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Novel Assessment Tools for Osteoporosis Diagnosis and Treatment

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Abstract

This review describes new technologies for the diagnosis and treatment, including fracture risk prediction, of postmenopausal osteoporosis. Four promising technologies and their potential for clinical translation and basic science studies are discussed. These include reference point indentation (RPI), Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, and magnetic resonance imaging (MRI). While each modality exploits different physical principles, the commonality is that none of them require use of ionizing radiation. To provide context for the new developments, brief summaries are provided for the current state of biomarker assays, fracture risk assessment (FRAX), and other fracture risk prediction algorithms and quantitative ultrasound (QUS) measurements.

Keywords

Reference point indentation; Fourier transform infrared spectroscopy; Raman spectroscopy; Magnetic resonance imaging

Conflict of Interest

B. Gong, G.S. Mandair, and F.W. Wehrli all declare that they have no conflicts of interest. M.D. Morris has received research support from the National Institutes of Health; consultant fees from Kaiser Optical Systems, Inc.; and is a member of Biomatrix Photonics, LLC.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

All studies by B. Gong, G.S. Mandair, and M.D. Morris involving animal and/or human subjects were performed after approval by the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

Introduction

In this review we describe emerging approaches to diagnosis of postmenopausal osteoporosis and evaluation of fracture risk in patients previously diagnosed with osteoporosis. Coverage includes the period from January 2008–November 2013, with a small number of late-breaking reports through January 2014. PubMed was searched for English language publications using the terms: osteoporosis, fracture, fracture risk, prediction fracture risk assessment tool (FRAX), spectroscopy, quantitative ultrasound (QUS), reference point indentation (RPI), infrared Fourier transform infrared spectroscopy (FTIR), Raman, magnetic resonance imaging (MRI). The review includes brief sections on the current state of biomarker assays, FRAX, and other questionnaires and ultra-sound measurements. These provide context for discussions of recently developed measurement technologies. Coverage is limited to technologies that do not use ionizing radiation. This limitation precludes discussion of some promising approaches, including use of finite element analysis of fracture risk based on quantitative computed tomography (QCT), as well as newer methods of evaluating DXA scans and plain X-ray images. However, in view of increasing professional and public concern about tissue damage, it was felt that computed tomography advances might better be given their own review, where radiation effects to tissue could be fully addressed.

Biomarker Assays

Most bone turnover markers are noncollagenous matrix proteins, fragments of collagen, or proteins involved in osteoclast or osteoblast function [1, 2]. Common resorption markers include carboxy terminal telopeptide of type I collagen (CTX), amine terminal telopeptide (NTX), either measured in serum (s) or urine (u), and the cross-link fragments deoxypyridinoline (DPD) and pyridinoline (PYD), which are measured only in urine. Enzymes involved in osteoclast function such as tartrate-resistant acid phosphatase (TRAP) and cathepsin K are also included. Formation markers include procollagen N-terminal propeptide (PINP) and procollagen C-terminal propeptide (PIC) and proteins involved in osteoblast function, most commonly bone-specific alkaline phosphate (BAP) and osteocalcin (OC).

In contrast to clinical imaging modalities, which are insensitive to early stage events associated with bone turnover, biochemical assays have the potential to detect the earliest responses to bone turnover or therapeutic interventions. Despite the many advantages of biomarker assays, several issues complicate their routine use.

Bone turnover markers are successfully used in clinical trials of new therapeutic agents for postmenopausal osteoporosis. These studies demonstrate that the assays can be responsive to early changes to bone from therapeutic interventions. The long-term utility of these tests has not yet been demonstrated. Although some research has shown that inclusion of bone marker data into an epidemiologic algorithm may improve fracture risk prediction at the population level, the FRAX Position Development Conference Members group cautioned in 2011 on the use of biomarkers for fracture risk assessment [3]. The group cited methodological inconsistencies, inattention to circadian effects, different statistical treatments, selection of

different fracture outcomes and the possibility of false positives through use of multiple markers.

One hurdle to clinical acceptance is the variability in how data are reported. For example, a recent study of a German population established age-specific reference ranges for s-PINP and s-CTX [4]. The data are reported as median and 1st and 3rd quartiles. However, recent Japanese guidelines propose the same assays but with 95% confidence intervals as a reference range [5]. Moreover, for most bone turnover markers, there is still only fragmentary data on normal levels. Usually the reference ranges are the biomarker levels for healthy young adults. More data is needed on geriatric populations to account for the variability that may arise from ethnicity or country of origin [6].

To address these concerns, a joint International Osteoporosis Foundation (IOF) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) working group on Bone Marker Standards has advocated inclusion of reference bone resorption and bone formation markers in all observational and prospective clinical trials [7•]. They recommended s-CTX as the reference bone resorption marker and s-PINP as the reference bone formation marker. These are also among the biomarkers recommended by an ad hoc Austrian working group [8]. These markers are well-characterized, specific to bone, and show good performance in fracture risk prediction and treatment efficacy, are measurable in blood, and with creatinine correction, in urine. Standardized assays would ensure consistent sampling and statistical treatment of the data.

Commercially available assay kits have simplified and standardized marker analysis [9]. Most kits work with automated instruments and have intra-individual coefficients of variation of 5%–10%. Surprisingly, despite the advantages of specimen size, compact instrumentation, and short reaction times, there has been little attention to translating these and other bone biomarker assays to microfluidic formats [10, 11].

Biochemical markers of musculoskeletal tissue turnover exhibit diurnal variations, complicating monitoring. They are also influenced by diet, exercise, and medicines for unrelated conditions. The literature suggests that serum markers may be more sensitive to diurnal variations than urine markers. Some collagen metabolites in urine do not show strong diurnal variations, and urine specimens can be taken at times convenient for the patient [12].

The literature on sclerostin as a biomarker for osteoporotic fracture risk illustrates another problem with current assay technologies. Commercially available sclerostin immunoassays from three vendors each measure different epitopes of serum sclerostin [13•]. In a study of sclerostin levels in healthy older (65.1 ± 1.4 years) men and women [13•], the three assay results were linearly correlated with each other, but only for one was the biomarker strongly inversely proportional to concentrations of bone resorption markers and trabecular spacing (CT) and strongly positively correlated with BMD (DXA), BV/TV, trabecular number, and thickness. These results could explain why some recent studies report that there is no correlation between serum sclerostin levels and osteoporotic fracture risk [14] while others report that high serum sclerostin is a good predictor of fracture risk [15]. We caution that

differing outcomes could also result from different study designs, including statistical treatments and endpoints.

Overall, the future of biochemical assays to improve fracture risk assessment is promising. A new approach always faces technological, scientific, and acceptance issues. We think these issues are surmountable with additional funding and research efforts. We are especially enthusiastic about the benefit to the patient that is represented by a simple blood test for rapid treatment efficacy or improved fracture risk estimation.

Fracture Risk Prediction Models

There are several risk prediction tools currently available to assess a patient's 10-year probability of having a fracture, including the FRAX tool that is used worldwide, the Garvan Fracture Risk Calculator (Garvan), and QFracture Scores calibrated for use in the UK. The design, performance, and transparency of these prediction tools have been critically reviewed [16••] and systemically compared with other risk prediction models [17].

Developers of QFracture Scores have recently updated their algorithm to include different ethnic minorities and dichotomous responses to a dozen new risk factors, including previous fracture, diabetes, and Parkinson's disease [18]. The FRAX algorithm has also been updated to include a dichotomous response to prior fracture [19•]. Although risk prediction tools allow clinicians to have more informed discussions with their patients, they do not replace clinical judgment [19•]. A comprehensive physical and patient history examination, congruent with country-specific guidelines [20•], are still required to select individuals for intervention.

While current prediction tools continue to be optimized for cohort [21, 22] and country-specific populations [23•, 24], several risk factors with predictive ability have been reviewed [25] and a few have been proposed for inclusion in fracture risk prediction models. For example, inclusion of quantitative ultrasound (QUS) could overcome the limitation of femoral neck BMD measurements used in FRAX. A recent multisite QUS study found a significant 5-year clinical fracture prediction capability at distal radius and tibial sites in women. The women involved in this study were selected on the basis of risk factors used in FRAX algorithm [26]. For vertebral sites, trabecular bone scores (TBS) obtained from high-quality 2D DXA scans could enhance the predictive performance of FRAX [27•]. Further validation of TBS in prospective cohort studies are still required, including a clear definition of normal and abnormal TBS cutoff points.

In a recent retrospective study involving more than 29,000 postmenopausal women, TBS captured a larger portion of diabetes-associated fractures than BMD [28]. Despite the paradox of higher BMD found in women with diabetes than in women without diabetes, combining TBS with BMD improved fracture risk prediction. Since FRAX underestimates fracture risk in patients with diabetes [29, 30], future iterations of the algorithm might consider including diabetic status to improve their fracture risk prediction [30].

Quantitative Ultrasound

QUS has advantages over DXA or CT scans not only for its low cost, easy access and radiation-free operation, but also in its ability to provide comprehensive information on both bone mechanical and structural properties in addition to bone mass [31–34]. QUS measures bone properties using an ultrasonic wave with center frequencies in the range 0.5–1.25 MHz. Two key parameters, speed of sound (SOS) and broadband ultra-sound (intensity) attenuation (BUA) are commonly measured. SOS is correlated with bone material properties such as elastic modulus and compressive strength [32]. BUA is mainly related to bone density. Other parameters, including quantitative stiffness index (SI), ultrasound index (QUI) and amplitude-dependent speed of sound (AD-SOS) are derived from SOS and BUA and are generally more useful for study of pathologic bone tissue. Multiple skeletal sites, such as the calcaneus, phalanx, radius and tibia, have been examined by QUS. Among them, the calcaneus is most frequently studied because it is easily accessible and has high trabecular bone content (95%), high metabolic turnover, and similarities to spinal bone tissue.

While it has been shown that device-specific calcaneal QUS is as effective as DXA in predicting osteoporotic fracture risk [35–38], diagnosis of osteoporosis using QUS alone is less well supported [33]. The gold standard DXA T-score (≤ -2.5) cannot be compared directly to a QUS T-score because DXA and QUS measure different bone properties and use different reference populations. However, QUS can potentially be used to prescreen patients who are at risk for osteoporosis by using device-specific and parameter-specific calcaneal QUS cutoff T-scores [31, 33, 34, 39, 40]. Prescreening may be cost-effective and be accessible to patients for whom DXA may not be available or affordable. The use of QUS for monitoring osteoporosis patients has been studied [34, 41, 42]. At present, there have been no large-scale, randomized, double-blind, and placebo-controlled clinical trials.

Accuracy, precision, and reproducibility of QUS are still problematic. Different QUS instruments use different algorithms for data reduction, and there is no standardization of derived parameters or validation of methods [32]. The lack of standardization impedes both interpretation and comparative studies of different devices and limit the use of QUS in clinical practice.

In conclusion, QUS cannot currently be used as a stand-alone tool in the diagnosis of osteoporosis or to monitor osteoporosis treatment. The future for QUS might involve using calcaneal QUS in conjunction with clinical risk factors for large scale prescreening followed by DXA confirmation [31, 34, 43].

Reference Point Indentation

Reference point indentation (RPI) is a novel microindentation method that can be used to assess mechanical properties of bone both in vivo and ex vivo in variety of situations (e.g., pathologic bone conditions) and might be applied to diagnosis and management of osteoporotic patients in the future [44, 45–49]. RPI measures the indentation distance (ID) into the bone, reflecting bone resistance to microindentation fracture. In human subjects, the measurement site is usually the tibia plateau because it is readily accessible. Held

perpendicular to the bone with a guidance arm, the BioDent-RPI instrument pierces the bone to a depth of a few microns, following a predefined protocol [44]. After local anesthesia and displacement of the periosteum, a 20-cycle indentation is performed at each preselected location. The average value of an RPI parameter is computed from five or more locations, each separated by at least two mm. Indentation damage is minor (indents ~200 μm wide, ~80 μm deep) and does not appear to harm patients. RPI parameters, including total indentation distance (TID), indentation distance increase (IDI), and creep indentation distance (CID), are directly calculated from load-displacement data. TID represents the total indentation depth from the surface of the bone to the maximum displacement in the final cycle. IDI is the increase of indentation distance from the first to the last cycle. CID is the average of the indentation creeping distance of the probe while it is held at the peak force during each cycle [48].

Preliminary correlations between RPI parameters and standard bone mechanical parameters have appeared. A recent study found that IDI over all indentation cycles is inversely related to toughness measured by 3-point bending of whole bones [50•]. The first cycle indentation distance is related to hardness, while the slope of the unloading portion of the first cycle is a measure of elastic modulus. A small scale clinical study has shown that RPI can distinguish between patients with and without osteoporosis-related fractures [44]. The TID and the IDI of patient with fractures were increased relative to controls. In another study with small patient cohort, similar results were observed in patients with atypical femoral fractures [45]. RPI has been used in vivo in female beagle dogs to detect alteration of bone mechanical properties from raloxifene. Six-month raloxifene treatment decreased IDI and improved the material-level ability of the bone to resist production and propagation of indentation damage [47].

While RPI is feasible in vivo and may eventually be suitable for clinical use [44, 45–47], further validation is required. There have been no large scale trials of RPI and no reference values are currently available. At the basic research level, a fuller understanding of the relation between RPI-measured parameters and bone biomechanical and material properties is needed.

Fourier Transform Infrared Spectroscopy and Raman Spectroscopy

Fourier transform infrared (FTIR) and Raman spectroscopy are recognized as important aids to understanding bone quality and fracture risk in osteoporotic patients [51•, 52••]. These techniques have been used in small-scale laboratory studies only, and usually as microscopy techniques, because there are currently no noninvasive methods for obtaining spectroscopic measurements at clinically relevant sites. In vivo bone Raman spectroscopy has been reported for small animals, but not for human subjects [53].

Both spectroscopies are used to assess bone compositional parameters related to bone quality, such as mineral-to-matrix ratios, carbonate-to-phosphate ratios, mineral crystallinity, and/or collagen maturity [54, 55•]. Collagen maturity is a ratio of the amide secondary structure and is related to enzymatic collagen crosslink content. In the absence of both pathologic and nonenzymatic cross-links, collagen maturity is directly proportional to

pyridinoline/divalent crosslink ratios. Although indirect, it is a spectroscopic predictor of bone fragility and has been validated for FTIR spectroscopy [56], but not yet for Raman spectroscopy. Bone heterogeneity parameters have also proven useful [57]. Caution is needed when comparing compositional properties across different tissue types [55•] or anatomic locations [58].

FTIR studies of Iliac crest bone biopsies generally show a relation between collagen maturity and prior fracture history [59, 60, 61••]. Higher collagen maturity has been found in women with osteoporotic fractures. Lower mineral-to-matrix ratio and ratio heterogeneity have been found in women with femoral fractures than in women without [62]. Lower carbonate-to-phosphate heterogeneity and crystallinity variability have also been found in fracture cases. Studies of correlations between bone material and mechanical properties using vertebral biopsies from elderly patients are currently underway [63].

Short-term and long-term treatment with the anti-resorptive drug risedronate (RIS) reduces crystallinity and collagen crosslink ratios more than does another bisphosphonate (BP) alendronate (ALN) [64]. A trend of increased mineral-to-matrix ratio was found with treatment duration with either ALN or RIS. A significant increase in mineral-to-matrix ratio in trabecular bone was found when osteoporotic women were given teriparatide (TPTD) following ALN and RIS treatment [65]. Differential effects have also been found with zoledronate (ZOL) and raloxifene (RAL) in animal studies [66]. ZOL primarily increased mineral-to-matrix on trabeculae surfaces, while RAL increased mineralization in existing trabecular tissue, as evidenced by increased mineral-to-matrix ratio throughout the trabeculae. In a placebo-controlled human subjects study, ZOL increased mineral-to-matrix ratios at actively forming trabeculae, with no significant changes at osteonal surfaces [67].

In a small study, atypical femoral fracture (AFF) patients with prolonged BP use had higher mineral-to-matrix ratios and reduced mineral-to-matrix heterogeneity compared with age-matched controls [68]. Increased mineral-to-matrix ratios were also found in BP-treated patients with AFFs than in those with typical femoral fractures [69]. Heterogeneity of crystallinity and collagen maturity were also reduced, indicating diminished tissue-level toughening. BP-induced changes to bone composition and/or heterogeneity have also been reported in postmenopausal women [70, 71] and in canine tibia [57] even without AFFs. In these studies, modifications to bone quality were attributed to ALN use but its origins or the etiology of AFFs has yet to be clarified.

Magnetic Resonance Imaging (MRI)

The focus of much recent work has been on quantifying trabecular architecture by high-resolution imaging at the distal extremities, analogous to high-resolution peripheral QCT. General-purpose clinical MRI systems have been used, requiring only minimal customization of radiofrequency coils, imaging pulse sequences and processing and analysis software (for a recent review see [72]). Unlike X-ray based modalities that create images based on the much greater density of bone relative to soft tissues, MRI detects protons in bone marrow and adjacent soft tissue. Under ordinary imaging conditions, bone signal is close to the background intensity.

From a set of contiguous image slices the three-dimensional (3D) trabecular network can be reconstructed and parameters representative of scale (e.g., bone volume fraction), topology (e.g., plate vs rod character of the network) and orientation (e.g., by exploiting the direction dependence of trabeculae) can be quantified. Alternatively, structure analysis is bypassed altogether and a 3D voxel model of the MRI data is generated, which is fed into a finite-element solver that provides output measures of the bone's mechanical competence, such as stiffness, elastic modulus or predicted failure strength [73].

Several high-resolution MRI patient studies demonstrating the method's potential for assessing structural and mechanical implications in response to intervention have been published in recent years [74–79, 80•]. Patients treated with antiresorptives (estrogen, testosterone, or synthetic osteoclast inhibitors) showed improvement in trabecular network connectivity and plate architecture [74, 77, 79] evaluated on the basis of topological measures, and increases in estimated mechanical competence [76, 78].

The technical requirements for detection of treatment effects (typically a few percent over 12–24 months), are rather stringent. A recurring question is whether the achievable resolution, given the typical thickness of trabecular struts and plates of 50–150 μm , is adequate. Currently, there is no in vivo imaging technology to fully resolve trabeculae. However, it has been shown that some partial volume blurring is tolerable. Even though the derived structural and mechanical measures deviate from those obtained in the very high-resolution regime they are typically highly correlated with each other [81, 82].

For a given receiver coil design and magnetic field strength the signal-to-noise ratio (SNR) scales linearly with voxel size and square root of total scan time, so the practically achievable resolution, often expressed in terms of image voxel size, is limited. Recent treatment studies carried out at the distal extremities report voxel sizes of $137 \times 137 \times 410 \mu\text{m}^3$ ($7.5 \times 10^{-3} \text{ mm}^3$ voxel volume [80•]) and $156 \times 156 \times 500 \mu\text{m}^3$ ($1.2 \times 10^{-2} \text{ mm}^3$ voxel volume) [79]) with the largest dimension along the bone's long axis. Nevertheless, voxel dimensions alone do not determine actual spatial resolution, which also depends on the imaging method.

Depending on the method used scan times range from 6–15 minutes. Image degradation from involuntary subject motion can corrupt images. This problem is typically addressed by tight immobilization of the limb (wrist, tibia, foot). Retrospective motion correction techniques have been developed to correct for small (often sub-millimeter) displacements during the scan [83]. Further, given the heterogeneity of the trabecular architecture in both axial and transverse direction, accurate serial three-dimensional image registration is critical [84, 85].

The recognition of the role of cortical bone (CB) architecture as a modulator of fracture susceptibility has spurred interest in image-based assessment of CB quality [86]. MRI has shown potential to study CB, in terms of macrostructure and applicability to any anatomic site, including the femoral neck [87]. One confounding factor in visualization and quantification of macrostructural parameters such as cortical thickness and area is that highly collagenated soft tissues, notably ligaments contiguous to bone have very low inherent signal intensity. Nevertheless, recent work aimed at establishing the 3D geometry of

the proximal femur and its relationship to experimentally measured failure strength are promising [88].

Even though water makes up about 20%–30% of cortical bone volume, water is almost invisible in conventionally acquired images, largely due to its short transverse MR relaxation time T_2 . Detection and quantification are possible using ultra-short echo-time imaging techniques that allow capture of the proton signals from bone water. While measurement of bulk bone water has been shown to be clinically relevant [89], evaluation is complex because water resides in several compartments. Recent MR studies of excised human cortical bone show that the dominant bone water fractions are those of the hydration sphere of the collagen matrix (60%–80%), and the fraction occupying the pore structure of the Haversian system [90, 91]. The different lifetimes of the water MR signal in the two micro-environments allow quantification of pore water fraction in intact cortical bone [92] and in vivo [93], although measurements are not yet clinically feasible. These developments will allow measurement of pore volume fraction (ie, porosity) without the need to spatially resolve pores themselves.

MRI is likely to have a major impact on evaluation of bone marrow adiposity, because it is the method of choice for quantifying the composition of soft tissue fractions of water and fat. There is growing interest in this field because of connection between adipogenesis and osteogenesis. Mesenchymal stem cells can differentiate into either adipocytes or osteoblasts (recent review, see [94]). By means of spectroscopic imaging Wehrli et al. first observed in osteoporotic women that vertebral marrow fat fraction was a significant independent discriminator of fracture status (marrow fat volume fraction (0.55 ± 0.08 vs 0.45 ± 0.10 ; $P < 0.001$) [95]. These observations have since been corroborated in studies using vertebral marrow MR spectroscopy [96, 97, 98•] that also show low BMD positively associated with lower fatty acid unsaturation [96, 98•].

MRI, then, may become the technique of choice in certain osteoporosis diagnostic and patient management applications. Spatial resolution is adequate, and the absence of ionizing radiation is a major advantage. At present, there are no specialized instruments for bone MRI, and their absence may be hindering further exploration of the technique, as well as the necessary large scale trials and definition of parameters and their reference ranges for different populations.

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