Biochemical markers in osteoarthritis with lessons learned from osteoporosis

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ABSTRACT

Osteoarthritis (OA) is a disease of the whole joint, including synovium, bone and cartilage. OA is a slow degenerative and very heterogeneous disease, with both varying levels of disease activity and progression. Biomarkers are urgently needed to assist drug developers in selecting and developing the projects with the highest chance of success. Biomarkers for enrichment of clinical studies, early efficacy as well as other diagnostic tools are needed. Osteoporosis and OA have many similarities. In osteoporosis an armamentarium of treatments are now available with high clinical efficacy and well-described effects on biomarkers. Possibly, lessons learned from the osteoporosis field in the use of biomarkers may be applied in the OA field, from both technical and scientific perspectives. To help guide the way, the FDA has recently published the BEST guidelines, to facilitate obtaining a common vocabulary to assist biomarker researchers. In the current review, we will review the biomarkers of OA, with emphasis on bone, cartilage and synovial biomarkers, and draw clear perspectives to the use of biomarkers for drug development and clinical practice in the osteoporosis field.

Introduction

Osteoarthritis (OA) is the most common form of arthritis and one of the leading causes of disability in the world, with more than 10% of the elderly population having symptomatic disease (1, 2). The hallmark of the disease is joint pain and progressive degeneration of articular cartilage involving remodelling of all joint tissues (bone, synovium, ligaments) with subsequent JSN (3). Several lines of evidence suggest that the structural integrity of articular cartilage is dependent on normal subchondral bone turnover, synovial inflammation, intact chondrocyte function and physiological biomechanical stresses (4).

In addition, cartilage and bone metabolism may be partly linked, particularly subchondral bone turnover and its interaction with articular cartilage (4). This suggests that biomarkers of OA may be from several different compartments, as we consider OA a whole joint disease.

Osteoporosis and osteoarthritis share many similarities that may enable use and interpretation of biomarkers in the OA field. Both are slowly progressive diseases which pose a range of drug development challenges, particularly in Phase II dose-finding studies, and even in Phase 1b decision-enabling studies (5).

In osteoporosis, an armamentarium of treatments are available and the relationship between potential efficacy and delta biomarker change is well understood (6). These challenges remain to be addressed for OA, to optimise drug development as well as personalised and precision medicine (7, 8).

In osteoporosis, the standard biomarkers of bone resorption (CTX-I & NTX-I) (9-11) as well as bone formation (PINP, osteocalcin & BSAP) (10, 12, 13), provide optimal assessment tools when calculating the tissue balance (14). Interestingly, osteoporosis is a disease with both increased bone formation and bone resorption, as exemplified in figure 1, extracted from (15-17) and key data from (18). In contrast, the OA field is still exploring the diagnostic and prognostic capacity of biomarkers allowing deconstruction of the tissue turnover, and thereby diagnosis, prognosis and monitoring response to treatment.

The osteoporosis field has long benefitted from a host of sensitive and reliable methods, which have documented utility as surrogate markers of pharmacodynamic effects targeting the bone com-

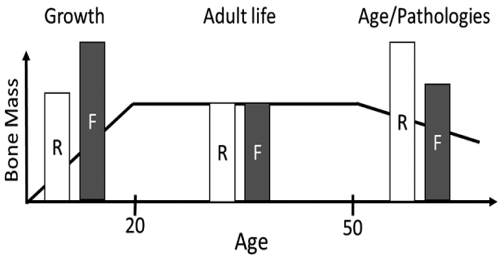

Fig. 1. During growth bone formation (**F**) exceeds bone resorption (**R**) leading to accrual of bone. In healthy adults, bone resorption is tightly linked to bone formation ensuring bone homeostasis. In osteoporosis or as a function of age bone resorption increases, and while bone formation also increases, the increase does not match that of bone resorption, resulting in a net loss of bone.

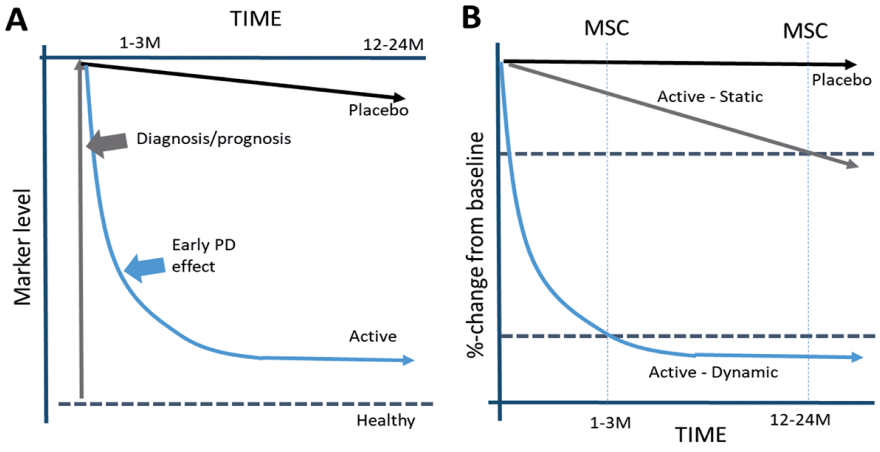

Fig. 2. Different applications of biomarkers.
A: Using a biomarker to identify the subgroup of a disease population that progresses (Diagnosis/Prognosis), and the same or an alternative biomarker to monitor the early PD response to a given treatment. **B**: For drug development the increased magnitude of the dynamic biochemical marker provides a clear advantage compared to a static biomarker such as imaging by MRI, DXA or alike. Modified from (22). MSC, minimal significant change.

partment (12). These biomarkers have provided drug developers with a series of advantages leading to rapid and effective development of drugs, and the osteoporosis market has, accordingly, a series of efficacious drugs that clearly sets it apart from a field such as OA, where fast and efficacious markers are still being investigated and developed (7, 19-21).

Figure 2 presents the ability of the dynamic bone markers to provide an indication of early efficacy, as opposed to the classical and more static biomarkers. As illustrated, the time up to a minimal significant change (MSC) using imaging, such as joint space width (JSW) assessed by x-ray, is often 12–24 months, whereas the biochemical markers often show immediate responses resulting in significant changes within 1–3 months. Such biomarkers allow drug developers more confidence and information to invest in the project with the best possibility for success. This type of biomarker is what is needed for the OA field.

Common confounders in the assessment of biomarkers

Measurement of biomarkers is in many cases dependent on a whole range of parameters (23, 24). Among these are assay technology, fasting status, time of sample, type of sample (plasma, serum etc.), handling of the sample, the population (males/females, age, disease status, treatments) and many more. These parameters have been extensively discussed in several papers (23, 24), including in the rheumatology field (23), and for the ease of reading we have included them in Table I, which is modified from (23).

Overall, there is an ever-expanding portfolio of biomarkers applied in a plethora of diseases, both for identification of patients, identification of fast progressors and for monitoring responses to therapy. With this in mind, there is a continuous need to ensure that these tools are as comparable as possible.

Biomarker nomenclature

Biomarkers are utilised for assessment of many different parameters in clinical studies. Hence, having a glossary ensuring broad understanding of the clinical relevance and thereby the application of the biomarkers is critical. In this regard, the FDA and NIH have proposed the BEST Resource, which captures these aspects in a simple and easy to follow format. The Osteoarthritis Research Society International has proposed the BIPED criteria (25); assessing Burden of disease, Investigative, Prognostic, Efficacy of intervention and Diagnostics use. However, the BEST definitions include a more detailed glossary around biomarker functions and surrogate endpoints, such as pharmacodynamics and safety, and thereby ensures both consistency and common understanding of issues related to use/interpretation of biomarker data from clinical trials (26).

Introduction to the bone field

Bone remodelling in healthy adults is a process occurring at all time points, and it is key to safeguarding bone integrity (17). At the cellular level, the bone resorbing osteoclasts and the bone forming osteoblasts, which work in synchrony to ensure calcium homeostasis and repair of damage occurring in the bone remodelling units, conduct bone remodelling (17).

In many cases, pathologies in bone arise from imbalance in bone remodelling, as exemplified by post-menopausal osteoporosis, as well as a host of other metabolic bone disorders (17, 27). These disorders are characterised by increased bone resorption, followed by increased bone formation, albeit to an insufficient extent. This results in loss of bone, and potentially osteoporosis and a high risk of fractures which are known to be detrimental to both quality of life and life expectancy (17).

Post-menopausal osteoporosis is the most common bone pathology, occurring in as many as 1/3 of all women (28). In parallel, a larger number of men also experience bone loss as a function of age and cessation of gender hormone production (28). Osteoporosis, whether in men, women or induced by glucocorticoids, is a low bone mass disorder, characterised by thinning of the bone structures, leading to increased risk of

Table I. Common parameters influencing the evaluation of novel non-invasive biomarkers.

BIOLOGICAL PARAMETERS	Food intake	Diurnal variation	Seasonal variation	Disease activity	Medical condition and treatments
SAMPLING PARAMETERS	Needle gauge, site of draw, handling	Shipping and storage condition	Freeze-thaw cycles	Matrix (serum, plasma, urine, CSF, saliva etc.)	Anti-coagulant (EDTA, heparin citrate)
ANALYTE FEATURES	Total protein	Protein fragment	Active enzyme	Latent enzyme	Biological role
ASSAY FORMAT	Competitive	Sandwich	Mono- or poly-clonal antibody	Multiplex or singular	Sample volume
ASSAY PARAMETERS	Analyte recovery and precision	Buffer robustness	Measurement-range	Sensitivity	Specificity and accuracy
STUDY PARAMETERS	Patient population and confounders	Mode of action	Duration of study	Onset of action	Number of samples

Adapted and updated from references (23) and (24).

fragility fractures. With an aging world population (28, 29) osteoporosis is becoming a global problem.

WHO defines osteoporosis based on DXA scans of the lumbar spine. The mean T-score in the lumbar spine of young adults (Young Adult Mean (YAM)) serves as the reference, and having a YAM between -1.0 and -2.5 indicates low bone mass (osteopenia), while a T-score below -2.5 of YAM is osteoporosis (29).

The major challenge using bone mineral density (BMD) as the definition of osteoporosis is the poor ability of BMD to predict the risk of fractures; a phenomenon underscored by a large amount of fractures occurring in individuals who are not in the osteoporosis category of BMD T-scores (30, 31). The predictive ability of BMD is markedly improved when utilising changes BMD over time; however, changes in BMD are small and rather slow, and the time to a predictive change is quite long, *i.e.* more than a year in many cases (30, 31).

To account for the rather poor predictive ability of BMD, the FRAX® algorithm was developed (32). FRAX® includes additional risk factors for fractures, such as age, gender, prior fractures, BMD, family history of fractures, BMI, smoking, alcohol, rheumatoid arthritis and glucocorticoid use (32). Unfortunately, implementing FRAX® has not improved the fracture risk prediction dramatically (33). An important and interesting point of discussion relating to FRAX® is the lack of use of bone turnover markers (BTMs) in the algorithm. The decision not to include them is likely driven by the rather variable nature of the BTMs, a point of discussion in the coming sections (34).

Table II. BEST resource for biomarkers (26).

Biomarker	Definition
Diagnostic biomarker	• Detect or confirm presence of the medical condition of interest. • Identify individuals with a subtype of the medical condition of interest.
Monitoring biomarker	• Monitoring status of a medical condition by repeated measurements. • Assessing possible effect of exposure to a drug or an environmental agent.
Pharmacodynamic/response biomarker	• Display if a biological response has occurred after exposure to a drug or an environmental agent
Predictive biomarker	• Identify those subjects who are more prone than similar subjects, to experience a favorable or unfavourable effect after exposure to a drug or environmental agent.
Prognostic biomarker	• Identify probability of a clinical event, disease recurrence or progression in patients with the medical condition of interest.
Safety biomarker	• Measure before and/or after exposure to a drug or environmental agent to asses possible toxicity as an adverse effect
Susceptibility/Risk biomarker	• Assessing the potential for developing a medical condition in a subject who does not currently have any symptoms

Bone turnover markers: what they can and cannot do

As mentioned previously, the challenge with BMD is the slow rate of changes, which limits the ability of using it to identify those who lose bone fast and those who respond well to treatment, thereby pin-pointing the individuals who are in most need of treatment and those who should be enrolled in clinical trials of anti-osteoporotic drugs (12, 35). These points underscored the need for being able to measure the bone turnover balance with a higher sensitivity, *i.e.* through the use of biochemical markers that reflect bone resorption and bone formation rates, and as such provide higher resolution information about the changes in bone, and thereby help identify patients in need (12, 35).

In relation to bone turnover markers, collagen type I turnover has proven highly relevant. Collagen type I is, by

far, the most abundant protein in the bone matrix, and accordingly early biomarker development focused on monitoring changes in type I collagen synthesis and degradation (36, 37). Importantly, studies of collagen type I formation and degradation biomarkers have shown that monitoring these processes provides a unique ability to measure the actual bone turnover rate. When these assessments of rate are combined with the bone status provided by BMD measurements, it presents the possibility to identify those in need of treatment, *i.e.* fast bone losers (38-40).

For monitoring bone resorption, collagen type I degradation by the bone resorbing osteoclasts is a key process. Accordingly, studies have shown that CTX-I, which is a collagen type I fragment generated by the osteoclast protease cathepsin K, is a very sensitive measurement of bone resorption (12, 41). Accordingly, CTX-I has been shown to respond to anti-resorptive therapies, including bisphosphonates, denosumab, oestrogen, SERMs (selective oestrogen modulator), cathepsin K inhibitors, as well as other compounds at various stages of clinical development (17, 42).

The major breakthrough for CTX-I was a study of the bisphosphonate, alendronate (43, 44). The key results are shown in Figure 3, and they highlight the CTX-I and BMD changes as a function of alendronate dose. The data clearly illustrate a fast and large suppression of CTX-I, early after onset of treatment. In contrast, changes in BMD occurred more slowly, and the magnitude of change was markedly smaller (Fig. 3). Most importantly, the BMD change induced by alendronate was predicted by the early CTX-I alterations, clearly highlighting the utility of CTX-I alteration in terms of assisting drug development, both at the level of dose selection, as well as the overall chances of obtaining an efficacious drug on bone resorption.

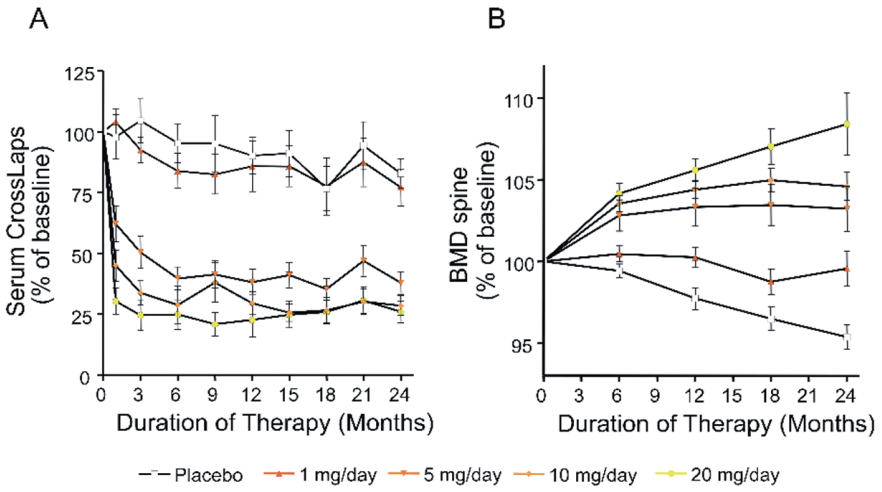

Fig. 3. (**A** & **B**) Dose-response in biochemical marker CTX-I in response to alendronate in a phase II clinical trial. The sensitivity of response of the biochemical marker CTX-I compared to that of the "gold standard" BMD provides evidence of efficacy faster and in a smaller study population. Modified from (43) and discussed in (11).

Existing bone markers and their utility

Three major categories of bone markers are of relevance: (1) bone resorption markers, *i.e.* the activity of the bone resorbing osteoclasts, (2) markers that reflect the number, but not the activity, of osteoclasts, and (3) bone formation markers providing insights into the activity of the osteoblasts. For overview, we have summarised the bone turnover biomarkers which are still of clinical utility in Table III, including a brief description of their use with the appropriate references.

A few biomarkers not falling into the three above-described categories exist. These include assessment of the levels of the bone formation inhibitors, DKK1 and sclerostin, as well as the pro-osteoclastic cytokine RANKL (12). In bone, these biomarkers still do not have a clear-cut additional value on top of the classical biomarkers. On the other hand, in rheumatic diseases, including osteoarthritis and rheumatoid arthritis, they may still be of quite some interest, although more studies are needed.

The bone field has benefitted substantially from having truly well-characterised biomarkers reflecting bone resorption and bone formation (10, 17). Utilising these biomarkers has allowed drug development for osteoporosis by identification of novel drug targets and candidates. The BTMs have also shown significant clinical utility as markers of bone safety as a function of various drugs, include glitazones, anti-psychotics and more (41). However, the most significant contribution of the BTMs has been to ramp up the speed of drug development through the implementation of earlier indications of efficacy, or lack thereof, on BMD and subsequently fracture reduction in the clinical studies.

The utility of BTMS for diagnosis and prognosis

While CTX-I is one of the most, if not the most sensitive marker of bone resorption, it is not a suitable tool for measurement of BMD in patients, as the correlation between CTX and BMD, at any given time point, is modest (41, 60). Overall, there is a slight elevation of the BTMs following menopause, which corresponds to the overall increase in bone turnover, both at the level of bone resorption and bone formation; however, the increases are not large enough to provide a stand-alone diagnostic value at any given time point (35, 52). There are indications that the inverse relationship between bone turnover markers and bone mineral density becomes stronger with age, an association that is particularly good for bone resorption markers (61, 62).

In terms of prognostic ability, the BTMs, due to their direct relation to the activities of the bone cell populations, have a clear-cut value, as underscored by the large studies called EPIDOS and OFELY (63-66). In EPIDOS and OFELY, the relationship between baseline levels of the BTMs and fracture risk was investigated, and the BTMs provide an independent ability to predict fracture risk in these populations

Table III. Biomarkers in the bone field and their application according to BEST (26).
*Recommended by The International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) as the biomarkers to use in clinical studies (35).

Biomarker	Process	Description	BEST classification	Ref.
αCTX-I	Bone resorption particularly in cases with very high bone turnover, such as Paget's, Osteolytic metastases, OA and RA.	A c-terminal crosslinked fragment of collagen type I generated by cathepsin K cleavage during osteoclast-mediated bone resorption of newly synthesised collagen	Prognosis, Pharmacodynamic	(45)
βCTX-I*	Bone resorption	A C-terminal crosslinked and isomerised fragment of collagen type I generated by cathepsin K cleavage during osteoclast-mediated bone resorption of mature collagen	Prognosis, Pharmacodynamic, Safety	(12, 41, 46, 47)
NTX	Bone resorption	An N-terminal crosslinked fragment of type I collagen generated by cathepsin K during osteoclastic bone resorption	Prognosis, Pharmacodynamic	(48)
TRACP 5b	Osteoclast number	TRACP 5b (Tartrate resistant acid) is an enzyme produced specifically by the osteoclasts	Pharmacodynamic	(10, 49, 50)
Cathepsin K	Osteoclast number	An enzyme produced specifically by the osteoclasts. Present in both a pro and active form	Pharmacodynamic	(10, 51)
PINP* PICP	Bone formation	The propeptides are trimeric, globular peptides enzymatically released from newly synthesised pre-pro-collagen prior to incorporation of the collagen molecule into the extracellular bone matrix	Prognosis, Pharmacodynamic, Safety	(35, 46, 47, 52)
BSAP	Bone formation	BASP (bone specific alkaline phosphatase) an enzyme specifically produced by osteoblasts	Pharmacodynamic	(35, 52)
Osteocalcin	Bone formation	OC is a 49-amino acid bone-specific protein produced by the osteoblast and inserted into the bone matrix	Pharmacodynamic	(35, 52)
RANKL	Not clear yet	RANK-L (receptor nuclear activator of NFkappabeta- ligand)Pro-osteoclastic cytokine produced by osteoblasts, primarily expressed on the cell surface	Not clear	(53)
ICTP	Matrix destruction in cases of high turnover, such Paget's, Osteolytic metastases and RA. Not bone resorption	Matrix Metallo Proteinase cleaved type I collagen generated by many cell types including osteoclasts	Pharmacodynamic	(54)
Sclerostin	Not clear yet	Inhibitor of wnt signalling and thereby bone formation produced by the osteocytes	Not clear	(55-58)
DKK1	Not clear yet	DKK1(Dickkopf-related protein) Inhibitor of wnt signalling and thereby bone formation	Not clear	(59)

of elderly or post-menopausal women. Furthermore, in combination with other risk factors for future fractures, such as BMD and/or prior fractures, the prognostic capacity was enhanced (63-67), albeit with some fracture sites being better predicted than others (64, 65, 68).

However, as mentioned before, the ability of the BTMs, and particularly CTX-I to reflect the cellular activity in bone, *i.e.* the bone resorptive activity of the osteoclasts assessed by CTX-I, is what sets the BTMs apart in terms of drug development (13, 41).

BTMS as pharmacodynamic markers

As alluded to earlier in this review, one aspect of the BTMs has aided the osteoporosis field more than anything and that is their ability to monitor efficacies of intervention (41). This holds true whether it is the suppression of bone resorption by bisphosphonates or the induction of bone formation by PTH analogues, as well as for the other osteoporosis drugs and drug candidates (41).

In early clinical trials, the BTMs are used to provide an early indication of treatment efficacy for osteoporosis, as illustrated by the earlier mentioned alendronate example, but also by studies of the cathepsin K inhibitor odanacatib (69-71). In these studies, the BTMs are applied to provide surrogate measures of the BMD changes, as well as to provide an indication of fracture risk reduction, already at the early stages of development, thereby allowing go-no-go decisions based on these data (13).

As an illustration of the utility and the magnitude of the responses measured using BTMs, Table IV provides an overview of the therapy-induced

changes in CTX-I and PINP, which are the markers recommended by the IOF-IFCC (35), and as such the ones applied in most trials.

For CTX-I there is a very clear relationship to the suppression of bone resorption, with the most potent resorption inhibitors, such as denosumab, virtually eliminating CTX-I from the circulation, clearly indicating specificity for bone resorption (72, 73). On the other hand, less potent anti-resorptives, such as raloxifene suppress CTX-I to a lower extent (74), but also have a less pronounced effect on BMD, once again underscoring the strong relationship between changes in CTX-I and changes in BMD. An important aspect in addition to the suppression of CTX-I by the anti-resorptives, is the extent of the suppression of bone formation also induced by anti-resorptives (17, 41). This is illustrated by the prominent suppression of the bone formation marker PINP by bisphosphonates and denosumab (17, 41), which is consequent to the tight coupling of bone formation to bone resorption during bone remodelling.

PINP has also been applied extensively during the development of bone anabolic drugs, such as PTH and PTHrP analogues, and romosozumab (75-78). In these studies, clear PINP responses were observed as a function of the drugs, and when combining the changes of PINP with alterations in CTX-I, a good indication of the expected BMD changes was obtained (75-78).

As indicated earlier, one important point about the BTM responses needs to be underscored: suppression in BTM observed after three months of treatment is predictive of long term change in BMD (43, 44). Additionally, the amplitude of the BTM response (30–100% change in the majority of studies) clearly exceeds that amplitude of BMD (1–10%) and fracture rates (1–5%), and therefore the number of study subjects in early trials can be reduced, allowing faster and more efficient determination of whether it is a drug candidate worth pursuing (11). Finally, the ability to monitor both bone resorption and bone formation simultaneously and frequently, also allowed investigation of novel drug candidates with alternative modes of action, *i.e.* drugs that only inhibit bone resorption without effects on bone formation (79-81), or drugs with a pure anabolic effect, as opposed to the PTH analogues which augment overall bone turnover (75-78). At present, there are drugs only inhibiting bone resorption, while BTM studies of the PTHrP analogues abaloparatide and the anti-sclerostin antibody have shown that a purer bone anabolic effect can be obtained with these drugs (75-78). These data again highlight the importance of having these types of biomarkers available during drug development to ensure successful development and differentiation from other drugs.

The safety aspect: BTMS as indicators of bone adverse effects

During clinical development of several different types of drugs for indications ranging from cancer and rheumatoid arthritis, through type 2 diabetes to viral infections and psychosis, warning signals in terms of reports of increased fracture rates have been presented (46, 47, 99, 100-105). While the mode of action underlying the increased fracture rates in some cases is poorly understood, it has become clear that the increased fracture rates reside in detrimental effects of the drugs on bone turnover (46, 47, 99, 100-105).

In many cases, the detrimental effects of these drugs on bone can be monitored using BTMs, as has clearly been shown for glitazones and glucocorticoids (99, 100). While the benefits of these drugs often clearly exceed the increased risk of fractures, application of the BTMs could allow deselection of those losing bone the fastest, as illustrated by studies of glitazones (78, 106), and as such complications can be reduced by treating subjects at risk of bone loss with other alternatives (13). Furthermore, the BTMs have also been applied during the development of non-bone harming TZDs, such as the partial PPAR gamma agonist balaglitazone, again highlighting the potential of BTMs for monitoring adverse bone responses during drug development (47). More recently, measurements of BTMs have been applied in the development of FGF-21 analogues, where they indicate a modest reduction in bone formation accompanied by a minor increase in CTX-I (107), and as such can help guide the future development of drugs with the same mode of action, as described for Pegbelfermin (105, 108).

Limitations

The main limitation in the interpretation and utility of the BTMs is variation. Importantly, it is well known how to control the majority of the variation (24, 23,109). A series of studies shed light on the impact of diurnal variation and food intake on the BTMs, and these clearly showed that it is essential to collect blood samples for BTM analyses in the fasting state and in the morning, as this circumvents the impact of these parameters on the read-out (35, 109).

At the individual level, the application of BTMs is still rather flawed unless the samples are collected longitudinally, with multiple samples collected over time and thereby studying the fluctuation of the BTMs between individuals exposed to various stimuli. This has been neatly demonstrated in clinical studies involved in the optimisation of dosing time and frequency of oral calcitonin (79, 80, 110, 111), where alterations were provided a way of monitoring response to treatment at specific time points, but also compliance with the treatment (11, 35).

There are two primary types of variation: The one that cannot be controlled but needs to be carefully reported: age and gender, menopause, diseases and drugs, fractures, prolonged bedrest, and others (see Table I) (35, 109, 112). As mentioned previously, circadian rhythm and food intake, which, in addition to a host of technical parameters, such as needle gauge, tubes and location of the blood draw also need to be controlled to obtain good BTM data (35, 109, 112).

Cartilage turnover markers

Joint destruction has been suggested to follow a pattern of inertia accelerating disease progression (113). This poses high demands on the understanding of the clinal representation in the interpretation of image-based biomarkers. Highly important, as depicted in Figure 4, the level of a biomarker may be

Table IV: Summary of the changes in bone resorption and bone formation marker measured in drug treatment studies using the IOF-recommended markers CTX-I and PINP (35).

MOA	Treatment	CTX*	PINP*	
Anti-resorptive	Nasal calcitonin	-10%		(82)
	Oral calcitonin#	-20%		(83, 84)
	Alendronate	-71 – -81%	-64 – -70%	(74, 85, 86)
	Risedronate	-55%	-48%	(87)
	Ibandronate	-58% – -73%	ND	(87-89)
	Zoledronate	-58%	-59%	(90)
	Denosumab	-70 – -75%	-50 – -60%	(72, 73)
	HRT (s.c. pellet)	-40%&	-35%	(91)
	Raloxifene	-21 – -28%	-34%	(92, 93)
	Strontium Ranelate	-12%	-6.3%	(94, 95)
	Odanacatib#	-72%	-40%	(70, 71)
	ONO-5334	-41%	-27%	(96)
Anabolic	PTH(1-34)	5%	111–135%	(74, 75)
	PTH(1-84)	10–100%	90–150%	(97, 98)
	Abaloparatide	20%	95%	(78)
	Romosozumab	-50%	+180%	(76, 77)

The table was inspired by (12). *The responses are presented as ranges depending on different doses, treatment strategies, and different cohorts. &uNTX, not CTX-I.

low or high independent of the disease status if the biomarker is related to disease activity such as the tissue turnover biomarkers described in Table III (114). In direct alignment, a bathtub analogy may be applied. If we are to predict how much water is in a bathtub tomorrow, we need three measures. How much water is in it today (this could be an image of the knee); how much water is running in (this could be a cartilage formation biomarker such as PRO-C2 or PIIANP); and finally how much water is running out (this could be a cartilage degradation biomarker such as CTX-II, C2C, or C2M) (115, 116). With this balance and refinement, an increased understanding is achieved. The level of a biomarker does not necessarily need to correlate to the status of the disease, that is KL score, but rather to the velocity in which the disease is progressing (117). In addition, as a direct consequence, we possibly need to combine the imaging and soluble biomarker modalities rather than making simple correlations (118).

Cartilage consists mainly of type II and X collagen, as well as other minor collagens (119) in addition to a host of non-collagenous proteins such as COMP and HA (120) and aggrecan which is the other main macropmolecule next to type II collagen. During cartilage formation of degradation, cartilage turnover changes, either in response to a pathological insult or to pharmacotherapy. Cartilage consists of type II collagen (60–70% of the dry weight) and proteoglycans (10%) of which aggrecan is the most abundant (119). The key mediators of cartilage degradation include matrix metalloproteinases (MMPs) and the closely related aggrecanases, which are members of the ADAM-TS family (a disintegrin and metalloproteinase with thrombospondin motifs) (121). Aggrecan is degraded by both MMPs and aggrecanases, whereas type II collagen is degraded mainly by MMPs. Since type II collagen is the most abundant protein in cartilage, several different degradation fragments of type II collagen have been identified for non-invasive and objective assessment of joint pathology (122).

If the delicate balance between cartilage formation and cartilage degradation is slightly tilted toward a loss of cartilage, an overall thinning of cartilage over time may be the result. With joint disease, this imbalance is accelerated leading to a measurable net loss of cartilage (123).

Table V provides a list of biomarkers used in direct research setting in the rheumatology field .

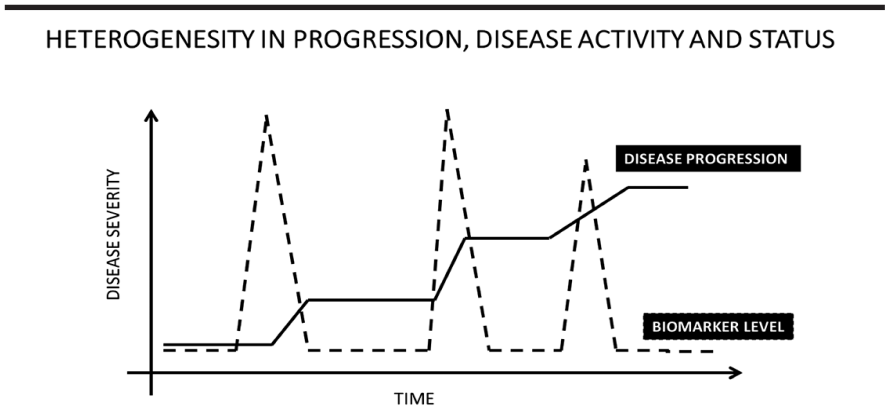

Fig. 4. The level of disease activity biomarkers may be independent of the status and level of disease, and as such clinical data and biomarker data may not always be interpreted as simple correlations, but rather in combination with synergy as demonstrated by Dam and colleagues (118). As OA progression is higher in some intervention, levels of prognostic biomarkers may be elevated during these periods, but low in period of slow progression.

Cartilage degradation

C-terminal telopeptide of type II collagen - CTX-II

The turnover of type II collagen is slow; however, in joint degenerative pathologies such as OA and RA an overproduction of proteases leads to increased type II collagen degradation (118). When MMPs act on type II collagen, small fragments of the protein are released into the synovial fluid and later into serum and excreted in urine, where it may be targeted as biochemical markers (150).

CTX-II is a cartilage degradation marker which has shown to be the best prognostic biomarker in the field (125, 117). Elevated CTX-II has, in multiple clinical studies, shown to be diagnostic and prognostic for OA. Reijman *et al*. showed that high levels of CTX-II were associated with increased risk (odds ratio of 5 in the upper quartile) of both knee and hip OA (117). In addition, elevated levels were highly predictive with an odds ratio of more than 8 for radiographic progression measured by joint

Table V. An objective list of biomarkers used in preclinical and clinical setting reflecting either the bone, cartilage or synovium component of the joint. Modified from Bay-Jensen *et al*. (123)

Biomarker/Protein	Description/Understanding	Selected references
	BONE	
Alpha CTX-I	Cathepsin K degraded newly formed type I collagen – subchondral bone turnover	Associated with subchondral bone turnover, JSN and osteophyte progression (124, 125).
CTX-I	Old type I collagen degraded by Cathepsin K degraded type I collagen	FNIH, CTX-I was associated with disease progression (126).
NTX	Cathepsin K degraded type I collagen	FNIH, NTX was associated with disease progression (126).
Osteocalcin	Bone formation	FNIH, osteocalcin was borderline associated with disease progression (126).
	CARTILAGE	
ARGS /NITEGE	Aggrecanase mediated degradation of aggrecan.	Serum and urine ARGS associated with OA (127) and response to therapy in cartilage explants (128).
C2C	MMP-mediated degradation of type II collagen.	C2C concentrations were correlated with CTX-II, ARGS, osteocalcin, osteopontin and IL-8, but not structural joint injury by MRI (129).
C2M	MMP-mediated degradation of type II collagen.	C2M was associated with KL-2 score and levels of chronic inflammation (130).
C-Col10	Type X collagen turnover.	C-Col10 was elevated in patients with significant OA (131).
Coll2-1	Protease-mediated degradation of type II collagen.	Curcumin treatment reduced Coll-2-1 serum levels (132).
Coll2-1 -NO2	Protease-mediated degradation of nitrosylated type II collagen.	Baseline levels were negatively associated with incidence of knee OA (133).
COMP	Cartilage oligomeric matrix protein turnover/degradation.	CTX-II and COMP were related to progression of OA and (134).
CTX-II	Protease-mediated degradation of type II collagen.	CTX-II was associated with progression of OA(117), and diagnosis, and responded to therapy(135-137).
Fib3-1 / -2	Protease-mediated degradation of Fibulin 3.	Fib3-1, Fib3-2 and Fib3-3 were associated with incidence of clinical knee OA (138).
PRO-C2	The pro-peptide of type II collagen – cartilage formation.	PRO-C2 was induced by different treatments in *ex vivo* cultures, and predictive of structural progression (139, 140).
PIIANP	Type IIA collagen formation.	FNIH, PIIANP was associated with structural progression (126).
	INFLAMMATION	
VICM	Macrophage activity – inflammation.	VICM was shown to be associated with radiographic progression of ankylosis spondylitis (141).
C1M & C3M	MMP mediated type I and III collagen. Inflammation mediated tissue degradation, associated with synovitis.	C1M was associated with radiographic progression in RA(142) and both were shown to be associated with synovitis (143) and respond to efficacious but not non-efficacious therapy (144).
MMP-3	Both total and active MMP-3 assays are available.	MMP-3 is highly produced by the inflamed synovium and response to anti-inflammatory treatments(145).
CRPM	During tissue inflammation, CRP produced in the liver, binds to inflammatory cells in the tissue and is metabolised into smaller fragments, of which one is CRPM (146).	CRPM was elevated under inflammatory condition in OA (130) and associated to progression, and response to anti-inflammatory treatments (147), predicting efficacy.
C4M	Basement membrane remodelling, associated with blood vessels.	Prognostic for progression in RA (148) and elevated in a range of inflammatory conditions (149).

space narrowing (117). These data have been confirmed in two other studies by Saberi *et al*. and Valdes *et al*., who also showed a significant risk of having OA and of progression (134). In a large Japanese OA cohort, CTX-II was found to be correlated with radiographic severity (117, 118, 151), as well as in the GARP study by Meulenbelt and colleagues to be directly associated with the number of skeletal sites (spine, hip, hands and knees) affected by radiographic OA (152). Furthermore, there are several studies showing that CTX-II may act as a marker of response (110), and finally CTX-II levels have been shown to be affected by several different treatments (111).

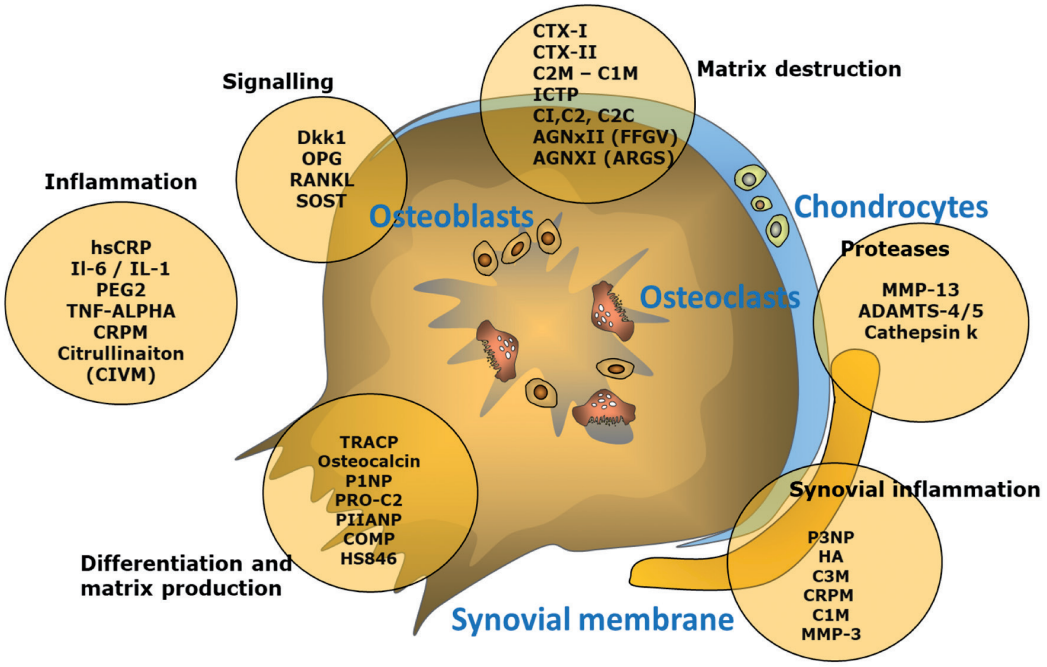

Fig. 5. Biomarkers of different tissues (bone, cartilage and synovium), as well as different processes; inflammation, signalling and cell differentiation/ matrix production. Each biomarker may be used for a specific purpose.
Modified and reproduced with permission from (184).

Aggrecanase degradation of aggrecan – ARGS

Aggrecan is the most abundant proteoglycan of the articular cartilage, and a significant amount of attention has been devoted to identification of pathophysiological relevant degradation fragments as well as developing antibodies and assay towards those (127, 153, 154). There is a suite of literature available on the "degradome" of aggrecan, of which some of these fragments may have more pathobiological relevance than others, and of which some may even have signalling capabilities (155). The ADAMTS-4/5 generated fragment with the N-terminus ARGS has received the most attention (156), and albeit an array of other aggrecan biomarkers are available which may prove useful in the future (11) ARGS has proven to be the most robust of the tested biomarkers.

ARGS measured in synovial fluid has been shown to be associated with improvements in KOOS symptoms and pain in patients with anterior cruciate ligament (ACL) trauma with concomitant articular cartilage injuries (154, 157). The link between ARGS and pain has recently been suggested to be mediated through the TLR receptor pathway, in which a high induction of IL-6 leads to a vicious cycle of inflammation, protease production, joint destruction and more TLR activation (155, 158). Two different ARGS assays (156, 159) have been reported, but further validation work is required to understand the pathophysiological relevance in OA, as well as upgrading their technical performance. Important for the current context, the ARGS assay has been applied in early drug development, in preclinical and clinical settings as well as human and bovine cartilage explants (121, 127, 160-162).

Cartilage oligomeric protein - COMP

COMP may well be the most used biomarker in the OA field, albeit with very varying data (163). For example, in a traumatic OA study no association was found between traumatic knee OA severity and concentrations of COMP (129, 164, 165), whereas a study in women showed that the highest levels of COMP were associated with increased risk of radiographic OA (166). The most used COMP assays do not discriminate between COMP degradation and turnover, and as COMP is controlling the fibrillar formation of collagens, a list of publications is arising in which COMP is associated with different collagens diseases, such as fibrosis of the lung and liver (167). Recently, a new COMP assay was presented which measures a specific COMP fragment – COMPneo (168, 169). Preliminary data showed that this biomarker was indeed released from human articular cartilage when stimulated with catabolic factors, which warrants further investigations.

Cartilage formation

Biomarkers of cartilage formation are urgently needed, and a range of biomarkers are becoming available such as CS846 (139) and PIIANP (170), CPII (PIICP) (171) and PRO-C2 (172). PRO-C2 quantifies the propeptide of type IIB collagen and in contrast, PIIANP (171, 173), PRO-C2 reflects formation of adult form of type II collagen. Data are emerging, and latest from the FNIH, that these biomarkers may be valuable in finding progressors (126).

Synovitis

The synovium is becoming increasingly investigated in OA and has been debated to be part of an inflammatory endotype (122, 130). The synovium is the structure surrounding the joint cavity. It is composed of two resident cell types: fibroblast-like synoviocytes (FLS) and macrophage-like synoviocytes (174). Synovitis is the inflammatory condition, which clinically is manifested by local warmth, swelling and tenderness in joint diseases such as RA, OA and SpA (175). Synovitis involves expansion of resident synoviocytes, fibrosis and infiltration of mononuclear cells resulting in an enlarged synovium with

increased cell numbers. Synovitis may not be the initiator of the disease, but at some point may become the driver of disease (176, 177), and could constitute an endotype that would warrant a targeted therapy.

CRP is often used as a biomarker for inflammation, although it is a simple acute phase reactant produced in the liver, not conferring any tissue specificity (146). In addition, erythrocyte sedimentation rate (ESR), IL-1β, IL-6, TNF-α and fibrinogen are also used as quantitative measurement of inflammation, however with limited success likely due to the non-joint related activity and production of these biomarkers, as well as an extreme variation, limiting the clinical applicability (175). As these simple inflammatory biomarkers do not reflect synovitis or joint tissue inflammation directly, another avenue to explore could be biomarkers associated with tissue turnover, possibly inflammation-driven. Some biomarkers associated with structural proteins are highly correlated with CRP; these include MMP-mediated type I and III collagen degradation markers C1M and C3M (175), and citrullinated and MMP-degraded vimentin (VICM) (147, 149, 178). C1M was associated with a phenotype of OA with high remodelling, KL radiographic grade, and high CRP and CRPM (130), and in other studies shown to be predictive of progression in RA(142), but most importantly, C1M was shown to provide dose resolution in response to anti-inflammatory treatment in RA (147), that was associated with clinical efficacy. These data combined may suggest that tissue inflammation biomarkers can provide the prognostic and efficacy of intervention capacities that standard CRP does not. When CRP is deposited in the tissue, it is metabolised by MMPs, resulting in smaller fragments of that protein (175). One such fragment is CRPM (146). CRPM, was recently shown to be associated with OA progression, with a small variation as compared to CRP.

Figure 5 reflects how several different biomarkers have been developed and used for OA, focusing on each of the three key tissues of the joint; bone, cartilage and synovium. How different biomarkers are associated with these three tissues is illustrated in Figure 5, modified with permission from (180)

The biomarker consortia: beginning of success and a much-needed joint effort

Recently two biomarker consortia have been established and have begun to report data. The FNIH biomarker initiative in OA and APPROACH. Very importantly, the FNIH/OAI cohorts have identified a set of biomarkers to be associated with prognostic for structural progression, which included the biomarkers CTX-I, NTX, alpha-CTX-I, CTX-II and PIIANP (126). In addition, a concerted effort to identify reference range of these biomarkers, which is much needed in clinic research, has been completed (181). In addition, a second wave of research has now been undertaken in this consortium, in which clinical studies are used to validate these findings and better investigate the suite of biomarkers to be used in clinical studies. Such data are much awaited.

In addition, the APPROACH consortium is well underway with the exact mission of delivering non-invasive biomarkers for patient endotyping and drug development tools. These data are highly needed in the field, and yet to be publicly available.

The future

There is an imminent need for reliable biomarkers in the OA field. At least 3 different classes of biomarkers are urgently needed to support drug development and delineate the clinical trajectory of patients.

Efficacy

Biomarkers which at an early timepoint carry the promise and hope of clinical efficacy, such as an early delta change in CTX-I associated with increased BMD in the osteoporosis field.

Endotype

All OA patients may not respond to the same intervention, and there is a need for identification of the OA patients which are fast progressors and respond to a given treatment at the same time. A potential breakthrough was recently found in the UK biobank with more than 3,500,000 patients analysed (186). Three genes associated with cartilage formation and repair were identified to be associated with OA. FGF-18, GDF-5 and TGF-beta (182). Interestingly, all these growth factors stimulate cartilage formation as measured by PRO-C2 in cartilage explants cultures (139, 183), and recently PRO-C2 was shown in 3 independent cohorts to be associated with structural disease progression. Whether this could be a low repair endotype which should receive additional attention, needs a further investigation.

Clinical trial enrichment

The landscape of clinical trial design in the OA field is changing. As OA has been acknowledged as a serious disease by the FDA the use of accelerated approval is now a possibility, much like the field of drug development to treat NASH and cancer has seen major advances. This means that biomarker enrichment for outcome which is either TJR or TKR, may be needed (184). Preliminary data was presented at the OARSI conference, in which CTX-II was prognostic for TJR in two combined phase III clinical studies. Additional data need to be presented.

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