover markers in young, healthy women. Bone 42:623–630
110. de Papp AE, Bone HG, Caulfield MP, Kagan R, Buinewicz A, Chen E, Rosenberg E, Reitz RE (2007) A cross-sectional study of bone turnover markers in healthy premenopausal women. Bone 40:1222–1230

111. Adami S, Bianchi G, Brandi ML, Giannini S, Ortolani S, DiMunno O, Frediani B, Rossini M, on behalf of the BONTURNO study group (2008) Determinants of bone turnover markers in healthy premenopausal women. Calcif Tissue Int 82:341–347
112. Garnero P, Borel O, Delmas PD (2001) Evaluation of a fully automated serum assay for C-terminal cross-linking telopeptide of type I collagen in osteoporosis. Clin Chem Lab Med 47:694–702
113. 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
114. Rogers A, Glover SJ, Eastell R (2009) A randomised, double-blinded, placebo-controlled, trial to determine the individual response in bone turnover markers to lasofoxifene therapy. Bone 45:1044–1052
115. Hannon R, Blumsohn A, Naylor K, Eastell R (1998) Response of biochemical markers of bone turnover to hormone replacement therapy: impact of biological variability. J Bone Miner Res 13:1124–1133
116. Eastell R, Krege JH, Chen P, Glass EV, Reginster J-Y (2006) Development of an algorithm for using PINP to monitor treatment of patients with teriparatide. Curr Med Res Opin 22:61–66
117. Greenspan S, Resnick N, Parker R (2005) Early changes in biochemical markers of bone turnover are associated with long-term changes in bone mineral density in elderly women on alendronate, hormone replacement therapy, or combination therapy: a three-year, double-blind, placebo-controlled, randomized clinical trial. J Clin Endocrinol Metab 90:2762–2767
118. Chen P, Satterwhite JH, Licata AA, Lewiecki EM, Sipos AA, Misurski DM, Wagman RB (2005) Early changes in biochemical markers of bone formation predict BMD response to teriparatide in postmenopausal women with osteoporosis. J Bone Miner Res 20:962–970
119. Cummings SR, Karpf DB, Harris F, Genant HK, Ensrud K, LaCroix AZ, Black DM (2002) Improvement in spine bone density and reduction in risk of vertebral fractures during treatment with antiresorptive drugs. Am J Med 112:281–289
120. Watts NB, Cooper C, Lindsay R, Eastell R, Manhart MD, Barton IP, van Staa TP, Adachi JD (2004) Relationship between changes in bone mineral density and vertebral fracture risk associated with risedronate: greater increases in bone mineral density do not relate to greater decreases in fracture risk. J Clin Densitom 7:255–261
121. Sarkar S, Mitlak BH, Wong M, Stock JL, Black DM, Harper KD (2002) Relationships between bone mineral density and incident vertebral fracture risk with raloxifene therapy. J Bone Miner Res 17:1–10
122. 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
123. Eastell R, Barton I, Hannon RA, Chines A, Garnero P, Delmas PD (2003) Relationship of early changes in bone resorption to the reduction in fracture risk with risedronate. J Bone Miner Res 18:1051–1056
124. Reginster J-Y, Sarkar S, Zegels B, Henrotin Y, Bruyere O, Agnusdei D, Collette J (2004) Reduction in PINP, a marker of bone metabolism, with raloxifene treatment and its relationship with vertebral fracture risk. Bone 34:344–351
125. Sarkar S, Reginster J-Y, Crans GG, Diez-Perez A, Pinette KV, Delmas PD (2004) Relationship between changes in biochemical markers of bone turnover and BMD to predict vertebral fracture risk. J Bone Miner Res 19:394–401
126. Bauer DC, Black DM, Garnero P, Hochberg M, Ott S, Orloff J, Thompson DE, Ewing SK, Delmas PD (2004) Change in bone turnover and hip, non-spine, and vertebral fracture in alendronate-treated women: the fracture intervention trial. J Bone Miner Res 19:1250–1258
127. Delmas PD, Munoz F, Black DM, Cosman F, Boonen S, Watts NB, Kendler D, Eriksen EF, Mesenbrink PG, Eastell R, The HORIZON-PFT Research Group (2009) Effects of yearly zoledronic acid 5 mg on bone turnover markers and relation of PINP with fracture reduction in postmenopausal women with osteoporosis. J Bone Miner Res 24:1544–1551
128. Bjarnason NH, Sarkar S, Duong T, Mitlak B, Delmas PD, Christiansen C (2001) Six and twelve month changes in bone turnover are related to reduction in vertebral fracture risk during 3 years of raloxifene treatment in postmenopausal osteoporosis. Osteoporos Int 12:922–930
129. Li Z, Meredith MP, Hoseyni MS (2002) A method to assess the proportion of treatment effect explained by a surrogate endpoint. Stat Med 20:7531–7588
130. Freedman LS, Graubard BI, Schatzkin A (1992) Statistical validation of intermediate endpoints for chronic diseases. Stat Med 11:167–178
131. Eastell R, Hannon RA, Garnero P, Campbell MJ, Delmas PD (2007) Relationship of early changes in bone resorption to the reduction in fracture risk with risedronate: review of statistical analysis. J Bone Miner Res 22:1656–1660
132. Bauer DC, Garnero P, Hochberg MC, Santora A, Delmas P, Ewing SK, Black DM (2006) Pretreatment levels of bone turnover and the antifracture efficacy of alendronate: the fracture intervention trial. J Bone Miner Res 21:292–299
133. Veitch SW, Findlay SC, Hamer AJ, Blumsohn A, Eastell R, Ingle BM (2006) Changes in bone mass and bone turnover following tibial shaft fracture. Osteoporos Int 17:364–372
134. Hansen M, Overgaard K, Riis B, Christiansen C (1991) Potential risk factors for development of postmenopausal osteoporosis—examined over a 12-year period. Osteoporos Int 1:95–102
135. Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J (2000) The use of biochemical markers of bone turnover in osteoporosis. Osteoporos Int 11:S2–S17
136. McKiernan FE, Berg RL, Linneman JG (2010) The utility of BMD Z-score diagnostic thresholds for secondary causes of osteoporosis. Osteoporos Int. doi:10.1007/s00198-00010-01307-00191
137. Seibel MJ, Naganathan V, Barton I, Grauer A (2004) Relationship between pretreatment bone resorption and vertebral fracture incidence in postmenopausal osteoporotic women treated with risedronate. J Bone Miner Res 19:323–329
138. Delmas P, Licata A, Reginster J, Crans G, Chen P, Misurski D, Wagman R, Mitlak B (2006) Fracture risk reduction during treatment with teriparatide is independent of pretreatment bone turnover. Bone Miner 39:237–243
139. Collette J, Bruyère O, Kaufman J, Lorenc R, Felsenberg D, Spector T, Diaz-Curiel M, Boonen S, Reginster J (2010) Vertebral anti-fracture efficacy of strontium ranelate according to pre-treatment bone turnover. Osteoporos Int 21:233–241
140. Blouin J, Dragomir A, Ste-Marie LG, Fernandes JC, Perreault S (2007) Discontinuation of antiresorptive therapies: a comparison between 1998–2001 and 2002–2004 among osteoporotic women. J Clin Endocrinol Metab 92:887–894
141. Blouin J, Dragomir A, Moride Y, Ste-Marie LG, Fernandes JC, Perreault S (2008) Impact of noncompliance with alendronate and risedronate on the incidence of nonvertebral osteoporotic fractures in elderly women. Br J Clin Pharmacol 66:117–127
142. Siris ES, Harris ST, Rosen CJ, Barr CE, Arvesen JN, Abbott TA, Silverman S (2006) Adherence to bisphosphonate therapy and fracture rates in osteoporotic women: relationship to vertebral and nonvertebral fractures from two US claims databases. Mayo Clin Proc 81:1013–1022
143. Clowes JA, Peel NF, Eastell R (2004) The impact of monitoring on adherence and persistence with antiresorptive treatment for

postmenopausal osteoporosis: a randomized controlled trial. J Clin Endocrinol Metab 89:1117–1123
144. Delmas PD, Vrijens B, Eastell R, Roux C, Pols HA, Ringe JD, Grauer A, Cahall D, Watts NB, Improving Measurements of Persistence on Actonel Treatment (IMPACT) Investigators (2007) Effect of monitoring bone turnover markers on persistence with risedronate treatment of postmenopausal osteoporosis. J Clin Endocrinol Metab 92:1296–1304
145. Foged N, Delaissé J, Hou P, Lou H, Sato T, Winding B, Bonde M (1996) Quantification of the collagenolytic activity of isolated osteoclasts by enzyme-linked immunosorbent assay. J Bone Miner Res 11:226–237
146. Panteghini M (2007) Traceability, reference systems and result comparability. Clin Biochem Rev 28:97–104
147. Müller MM (2000) Implementation of reference systems in laboratory medicine. Clin Chem 46:1907–1909
148. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John GW, Kobold U, on behalf of the IFCC Working Group on HbA1c Standardization et al (2004) IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem 50:166–174
149. Panteghini M, Bunk D, Christenson R, Katrukha A, Porter R, Schimmel H, Wang L, Tate J, The IFCC Working Group on Standardization of Troponin I (2008) Standardization of troponin I measurements: an update. Clin Chem Lab Med 46:1501–1506
150. Stamey TA, Chen Z, Prestigiacomo AF (1998) Reference material for PSA: the IFCC standardization study. Clin Biochem 31:473–481
151. Stenman U (2004) Standardization of assays for human chorionic gonadotropin. Clin Chem 50:798–800
152. Thienpont LM, van Uytfanghe K, Beastall G, Faix JD, Ieiri T, Miller WG, Nelson JC, Ronin C, Ross HA, Thijssen JH, Toussaint B, for the IFCC Working Group on Standardization of Thyroid Function Tests (2010) Report of the IFCC Working Group for Standardization of Thyroid Function Tests; part 1: thyroid-stimulating hormone. Clin Chem 56:902–911
153. Thienpont LM, van Uytfanghe K, De Leenheer AP (2002) Reference measurement systems in clinical chemistry. Clin Chim Acta 323:73–87
154. Morris HA (2009) Traceability and standardization of immunoassays: a major challenge. Clin Biochem 42:241–245
155. Panteghini M (2009) Traceability as a unique tool to improve standardization in laboratory medicine. Clin Biochem 42:236–240
156. Christenson RH, Duh SH, Apple FS, Bodor GS, Bunk DM, Panteghini M, Welch MJ, Wu AHB, Kahn SE, for the American Association for Clinical Chemistry Cardiac Troponin I Standardization Committee (2006) Toward standardization of cardiac troponin I measurements part II: assessing commutability of candidate reference materials and harmonization of cardiac troponin I assays. Clin Chem 52:1685–1692
157. Klee GG (2010) Harmonization and standardization of thyroid function tests. Clin Chem 56:879–880