THEME: Rheumatology

Biomarkers in the development of novel disease-modifying therapies for osteoarthritis

Identification and utilization of biomarkers is vitally important for the successful development of disease-modifying osteoarthritis drugs. Biochemical and imaging platforms hold great promise to deliver such biomarkers. Studies indicate a marked increase in biochemical products arising from the breakdown and biosynthesis of collagen, extracellular matrix and bone in osteoarthritis. These molecules have been associated with disease severity and may also have prognostic value as indicators of disease progression. However, issues including biological variability and lack of tissue specificity currently hinder the utility of these molecular markers in drug development. Imaging technologies hold great potential for sensitive and accurate measurement of disease-related structural damage. Drawbacks, including expense, need for validation and limited accessibility also limit the utility of these technologies. In this article, the potential value and challenges in developing and utilizing biomarkers in disease-modifying osteoarthritis drug development will be discussed.

KEYWORDS: biomarkers, cartilage metabolism, delayed gadolinium-enhanced MRI, disease-modifying osteoarthritis drugs, drug development, joint-space narrowing, molecular imaging, MRI, osteoarthritis, T1ρ magnetic resonance imaging, translational medicine

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Osteoarthritis (OA) is the most common form of arthritis, and it is estimated that 26.9 million Americans aged 25 years and older have clinical OA [1]. OA is a progressive degenerative condition characterized by multifactorial processes that lead to the breakdown of joint cartilage and surrounding tissues. Despite significant progress in identifying key molecular players and pathways involved in cartilage degeneration, the pathogenesis of OA remains inadequately understood. Current medical treatments are mostly palliative, aimed at relieving pain, but having no impact on the disease course. While tremendous efforts have been put toward the development of disease-modifying OA drugs (DMOADs), to date such efforts have been unsuccessful. The difficulty in successfully developing such drugs lies in large part with the heterogeneous and slowly progressive nature of OA. Currently, joint-space narrowing (JSN) as determined by X-ray is the only surrogate endpoint for efficacy accepted by the US FDA and the European Medicines Agency. However, abnormal metabolic processes associated with destruction of the articular surface occur years before the disease can be detected radiologically. Moreover, the difficulty associated with obtaining reproducible images during successive patient visits make this a less than ideal method for monitoring slowly progressive cartilage damage and degradation. Such limitations in detecting early disease and in monitoring disease progression, therefore, necessitate large, long and complex clinical trials for demonstration of efficacy. Such trials, however, still continue to fall short of establishing proof of efficacy. In fact, nine large clinical trials with DMOADs carried out over the last decade have all been unsuccessful [2]. These failures underscore the need for identification and validation of additional biomarkers to monitor disease progression and identify those patients most likely to progress. Such biomarkers will not only guide patient selection in clinical trials but will also permit assessment of efficacy of treatment in a time- and cost-effective manner.

In this article, we will review the most widely researched candidate biomarkers for OA and provide our future perspectives on biomarker development and application in this field (**Box 1**).

Biochemical biomarkers

OA affects multiple tissues, including cartilage, bone and synovium. The structural proteins comprising these tissues, their degradation products and the proteases implicated in their destruction have all been implicated as candidate biomarkers for OA. Here we will review a number of studies that have examined altered expression or metabolism of proteins associated with these tissues.

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Box 1. Article outline.

- Biochemical biomarkers
- Biomarkers of cartilage turnover
  - Collagen-derived epitopes
    - Cleavage neoepitopes:
      C2C and C1,2C (P and E)
      uTIINE (P, E and B)
    - Denaturation epitopes:
      Coll 2–1 and Coll 2–1 NO_2 (P and E)
      HELIX-II (P and E)
      COL2–3/4m (P and E)
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      PIIANP (P and E)
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- Biomarkers of bone turnover
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  - PYD and DPD (E)
  - NTX (D) and CTX-I (P and E)
  - Bone sialoprotein (P and E)
- Biomarkers of synovitis
  - Hyaluronan (B, P and E)
  - Glc-Gal-PYD (P and E)
  - YKL-40 (B and E)
- Imaging biomarkers
  - X-ray
  - MRI
    - T1ρ MRI
    - dGEMRIC

This outline denotes each biomarker's potential usage, as assigned by either the authors of this review or by Rousseau and Delmas **[3]**, *in parentheses, within the context of the recently proposed burden of disease, investigative, prognostic, efficacy of intervention and diagnostic (BIPED) classification of OA* **[4]**. *These assignments appear as (B), (I), (P), (E) or (D).*
COMP: Cartilage oligomeric matrix protein; CPII: Carboxyl propeptide of type II collagen; CTX: Cross-linked C telopeptide; dGEMRIC: Delayed gadolinium-enhanced MRI; DPD: Deoxypyridinoline; HELIX-II: Type II collagen helical peptide; NTX: N-telopeptide of type I collagen; PIIANP: Procollagen type IIA N-propeptide; PYD: Pyridinoline; uTIINE: Type II collagen neoepitope.

For many biomarkers discussed below, a number of credible studies have been carried out with widely divergent results. For these biomarkers, such inconsistencies will be highlighted below, as will other potential limitations. Also, while studies of animal models of OA have contributed greatly to our knowledge and understanding of a variety of OA candidate biomarkers, for brevity this review will focus only on findings from human clinical studies.

Biomarkers of cartilage turnover

Cartilage contains two major proteins: type II collagen and aggrecan. The cartilage matrix continually undergoes a remodeling process in which a cycle of degradation is balanced by the synthesis of new collagen and aggrecan. In OA, there is an imbalance in this process, whereby inadequate synthesis results in a net degradation of collagen and aggrecan. As a result, the cartilage extracellular matrix is eroded, exposing articular cartilage and, eventually, bone.

Collagen-derived epitopes

An increase in a variety of type II collagen products is associated with cartilage degradation. These products can be categorized into four major groups.

Cleavage neoepitopes

Cleavage neoepitopes result from the initial cleavage of type II collagen into three-quarter- and one-quarter-length fragments by collagenases. Such fragments include Col2–1/4N1, Col2–1/4N2, C1,2C ($COL2–3/4C_{short}$), C2C ($COL2–3/4C_{long\ mono}$) and type II collagen neoepitope (uTIINE) (**Figure 1**).

A small study of male and female patients with symptomatic knee OA demonstrated a significant positive correlation between serum C2C levels and cartilage degeneration (as indicated by an increase in the MRI T2 parameter) in two compartments of tibiofemoral joint in the male group [5]. Similarly, another small study of patients with radiographic knee OA found a significant correlation between serum C2C and C1,2C levels and disease severity, as determined by Kellgren–Lawrence scoring [6]. An inverse correlation between elevated C2C levels and minimum joint-space width in patients with isolated hip OA has also been reported [7]. By contrast, however, other studies of patients with knee OA have found no significant correlation between baseline levels of either C2C or C1,2C and cartilage loss [8,9]. It is noteworthy to mention that diurnal variation in serum and urinary levels of both C2C and C1,2C have been examined. While no diurnal variation in serum levels of either C2C or C1,2C were detected, urinary levels of C2C increased significantly after a full day of normal activity [6].

Higher urine concentrations of uTIINE have been reported in patients with symptomatic radiographic OA of the hip or knee compared with asymptomatic patients and patients over the age of 55 years without radiographic OA [10]. Serial measurements of uTIINE reflected concurrent

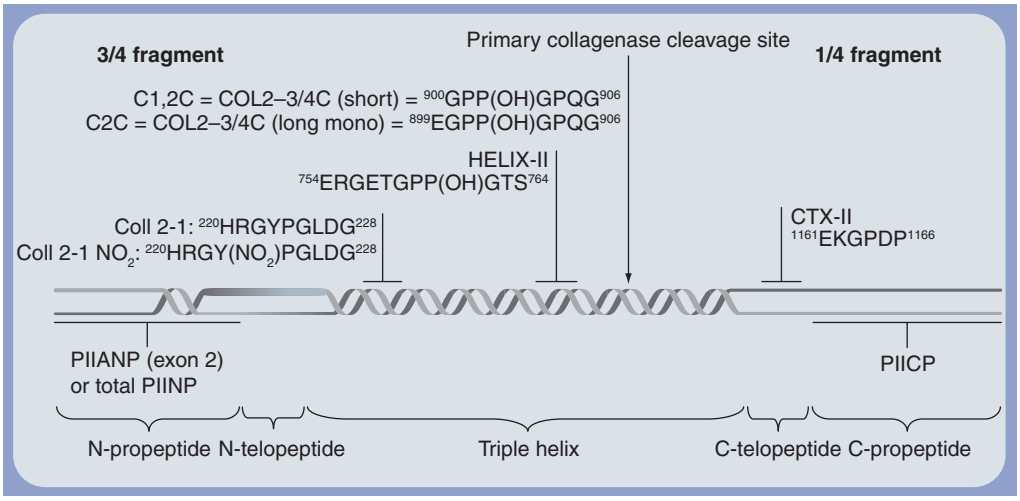

Figure 1. Collagen-derived epitopes. Adapted from [3] with permission from Macmillan Publishers Ltd., *Nature Clinical Practice Rheumatology* © 2007.

JSN in doxycycline-treated patients from baseline to 16 months and from 16–30 months, relative to placebo [11]. Baseline uTIINE, however, was not found to be a consistent predictor of JSN in subjects with knee OA [11]. However, a subsequent study on the same patient population demonstrated that uTIINE levels were highly variable from visit to visit, suggesting the limited utility of the previous findings [12].

Denaturation epitopes

Denaturation epitopes arise from second-round cleavage events and include COL2–3/4m, Coll2–1, Coll2–1 NO_2, HELIXII (type II collagen helical peptide) and collagen fragments containing glucosyl–galactosyl pyridinoline crosslinks (FIGURE 1) [13].

The Coll 2–1 neoepitope exists both in a native and nitrated form (Coll 2–1 NO_2), with the nitrated form being associated with inflammation in the joint and reflecting increased NO synthesis. Notably, the mean serum levels of Coll 2–1 and Coll 2–1 NO_2 in adults remain constant with age and do not demonstrate diurnal variation, making them good candidate biomarkers [14]. Available data suggest that these neoepitopes are both markers of OA clinical activity and are predictive of the radiological progression of disease. In OA patients, Coll 2–1 and Coll 2–1 NO_2 serum levels were significantly elevated compared with age-matched controls [14,15]. Furthermore, elevated urinary levels of these neoepitopes over a 1-year period was predictive of JSN in patients with knee OA after 3 years [16]. Interestingly, a recent study also found that following joint replacement Coll 2–1 levels drop to control values postoperatively and remain there up to 1 year following joint replacement [13].

HELIX-II is another degradation epitope located in the triple helical domain of type II collagen. Urinary HELIX-II levels have been shown to be increased in patients with knee OA compared with healthy controls [17]. In agreement, a retrospective study found that, as a group, patients with rapidly destructive and slowly progressive hip OA had urinary HELIX-II levels significantly higher than healthy controls [18]. Furthermore, this latter study showed that increased urinary HELIX-II levels were significantly associated with decreased joint-space width assessed by radiography of the most affected hip [18]. Recently, the correlation of HELIX-II with Kellgren–Lawrence (K–L) score as well as the potential diurnal variation in HELIX-II expression was investigated by Quintana *et al.* [19]. This study found a significant diurnal variation in serum concentrations of HELIX-II, but no association of serum levels with the sum K–L score. HELIX-II levels were elevated 1–2 h following the onset of morning activity and returned to baseline later in the day. The authors hypothesized that this change may be due to the accumulation of HELIX-II in the synovium of the OA joint during rest and its release into the circulation during physical activity. Larger studies are needed to further evaluate HELIX-II as a biomarker of disease severity.

Degeneration of carboxyl propeptide of type II collagen (CPII), or CII synthesis C-propeptide, by collagenases results in a three-quarter-length fragment and a one-quarter-length fragment. The COL2–3/4m epitope is localized to the three-quarter-length fragment. Expression profiling of COL2–3/4m in normal human cartilage found that levels do not change as a function of age [20]. However, increased levels of COL2–3/4m can be detected in the superficial layers of OA

cartilage early in the disease process; as the disease progresses, it is also increasingly present in deeper layers [21].

Telopeptidic epitope

Telopeptidic epitope is produced by the degradation of collagen telopeptide regions; type II collagen C telopeptide, also known as cross-linked C telopeptide (CTX)-II (FIGURE 1) [13].

CTX-II is a degradation fragment of the C telopeptide of type II collagen. Elevated urinary and synovial fluid levels of CTX-II have been widely reported in OA patients [22–26], and increased urinary CTX-II concentrations have been found to correlate with the radiographic severity of disease in a number of studies [6,23,25,27,28]. However, findings from studies examining the value of CTX-II as a biomarker of progression are less concordant. A number of studies support this association. For example, a 1-year prospective study of knee OA patients demonstrated a relationship between increased urinary CTX-II levels at 3 months and 1-year changes in mean medial and lateral tibia cartilage thickness as assessed by MRI [29]. Very recently, Dam *et al.* also reported a significant association between high CTX-II levels, in this case at baseline, and an accelerated loss of cartilage volume as determined by MRI [26]. A statistical association could be demonstrated, with just 21 out of 158 patients having early radiographic progression, and the association could be detected in just 21 months. Interestingly, however, they detected no association between baseline urinary CTX-II levels and actual cartilage volume as measured by MRI in either healthy or OA subjects [26]. Another prospective longitudinal study found a strong relationship between baseline CTX-II levels and radiographic progression of knee and hip OA [24]. Furthermore, sustained elevated levels of CTX-II have been associated with patients who show disease progression [30]. Interestingly, a large 2-year study examining CTX-II levels in knee OA patients receiving risedronate demonstrated that not only did CTX-II levels decrease with risedronate, but that those patients who had low CTX-II levels both at baseline and at 6 months were at lowest risk for progression [31]. The authors speculate that the decrease in CTX-II levels observed with risedronate could result from either indirect or direct effects of the drug on cartilage metabolism. It is possible that risendronate affects bone turnover via a reduction in the secretion of unidentified procatabolic factors, which may also indirectly influence cartilage degradation, leading to reduced CTX-II levels. Studies have also suggested that bisphosphonates can mediate inhibition of matrix metalloproteinase (MMP) activity, which might directly affect cartilage metabolism. Also, the possibility cannot be excluded that other tissues, such as bone matrix, contain small amounts of type II collagen and that inhibition of skeletal turnover by risedronate could contribute to decreased urinary CTX-II levels.

Despite an observed association between CTX-II levels and OA progression in the aforementioned studies, yet other studies have been unable to establish such an association. For instance, Dam *et al.*, despite establishing a clear association between baseline CTX-II levels and disease progression as determined by MRI, found no significant association between CTX-II levels and progression of disease as assessed by radiography, despite noting a clear trend. Other studies have also been unable to establish a significant association between CTX-II levels and radiographic disease progression. For example, a clinical trial of obese women with knee OA receiving doxycycline treatment showed no significant association between CTX-II levels and progression of JSN [8]. Likewise, a study of CTX-II levels in subjects with progressive radiographic or symptomatic knee OA versus those with stable disease showed no significant difference in CTX-II levels between these groups [32]. The reasons for the divergent findings among groups examining the association of urinary CTX-II levels and disease progression are unclear. It is possible that differences in urine collection procedure, assay antibodies, radiography protocols and diurnal variations in CTX-II levels may have contributed to divergent findings [32]. In addition, the use of MRI for monitoring disease progression may positively contribute to the detection of these significant associations in that it is a more sensitive modality than radiographs for quantifying progression of OA.

Propeptidic epitopes

Propeptidic epitopes are released during collagen synthesis, including CPII and procollagen type IIA N-propeptide (PIIANP) (FIGURE 1) [33].

Before mature collagen molecules are incorporated into fibrils in matrix, the N- and C-propeptides must be removed by specific proteases [34]. Unlike other biomarkers examined in this article thus far, studies have shown that levels of PIIANP were significantly lower in patients with knee OA [35–37], perhaps suggesting a disease-related impairment of type IIA collagen synthesis. By contrast, a longitudinal study

of patients with knee OA found that patients whose disease progressed over the 5-year follow-up period had higher serum PIIANP levels than nonprogressors, although the difference on baseline values did not reach statistical significance [30]. A recent study did find that, as with serum HELIX-II, PIIANP levels rise markedly for 1–2 h following the onset of morning activity and then return to baseline later in the day. While this may or may not explain the difference in findings described here, this should be an important consideration in future studies examining PIIANP levels. It is also worth mentioning that a study of hand OA found no differences in levels of PIIANP between OA and control samples [38].

Like the N-propeptide, the CPII is a marker of collagen synthesis and has been examined as a potential indicator of OA severity and progression. A cross-sectional study of patients with developing knee OA showed that CPII levels in synovial fluid were increased at all stages of OA development, except in the most advanced phases. This increase was maximal before radiographic changes became apparent [39]. A similar study by Kobayashi *et al.* published the following year supported these findings [40]. With regard to disease progression, a recent prospective 4-year longitudinal study demonstrated that JSN was positively correlated with baseline CPII levels in synovial fluid [41]. However, this finding was not corroborated in a study of knee OA patients followed over a 30-month period [8]. In this case, plasma levels of CPII were not found to be predictive of JSN. It has been suggested that CPII measurements in synovial fluid are most useful for diagnosis of early OA and in predicting disease progression. Furthermore, serum levels of CPII do not correlate with synovial fluid measurements, potentially explaining the incongruity of the aforementioned findings [42].

Aggrecan-derived epitopes

Aggrecan is a large protein, composed of a 210–250-kDa core protein and numerous chondroitin sulfate and keratan sulfate glycosaminoglycan (GAG) chains (FIGURE 2) [43]. Aggrecan has two globular domains (G1 and G2) at the N-terminus, and a third globular domain (G3) at the C-terminus. The globular G1 domain associates noncovalently with hyaluronan (HA) and the link glycoprotein, facilitating the aggregation of individual aggrecan monomers to form high-molecular-weight (>200 MDa) complexes. The region between the G2 and G3 domains is rich in chondroitin sulfate and keratan sulfate chains. The chondroitin sulphate chains on aggrecan are highly negatively charged and bind large amounts of water. This leads to an osmotic swelling pressure that provides a compressive stiffness to the cartilage, allowing it to resist deformation and compression. Loss of cartilage integrity in OA arises due to proteolytic cleavage of the aggrecan core protein (which decreases aggrecan charge) or cleavage of the HA (which decreases aggregate size). Neoepitopes of both cleaved aggrecan fragments and newly synthesized aggrecan have been examined as potential biomarkers of OA. GAG components of aggrecan (e.g., keratan sulfate and chondroitin sulfate) have also been investigated as biomarkers of OA, but will not be covered in this article.

Aggrecan cleavage epitopes

Aggrecan cleavage fragments are generated by the proteolytic activities of aggrecanases and MMPs. To date, two major aggrecanases have

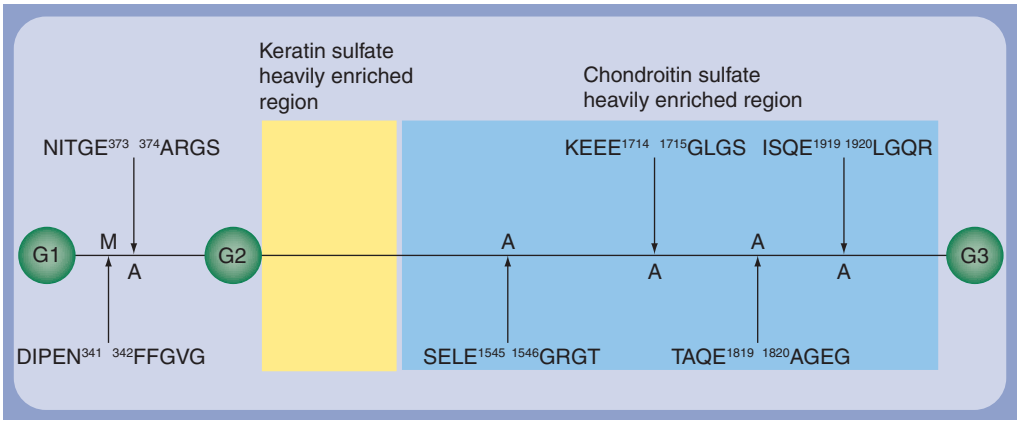

Figure 2. Aggrecan-derived epitopes.
CTX-II: Cross-linked C telopeptide; HELIX-II: Type II collagen helical peptide ; PIICP: Procollagen type IIC N-propeptide.
Adapted with permission from [43].

been studied and characterized: ADAMTS-4 and ADAMTS-5. While ADAMTS-5 has been shown to be the principal aggrecanase in mouse cartilage [44,45], whether this is true for human cartilage is still unknown. Aggrecanases cleave aggrecan at various sites within the core protein [46]. The most widely studied neoepitope results from cleavage in the interglobular domain between Glu^{373} and Ala^{374}, which generates fragments bearing –NITEGE^{373} and ^{374}ARGS–neoepitopes (Figure 2) [47]. Stromelysin-1 or MMP-3, along with many other MMPs, cleaves the interglobular domain of aggrecan between Asn^{341} and Phe^{342}, generating fragments bearing –DIPEN^{341} and –^{342}FFGVG neoepitopes (Figure 2).

Neoepitopes of fragments generated by both MMP and aggrecanase cleavage can be detected in human OA joint cartilage and synovial fluid [47–50]. The vast majority of fragments present in OA synovial fluid derives from aggrecanases [48]. In cartilage, both MMP- and aggrecanase-generated epitopes have been detected by immunostaining, with the areas of most intense staining corresponding to areas of extensive degeneration [47].

These catabolic neoepitopes have great potential as biomarkers of OA disease. Quantification of fragments bearing these neoepitopes could provide great insight into severity of disease, help to stratify patients for clinical trials and potentially be used to monitor pharmacodynamic responses of drugs targeting these enzymes in both preclinical and clinical settings. However, validation of these neoepitopes as OA biomarkers continues to be hampered by the lack of availability of sensitive assays that specifically recognize them. Sandwich ELISAs that claim to have high specificity have recently been described in the literature [43,51], but results on their use in human biological fluids, and in particular synovial fluid, are very limited thus far.

Neosynthetic epitopes

It has been shown that larger aggrecan molecules are present in degenerated cartilage, suggesting that new aggregan is synthesized to replace degraded aggrecan [52]. Neoepitopes of newly synthesized aggrecan have therefore been investigated as potential biomarkers of OA. For example, the 846 epitope present during fetal development is virtually undetectable in healthy adult articular cartilage, but reappears in OA [52,53]. Epitope 846 levels are elevated in synovial fluid over serum, and, in fact, the reactivity of the 846 epitope has been demonstrated to be 40-fold greater in human OA synovial fluid than in serum [54]. Levels of epitope 846 have been reported to be higher in knee OA patients, with the longest disease duration and greatest degree of cartilage degeneration [52,53,55], although one study reported significantly lower concentrations of the 846 epitope in synovial fluid from patients with late-stage OA [56]. Epitopes 3B3 and 7D4 are located on the chondroitin sulfate chains of newly synthesized aggrecan. Increased levels of epitope 3B3 were reported in serum and synovial fluid of OA patients [57]. However, no significant difference in synovial fluid levels of epitopes 3B3 and 7D4 could be detected between patients with radiographically progressive and nonprogressive knee OA [58]. Additional studies are needed to better assess the value of these epitopes as OA biomarkers.

Extracellular matrix protein

Cartilage oligomeric matrix protein (COMP) is a noncollagenous extracellular matrix protein that binds to collagens I, II and IX [59]. Increased serum COMP in patients with OA compared with controls has been reported in a number of studies [22,60–62]. In support of an association between disease progression and COMP, studies have shown that higher baseline COMP levels were associated with increased risk of occurrence of hip OA [63,64], as well as increased risk of hip OA progression [64]. Serum COMP levels were significantly higher in patients with symptomatic knee OA compared with healthy controls, and were elevated to a lesser, but still significant, degree in patients with nonsymptomatic narrowing of the articular space [65]. Prospective longitudinal studies have shown that serum concentrations of COMP were significantly elevated in individuals who showed radiographic signs of OA at follow-up, but not in nonprogressors [60,66]. Recently, Hunter *et al.* examined the relationship between disease progression as measured by loss of cartilage by MRI and expression of a number of markers of cartilage turnover, including COMP [9]. Of the six biomarkers profiled, only the baseline level of COMP correlated with cartilage loss. This study reported a sixfold increased odds of cartilage loss, even after adjustment for the known risk factors, with each unit increase in COMP [9]. Taken together, these studies suggest that baseline serum COMP may be important in selecting patients at high risk for cartilage loss for recruitment into clinical trials.

With regard to disease severity, COMP levels have been shown to be higher in OA patients with severe, but not mild, knee OA [67]. By contrast, a cross-sectional study of patients with

knee OA found that while levels of COMP in OA patients were elevated relative to controls, there was no significant correlation between COMP levels and the radiological status of the disease [62]. Such inconsistencies might be attributable to issues such as inherent patient variability of COMP levels based on age, ethnicity and even time of day of sample collection. Another disadvantage associated with use of COMP as a biomarker is the significant contribution of tissues other than cartilage to COMP production [68,69]. COMP is produced by tissues other than cartilage, including ligament, tendon and synovium. In fact, synovitis, or inflammation of the synovial membrane, has been shown to contribute to elevated serum COMP levels [3,27,70]. Since many patients with OA develop synovial disease, it may be difficult to interpret the significance of changes in COMP levels unless the presence of synovitis is assessed [70].

Biomarkers of bone turnover

Structural changes in OA patients are not limited to cartilage; changes are also observed in the bone. A number of studies have demonstrated a close relationship between progressive cartilage damage and underlying bone remodeling activity. However, alterations in biomarkers of bone metabolism have at times proven challenging to interpret and have yielded variable results. As with all biomarkers, lack of specificity to the tissue of origin is an important consideration when interpreting study findings. For instance, some biomarkers of bone metabolism, such as pyridinoline (PYD) and deoxypyridinoline (DPD) (see later), are highly expressed in bone, but are also expressed in cartilage and synovium. Abnormalities in the subchondral bone of affected joints, generalized skeletal alterations, or both, may also differentially affect bone turnover. Furthermore, depending on the severity of the disease, bone biomarkers may be either increased or decreased.

Biomarkers of bone formation

Osteocalcin

Osteocalcin is a marker of mature, fully differentiated osteoblasts [71]. The utility of serum osteocalcin as a biomarker of OA is not well established. Some studies examining levels of osteocalcin protein in serum of OA patients have found no significant difference in OA patients relative to controls [72,73]. This may be due, in part, to the fact that levels of osteocalcin in OA patients are highly variable [74]. However, in studies where a significant difference could be detected, patients with OA had lower serum osteocalcin levels than age- and sex-matched controls [22,74,75]. Correspondingly, a study of healthy Caucasian men with no symptoms of OA found that higher baseline serum osteocalcin levels tended to be associated with a decreased rate of cartilage loss over a 2-year period [76]. By contrast, Bruyere *et al.* found that increased serum osteocalcin correlated with radiographic progression of disease [77]. The latter findings are in line with the expectation that bone turnover should increase as the OA process becomes increasingly damaging. By contrast, other studies have found no significant difference in expression of osteocalcin between patients with rapidly destructive or slowly progressive OA [78]. Such discrepancies suggest that osteocalcin may have limited utility as an OA biomarker.

Bone-specific alkaline phosphatase

Bone-specific alkaline phosphatase (BAP) is a tetrameric protein localized in the plasma membrane of osteoblastic cells, which enters the circulation after enzymatic cleavage by phospholipase [79]. Circulating BAP has a relatively long half-life, and its levels are largely unaffected by renal clearance or diurnal variations [80]. Very little is known regarding the overall usefulness of BAP as a biomarker of OA presence, progression and/or severity. A study of patients with spinal and knee OA found that BAP levels were lower than in control patients [81]. With regard to disease severity, no significant differences in serum levels of BAP were detected between patients with rapidly destructive hip OA and in patients with slowly progressive hip OA [78].

Biomarkers of bone resorption

PYD & DPD

Pyridinoline cross-links, including PYD and DPD, stabilize new collagen fibrils in the bone extracellular matrix. Patients with OA have elevated levels of urinary PYD [82,83] and DPD [82] relative to healthy controls. These levels are higher in generalized OA, where they correlate with disease severity [84,85]. Significantly higher PYD expression was detected in degenerated cartilage from patients with preclinical early-stage knee OA compared with healthy cartilage from the same donor [86]. However, other studies have failed to establish a link between urinary PYD and/or DPD levels and either the presence of OA, its severity or progression [16,23,77,87].

NTX & CTX-1

N-telopeptide of type I collagen (NTX) and C-telopeptide (CTX-I) are specific and sensitive biomarkers of bone resorption. Increased serum levels of NTX were found in patients with radiographic hip OA relative to controls [64] in patients with erosive hand OA, but not in patients with nonerosive hand OA [88], as well as in patients with rapidly destructive hip OA relative to those with slowly progressive hip OA [78]. These findings suggest that elevated levels of these telopeptides are evident in patients manifesting the most severe forms of OA. Interestingly, higher baseline NTX levels also seem to correlate with increased risk of developing OA and increased risk of OA progression [64]. However, several studies of CTX-1 have shown no evidence that either baseline levels or changes in serum CTX-1 over time are informative with regard to disease presence, severity or progression [25,29,89]. A recent prospective 24-week study of patients with symptomatic knee OA observed no significant association between CTX-I and radiological features of OA [28]. This study did, however, note a modest but significant association between changes in serum CTX-I expression and clinical response over a 24-week period. Nonprogressors showed an increase of serum CTX-I while a slight decrease was seen in progressors.

Bone sialoprotein

Bone sialoprotein (BSP) is a phosphorylated glycoprotein synthesized by osteoblasts and osteoclasts [90]. BSP levels measured sequentially over a 3-year period in individuals with chronic knee pain were found to increase significantly in those patients with radiographic OA at follow-up but were unchanged in those with normal radiographs [66]. However, a 1-year prospective study of patients with symptomatic hip OA showed that serum baseline BSP concentrations were unrelated to disease progression [91].

In summary, while bone biomarkers might offer some value in assessment of OA severity and progression, the lack of specificity of these markers to the primary disease, the divergence of study findings to date and the need for more studies to better define their utility limit their usefulness at this time in OA drug development.

Biomarkers of synovitis

Hyaluronan

Hyaluronan is a member of the GAG group of polysaccharides. In cartilage, HA binds to other molecules, such as aggrecan and link protein, helping the cartilage to withstand the force of weight-bearing and movement of the joint. In the synovial fluid, HA plays a major role in lubricating the movement of the cartilage against the synovium, acting as a shock absorber. It has been well-demonstrated that the concentration of HA is decreased in the cartilage and synovial fluid of OA patients. However, growing evidence suggests a connection between higher HA levels and OA severity. A study of an ethnically diverse population found that serum levels of HA correlated positively with disease severity [92]. Similarly, an 18-month study of obese and overweight adults with knee OA found that levels of HA were negatively correlated with medial joint-space width and positively correlated with K–L score [93]. Furthermore, high baseline levels of HA were found to be predictive of disease progression, as determined by MRI, in knee OA patients [29]. These finding were corroborated by a recent systematic review of 37 observational studies to better define prognostic factors of knee OA [94]. Similar findings were reported in patients with hip OA [95].

Glc–Gal–PYD

Glucosyl–galactosyl PYD (Glc–Gal–PYD) is a glycosylated analogue of PYD, abundant in synovium and present in small amounts in cartilage and other soft tissues, such as muscle, but absent in bone [96]. Significant increases in urinary Glc–Gal–PYD and CTX-II, as well as a positive association between urinary Glc–Gal–PYD and K–L grade, total Western Ontario and McMaster Universities (WOMAC) index and JSN have been reported in knee OA patients [97].

YKL-40

YKL-40, also known as human cartilage glycoprotein-39, is secreted by a variety of human cells, including macrophages, and expressed in the synovium and cartilage of patients with arthritic disease [98]. YKL-40 is involved in remodelling and angiogenesis in a variety of inflammatory diseases. It has been hypothesized that YKL-40 interferes with the synthesis of HA, but no conclusive evidence has been reported to date in support of this hypothesis [98]. Patients with knee OA have high concentrations of YKL-40 in synovial fluid [99]. Likewise, serum levels of YKL-40 are significantly increased in patients with hip OA [100]. The link between YKL-40 levels and OA radiographic progression has been less well established. A study of postmenopausal women with primary knee OA did find a correlation between radiographic severity of hand/knee OA and knee/spinal OA

with YKL-40 levels [84]. However, a number of other studies have been unable to confirm this correlation [22,29,100,101].

In summary, biochemical biomarkers of OA may provide easy, rapid, noninvasive and relatively inexpensive means of diagnosing and monitoring progression of OA in patients. However, their impact on clinical development of DMOADs remains the subject of intense discussion. A key challenge in the application of these biomarkers to drug development is the high variability of their circulating levels, which can be influenced by multiple factors, and lack of tissue and/or disease specificity. For example, urinary CTX-II levels vary with age and can increase by 12–22% in the presence of ibuprofen. Similarly, COMP levels are also affected by age, as well as gender, body mass index and ethnicity (being higher in African–Americans), and have diurnal variations. HA levels are affected by food intake [3]. These biomarkers have also shown considerable variability across individuals and over time, reflecting the fact that structural damage progression in OA varies widely and nonlinearly over time. Only a better understanding of these variables to minimize their impact on disease activity assessment will allow optimal use in clinical trials with DMOADs. It is worth mentioning that in addition to biochemical biomarkers discussed above, novel biomarkers in the form of one or a cluster of genes or proteins have been discovered in recent years. Such discoveries are often the result of utilizing novel platforms in system biology such as gene transcriptional profiling, proteomics or metabolomics [102]. These exciting new platforms will continue to provide additional candidate biomarkers in the future.

Imaging biomarkers

X-ray is the standard radiographic technique used to diagnose OA and to quantify disease severity based on K–L scores. X-ray is the only FDA-approved surrogate end point for evaluating efficacy of a therapeutic intervention; however, its utility in clinical trials has presented significant challenges. For example, JSN has neither been shown to correlate with disease activity nor with abnormalities occurring relatively late in disease [103]. In addition, radiographic progress is too slow, and X-ray too imprecise, for the practical assessment of any therapeutic intervention. For a typical proof-of-concept trial for DMOADs, it has been estimated that more than 400 patients will be required for each arm, with greater than 1-year dosing needed to observe moderate efficacy. Therefore, a rapid surrogate marker (preferably between 3 and 6 months) for structural damage is critically needed. Currently, more sophisticated imaging modalities, such as MRI and molecular imaging, are being explored and adapted to overcome the limitations of standard X-ray.

MRI can evaluate multiple pathological processes with more accuracy in OA, such as fragmentation and generation of articular cartilage, bone sclerosis, osteophytes, cyst formation, menisci and ligament structure, synovitis and bone marrow edema. MRI can also accurately measure cartilage thickness and volume, surface smoothness and the distribution of disease changes. Cartilage thinning as measured by MRI most closely resembles, and potentially more precisely reflects, JSN on X-ray. Bone marrow abnormality is another feature of OA that can be captured and measured by MRI. It has been associated with both pain and CTX-II breakdown and can predict JSN and cartilage loss, especially at the area adjacent to the edema [104]. Furthermore, it is increasingly clear that early pathological changes that take place periarticularly are not captured well by X-ray, but are usually evident on MRI. As for the MRI technology itself, significant progress has been made in improving techniques for spin echo, gradient echo and fat suppression, which allows for better visualization of cartilage.

In numerous clinical studies using cross sectional and longitudinal OA patient cohorts, MRI has shown adequate reliability, specificity and sensitivity, as well as the ability to detect lesion progression within a reasonable observation time frame (1–2 years) [105]. This capacity, combined with the availability of robust imaging acquisition protocol for multicenter trials, indicates that MRI of cartilage has the tremendous potential to be widely used in clinical trials for monitoring treatment response to structure-modifying OA drugs. Experience accumulated from ongoing and future studies will serve to solidify MRI of the cartilage as the preferred method of choice to obtain reliable and quantitative data on cartilage morphology. With time, MRI of the cartilage may even serve as a surrogate marker for structure progression and clinical end point for DMOAD trials.

It should be pointed out that MRI provides only limited information on cartilage quality beyond the surface of the disease loci; novel molecular imaging technologies will be needed to provide this additional information by assessing the molecular composition of cartilage. To date, the most commonly used modality for molecular

imaging of cartilage has been T2 MRI. T2 is a magnetic resonance relaxation time, reflecting interactions between water molecules and between water and surrounding macromolecules, with increasing interaction leading to a decreased T2. So far, however, no relationship has been established between T2 changes and disease activity progression in clinical studies, despite intense research for the last decade. This can be primarily attributed to the fact that T2 is affected by many physiological and pathological processes that relate to the state of cartilage [106].

In addition to T2, several other molecular imaging technologies have been the focus of attention in the field of OA biomarker development. Among them, T1ρ MRI (T1 in the rotating frame) and delayed gadolinium-enhanced MRI (dGEMRIC) provide the most promise to potentially revolutionize OA biomarker development [107].

T1ρ MRI

T1ρ is a method designed to quantify low-frequency physicochemical interactions between water and surrounding macromolecules in the cartilage. It is highly sensitive to change in molecular content. Accumulating evidence demonstrates that MRI techniques based on this mechanism are a direct method to monitor proteoglycan loss and the integrity of cartilage [108]. The major advantages of this technique are that it is requires relatively little time, and it does not require contrast agent and delayed scan on patients. The key limitation is that it has less powerful resolution when compared with other techniques that are based on proton MRI. Furthermore, despite its larger dynamic range, T1ρ is sensitive to many nonspecific factors and interpretation of cartilage quality demands considerable caution. At the present time, proof-of-principle studies in animal models of OA have been completed [109], but limited information is available for human subjects (both healthy volunteers and OA patients) [110]. Current and future development efforts will focus on the following key areas: improvement of resolution by using the optimal coil, incorporation of 2D or 3D imaging to obtain cartilage thickness and volume information in conjuncture to PG content, and validation of the technique with human subjects – both normal healthy volunteers and OA patients.

Delayed gadolinium-enhanced MRI

Delayed gadolinium-enhanced MRI imaging is a noninvasive technique developed to sensitively and specifically measure cartilage GAG content *in vivo*. The main source of fixed charge density in cartilage can be attributed to the GAGs, which are negatively charged. Owing to this negative charge, intravenously administered gadolinium diethylenetriamine pentaacetate anion ($Gd\text{-}DTPA^{2-}$) equilibrates in a manner that is inverse to the fixed charge density and that relates directly to the GAG concentration. In the setting of dGEMRIC, T1 imaging thus directly reflects the GAG concentration in cartilage. This technique has the potential to monitor disease progression with precision and to assess therapeutic efficacy, reflecting the molecular mechanism of action of the disease-modifying therapeutics [111].

One of the key strengths of dGEMRIC is its capability to identify GAG loss in early-stage cartilage disease with high sensitivity [112]. More recently, Sharma and Burstein have shown that the biochemical information provided by dGEMRIC scans may augment standard radiography by improving the differentiation of disease status within a given radiographic grade, especially in early OA [113]. While conventional MRI has many advantages over X-ray, at least 6–12 months are needed to detect disease progression. To this end, novel molecular imaging modalities such as dGEMRIC and T1ρ MRI provide the most promising platforms to detect cartilage morphology and GAG content. In light of the fact that the focus of many of the current OA drug candidates is to maintain the integrity of cartilage, these technologies have tremendous potential to serve as ideal biomarkers for development of such drugs.

Despite the tremendous value these imaging modalities may add to OA drug development, several key factors limit their immediate application. Such limitations include: relatively high cost, increased procedure time and decreased availability compared with X-ray, increased difficulty in patient recruitment, complex data collection and analysis, lack of correlation with biochemical biomarkers, and uncertain predictive value for accepted structure end point (JSN) and clinical symptoms (pain and loss of functions). In addition, the prevalence and implication of changes detected by MRI among normal individuals are not clear (do the 'early pathological changes' always progress to OA damage?).

The future direction for the development of these technologies lies in the following areas:

- Technology optimization, such as 2D- and 3D-imaging incorporation, to allow concurrent evaluation of cartilage morphology and volume, synovium inflammation and GAG content;

- Validation in OA patients using cross-sectional and longitudinal cohorts.

Conclusion

Significant progress has been made in identifying potential biochemical biomarkers that reflect disease state and disease progression. One of the major focuses for OA biomarker development has been assessing their utilities in following up drug efficacy in clinical trials. So far the results have been inconclusive [8,9]. More work is needed in order to better understand the biological processes reflected by systemic biochemical biomarkers. It is essential to establish the linkage of biomarker changes to clinical outcome improvement.

Of the biomarkers that have been studied thus far, urine CTX-II and serum COMP hold the most potential value as biomarkers of disease. However, the further development of these biomarkers is limited by their lack of tissue specificity and correlation with disease activity at a single-joint level. Their use in combination with accurate measures of physical changes in joint cartilage by imaging may provide a more powerful assessment of disease. At the present time, there is no individual marker discriminative enough to prognose or diagnose OA. More prospective studies are required to validate the biomarkers. Furthermore, aggregate scores including imaging, biological or clinical parameters are probably required to increase the discriminative power of individual biomarkers. It is widely recognized that all biomarkers are not sensitive to one particular treatment and that all drugs can not be investigated with one single biomarker. Therefore, the use of a panel of biomarkers is required in clinical studies.

Currently, more sophisticated imaging modalities are being explored and adapted to overcome the limitations of standard radiography. MRI is poised to become the future surrogate end point in DMOAD development for measuring structure damage. However, molecular imaging techniques, such as dGEMRIC and T1ρ MRI have the greatest potential to be ideal imaging OA biomarkers, measuring disease activity and predicting 'rapid progressors' with precision.

Future perspective

The long-term goal for biomarker development is to deliver biomarkers that:

- Are detectable in blood and/or urine, allowing for repeated and frequent measurement;
- Would be detectable early in disease, prior to either JSN or appearance of bone abnormalities, permitting diagnosis of disease;
- Would correlate directly with severity of disease, permitting quick and sensitive assessment of therapeutic efficacy.

Once validated, these biomarkers would serve as surrogate markers for structure damage or overall disease status, and would be used in the go and no-go decision-making process during drug development. Ultimately, these biomarkers would be used for regulatory registration.

There is a long list of potential biochemical biomarkers. It is unlikely that any single marker can fulfill every need for successful drug development. Combinations of these markers will likely enhance their specificity in assessing various processes involving the development of the disease at different stages. The following areas will be key areas of focus in developing biochemical biomarkers and facilitating their incorporation in clinical trials:

- Further assessment of the correlation between biomarker changes and disease activity;
- Better understanding of the variations associated with age, sex, race/ethnic background, body mass index and concomitant medication; and
- Standardization of sample collection, processing and bioanalytical methods. In lieu of the complex processes involved in the pathogenesis of OA, it is conceivable that biochemical biomarkers could be used either independently or, preferably, in combination with each other or with imaging biomarkers to provide additional information during drug development.

In the meantime, imaging modalities will continue be the main focus for the development of biomarkers in OA. Among them, MRI is the most likely candidate to be used as a structural end point for DMOAD clinical trials. Furthermore, molecular imaging techniques, such as dGEMRIC and T1 MRI, have the potential to overcome the inability of standard imaging techniques, such as radiography, to reliably identify advancement of structural changes in OA and hence be used as surrogate markers for disease severity in future clinical trials.

Translational research in the biology and pathogenesis of OA that directly leads to discovery, characterization, validation and qualification of biomarkers for DMOAD development

is needed. Considering that significant resources are required to validate such biomarkers, public and private sector precompetitive collaboration and partnership is needed. The Osteoarthritis Initiative (OAI) coordinated through the US NIH is the best model of this kind. In addition, proactive engagement with regulatory agencies will be critical to qualify a biomarker as a surrogate end point in clinical trials for DMOAD development.

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Executive summary

Biochemical biomarkers

- Biochemical biomarkers are proteins (or their metabolic products) that comprise the target tissues in osteoarthritis (OA) and are present in blood and/or urine in a manner that is consistent with disease activity.
- Biochemical biomarkers may provide easy, rapid, noninvasive and relatively inexpensive means of diagnosing and monitoring progression of disease.
- Nonspecificity and numerous factors affecting the read-out are key limiting factors in OA biomarker development.
- Combinations of these biomarkers will likely provide more insight into disesase development and progression than a single biomarker alone.
- Biochemical biomarkers tend to be more responsive to therapeutic intervention; however, lack of correlation of these changes to clinical outcome has been the key obstacle in utilizing these biomarkers in disease-modifying osteoarthritis drug (DMOAD) development.

Imaging biomarkers

- MRI provides reliable and quantitative data on cartilage status in OA patients.
- Identifying patients who have rapid structure progression potential is the key attribute for MRI.
- Continued development of MRI should enable its utilization as a surrogate end point in clinical trials.
- Novel imaging technologies to measure the molecular composition of cartilage is an exciting new area of focus for OA biomarker development.

Conclusions

- Optimal biomarker application holds the key for successful DMOAD development.
- Significant progress has been made in the discovery and development of biochemical biomarkers. These biomarkers offer promising tools for patient selection, stratification and efficacy monitoring in OA drug development.
- Novel imaging technologies provide an exciting platform to further explore opportunities for monitoring disease progression and assessing therapeutic efficacy with precision. These exciting modalities could potentially replace X-ray to serve as surrogate clinical end points for DMOAD clinical trials.

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