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REVIEW

From biochemical markers to molecular endotypes of osteoarthritis: a review on validated biomarkers

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ABSTRACT

Introduction: Osteoarthritis (OA) affects over 500 million people worldwide. OA patients are symptomatically treated, and current therapies exhibit marginal efficacy and frequently carry safety-risks associated with chronic use. No disease-modifying therapies have been approved to date leaving surgical joint replacement as a last resort. To enable effective patient care and successful drug development there is an urgent need to uncover the pathobiological drivers of OA and how these translate into disease endotypes. Endotypes provide a more precise and mechanistic definition of disease subgroups than observable phenotypes, and a panel of tissue- and pathology-specific biochemical markers may uncover treatable endotypes of OA.

Areas covered: We have searched PubMed for full-text articles written in English to provide an in-depth narrative review of a panel of validated biochemical markers utilized for endotyping of OA and their association to key OA pathologies.

Expert opinion: As utilized in IMI-APPROACH and validated in OAI-FNIH, a panel of biochemical markers may uncover disease subgroups and facilitate the enrichment of treatable molecular endotypes for recruitment in therapeutic clinical trials. Understanding the link between biochemical markers and patient-reported outcomes and treatable endotypes that may respond to given therapies will pave the way for new drug development in OA.

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Biochemical marker; biomarker; bone remodeling; cartilage; endotyping; osteoarthritis; patient stratification; endotype

1. Introduction – molecular endotypes of osteoarthritis

Osteoarthritis (OA) affects over 500 million people worldwide and is a highly heterogenous disease [1–3]. Despite etiological differences, OA patients continue to be treated as a homogenous population. This 'one-size-fits-all' mentality has not only hampered the evolution of more effective patient care, but has likely contributed to the lack of clinically approved disease-modifying OA drugs (DMOADs) [4,5]. In medical practice, this approach could contribute to patients receiving ineffective, perhaps even harmful, treatments. In clinical trials, it can limit the ability to demonstrate efficacy of a novel therapy, leading to discontinuation of development. There is an urgent need to improve our understanding of the OA population to enable improved patient care and usher in a new wave of therapeutics.

The pathobiological drivers of OA and how these translate into identifiable phenotypes have not been fully elucidated, but subgroups driven by trauma-injury, subchondral bone, cartilage, and metabolic syndrome have been reported [6]. A clinical phenotype encompasses the observable traits that distinguish between groups of patients with a given disease [7]. While phenotypes are without mechanistic implications, endotypes are defined by distinct mechanisms which provide a link between the underlying disease drivers and the phenotypic traits to ascribe a more precise definition of patient subgroups [6]. Endotypes are often associated with clinical tests, such as biochemical markers, to align patients to specific subgroups for use in clinical stratification as a precursor to personalized medicine. A molecular understanding of OA phenotypes is therefore crucial and application of this knowledge can facilitate drug development and increase the potential for approval of effective and personalized treatments for OA patients [4].

Biochemical markers can uncover relevant subgroups of OA and facilitate development of DMOADs [2,8]. This has been the goal of the Osteoarthritis initiative from the Foundation for the National Institutes of Health (OAI-FNIH) and the Innovative

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Article highlights

- OA encompasses highly heterogeneous patient populations that display similar clinical manifestations but may have differing underlying pathobiological drivers of the disease.
- Endotypes of OA are defined by distinct biological mechanisms that describe the observable, phenotypic traits.
- Biochemical markers that reflect key pathologies of OA, including cartilage formation and degradation, bone formation and resorption, and connective tissue inflammation, can uncover clinically relevant, underlying disease drivers.
- Endotypes driven by mechanisms such as structural damage, inflammation, trauma-injury, metabolic syndrome, and low cartilage repair have recently been reported but are not fully elucidated.
- A molecular understanding of treatable endotypes can facilitate development of better and personalized treatment options for OA patients.

Medicines Initiative Applied Public-Private Research enabling Osteoarthritis Clinical Headway (IMI-APPROACH) [2,9]. IMI-APPROACH was a two-year, observational cohort that measured 16 molecular markers in 297 knee OA (KOA) patients [2]. The study identified endotypes of structural damage, inflammation, and low tissue turnover that were differentially associated with pain and structural outcomes at baseline [10]. To utilize such markers for future discovery of OA endotypes, it is imperative to understand what they measure and what processes they reflect [4]. As applied in IMI-APPROACH, this review provides an in-depth understanding of a panel of validated markers for endotyping of OA. This review will focus on markers of key OA pathologies, namely cartilage formation and degradation, bone formation and resorption, and connective tissue inflammation (Figure 1). The aim is to elucidate how such molecular markers may facilitate the enrichment of patients with clinically relevant endotypes for recruitment in future therapeutic clinical trials.

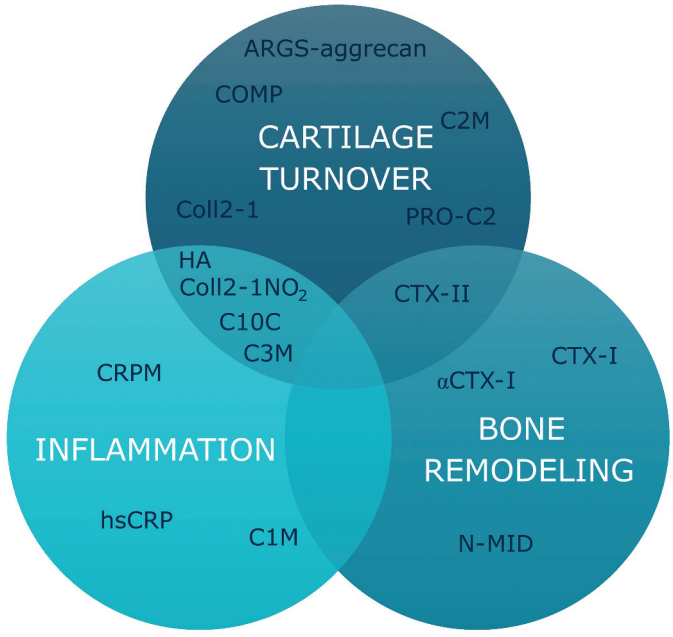

Figure 1. Association between biochemical markers and cartilage turnover, connective tissue inflammation, and bone-remodeling endotypes of osteoarthritis. This illustrates the overlapping and complex nature of their pathobiological relationships described in this review. It should be noted that other endotypes have been described, reflected by molecular markers not covered in this review [6,11]. αCTX-I, C-terminal cross-linked, α-isomerized telopeptide of type I collagen; ARGS-aggrecan, N-terminal neoepitope of aggrecanase-mediated degradation of aggrecan; C1M, matrix metalloproteinase (MMP)-mediated degradation fragment of type I collagen; C2M, MMP-mediated degradation fragment of type II collagen; C3M, MMP-mediated degradation fragment of type III collagen; C10C, C-terminal epitope of type X collagen; Coll2–1, N-terminal epitope on type II collagen ¾ degradation fragment; Coll2-1NO_2, nitrated form of Coll2–1; COMP, cartilage oligomeric matrix protein; CRPM, MMP-mediated degradation fragment of C-reactive protein (CRP); CTX-I, C-terminal cross-linked, β-isomerized telopeptide of type I collagen; CTX-II, C-terminal cross-linked telopeptide of type II collagen; HA, hyaluronan; hsCRP, high-sensitivity CRP; N-MID, N-terminal middle fragment of osteocalcin; PRO-C2, type IIB N-terminal propeptide of type II collagen.

2. Cartilage formation and degradation

A cornerstone of OA is the dysregulated balance between tissue formation and degradation. Affected tissues include articular cartilage, bone, and synovium, all of which are collagenous tissues with an extracellular matrix (ECM) [5]. As collagens and proteoglycans are the main structural proteins of the joint ECM that are continuously turned over, they are of special interest in OA [5,9,12]. Quantification of degradation as well as formation of such proteins can reflect overall joint disease activity in OA and can provide insight into the pathophysiology of a given patient and drive the choice of therapy [13]. Measurement of ECM degradation and formation products may in some cases provide more stability compared to whole proteins. As ECM turnover fragments are often denatured, they do not rely on correct protein conformation for quantification, which is often the case when measuring whole proteins such as cytokines and interleukins (ILs) [14].

2.1. Type II collagen and PRO-C2

Type II collagen contributes to the integrity of articular cartilage and its tensile strength [13,15]. It makes up ~60% of dry weight of healthy, adult articular cartilage, and its expression is regulated by chondrocytes (cartilage cells) [16,17]. The fibrillar collagen consists of a homotrimeric α1(II) chain and *N*- (PIINP) and C-terminal propeptides (PIICP) that are cleaved off by matrix metalloproteinases (MMPs) and N- and C-proteinases to form mature collagen. Thus, the amount of free propeptides reflects the formation of mature type II collagen [12]. Alternative splicing in the second exon of the gene *COL2A1* gives rise to two PIINP variants. Type IIA (PIIANP) is the embryonic variant that is replaced by type IIB (PIIBNP), transcribed without the second exon in mature articular cartilage. The competitive PRO-C2 electro-chemiluminescence immunoassay (ECLIA) (Nordic Bioscience, Herlev, Denmark) measures PIIBNP by targeting the epitope ^{26}QDVRQPG^{32} located between the first and third exons in type II collagen (accession no. P02458–1). PRO-C2 is a biochemical marker of cartilage formation [12] (Table 1). Studies have found lower serum levels of PRO-C2 in KOA patients with Kellgren-Lawrence (KL) grades between 2–4 compared to healthy controls, and low levels of PRO-C2 may predict radiographic progression and indicate a low cartilage repair endotype [6,12].

Fibroblast growth factor-18 (FGF-18) has previously been genetically associated with OA and has been found to

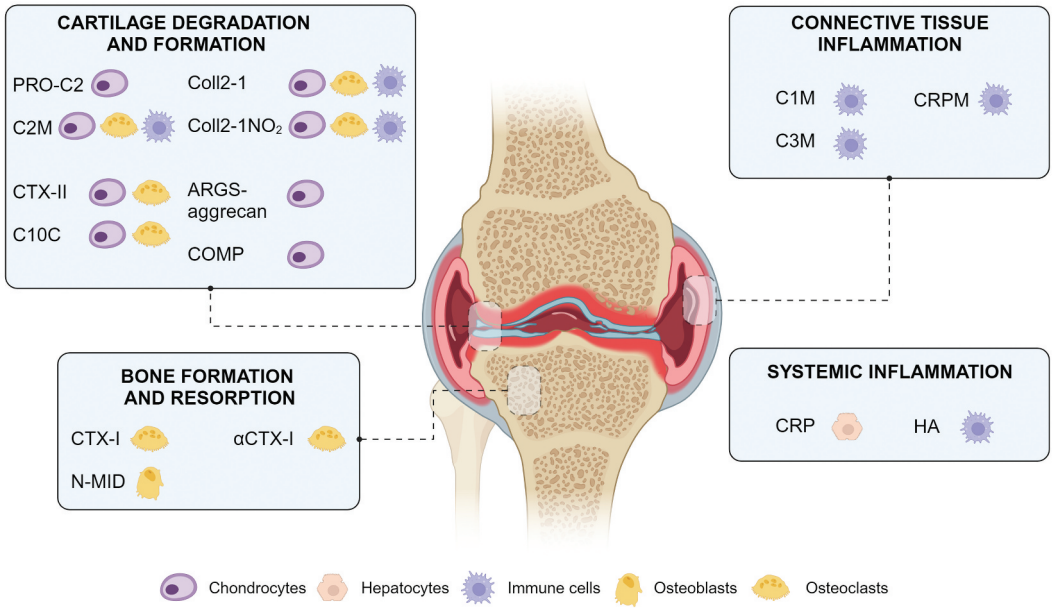

Figure 2. Illustration of the 16 biochemical markers covered in this review and the primary pathologies of osteoarthritis they reflect. The osteoarthritic knee joint and the main cell types believed to be responsible for the generation of the biochemical markers have been illustrated with BioRender.com. These include chondrocytes (cartilage cells), hepatocytes, immune cells (including but not limited to macrophages), osteoblasts (bone-forming cells), and osteoclasts (bone-resorptive cells). αCTX-I, C-terminal cross-linked, α-isomerized telopeptide of type I collagen; ARGS-aggrecan, N-terminal neoepitope of aggrecanase-mediated degradation of aggrecan; C1M, matrix metalloproteinase (MMP)-mediated degradation fragment of type I collagen; C2M, MMP-mediated degradation fragment of type II collagen; C3M, MMP-mediated degradation fragment of type III collagen; C10C, C-terminal epitope of type X collagen; Coll2–1, N-terminal epitope on type II collagen ¾ degradation fragment; Coll2-1NO_2, nitrated form of Coll2–1; COMP, cartilage oligomeric matrix protein; CRPM, MMP-mediated degradation fragment of C-reactive protein (CRP); CTX-I, C-terminal cross-linked, β-isomerized telopeptide of type I collagen; CTX-II, C-terminal cross-linked telopeptide of type II collagen; HA, hyaluronan; N-MID, N-terminal middle fragment of osteocalcin; PRO-C2, type IIB N-terminal propeptide of type II collagen.

stimulate cartilage formation, quantified by PRO-C2, in cartilage explant cultures [14,54]. Serum PRO-C2 was used to stratify 225 radiographic KOA patients from the phase II clinical trial FGF-18 Osteoarthritis Randomized Trial with Administration of Repeated Doses (FORWARD) of sprifermin [55]. Patients with low PRO-C2 lost more cartilage over time but grew more cartilage in response to sprifermin relative to placebo, when compared to patients with high levels. PRO-C2 was also measured in synovial fluid (SF) of 59 patients and increased in a biphasic manner over time in response to sprifermin. SF PRO-C2 was thus suggested as a pharmacodynamic (PD) indicator of the effect of sprifermin [55].

2.2. C2M

The stability of collagen fibrils is compromised in OA by their extensive degradation, leading to loss of cartilage tissue [22]. Proteolytic cleavage of type II collagen gives rise to an array of molecular markers, depending on the type of protease. MMPs (MMP-2, –8, –9, and –13) are vital for the pathogenesis of OA and type II collagen degradation [56]. However, cathepsin K also degrades type II collagen in articular cartilage [57]. The proteinases are secreted by chondrocytes, osteoclasts, and synovial cells in response to pro-inflammatory cytokines and growth factors [18,58]. Initial cleavage of type II collagen is typically mediated by MMP-1, –8, –13, and –14 and is preferentially cleaved between ^{975}Gly and ^{976}Leu (accession no. P02458–1). This generates two fragments that are ¾ and ¼ the size of mature collagen, resulting in unwound, denatured fragments with increased susceptibility to further proteolytic cleavage [22]. Subsequently, cleavage by MMP-8, –9, and –12 can occur on the ¼ fragment of type II collagen. This includes cleavage between ^{1053}Gly and ^{1054}Val, generating the C-terminal neoepitope N-RDGAAG^{1053} [18,19]. The competitive C2M enzyme-linked immunosorbent assay (ELISA) (Nordic Bioscience, Herlev, Denmark), measured on the automated assay platform IDS-i10 (IDS, Boldon, United Kingdom), targets the neoepitope and reflects type II collagen degradation [18] (Figure 2). Serum C2M was measured in a study of 81 subjects with mild (KL 1–2) or severe OA (KL 3–4), and 75 without OA, and was elevated in OA patients [18]. Serum C2M was also measured in a study of 103 ankylosing spondylitis (AS) patients, 47 rheumatoid arthritis (RA) patients, and 56 healthy controls. C2M was higher in both disease groups compared to controls, indicating an involvement in other rheumatological disorders with pathological collagen degradation as well [51].

2.3. CTX-II

Urinary C-terminal cross-linked telopeptide of type II collagen (uCTX-II) is one of the most well-described biochemical markers of type II collagen degradation [20]. Cleavage by cathepsin B and MMP-1, –3, –7, –9, and –13 on the type II collagen ¼ fragment in the C-terminal telopeptide region between ^{1235}Pro and ^{1236}Leu (accession no. P02458–1) generates the C-terminal neoepitope N-EKGPDP^{1235} [58]. The competitive CartiLaps (CTX-II) enzyme immunoassay (EIA) ELISA (IDS, Boldon, United Kingdom) targets the neoepitope which is exclusively found on type II collagen [20]. uCTX-II has been

Table 1. Panel of 14 serum and two urine (u) biochemical markers utilized for endotyping of osteoarthritis (OA) patients in IMI-APPROACH [2,10], and their association with OA.

Marker	Type	Molecular origin	Association with OA	Ref.
PRO-C2	Cartilage formation	Type IIB N-terminal propeptide of type II collagen.	Low levels predict structural progression and indicate low cartilage repair endotype.	[6,12]
C2M	Cartilage degradation	MMP-8, −9, −12 cleavage of type II collagen ¼ fragment.	Higher in mild (KL 1–2) and severe OA (KL 3–4) compared to no OA.	[18,19]
uCTX-II	Cartilage degradation, bone remodeling	Cleavage by cathepsin B, MMP-1, −3, −7, −9, −13 of C-terminal telopeptide of type II collagen ¼ fragment.	Prognostic marker of pain and structural progression, associated with bone metabolism.	[9,20,21]
Coll2–1	Cartilage degradation	Cleavage of type II collagen ¾ fragment.	Correlates with subarticular cysts and bone attrition WORMS, associates with JSN progression.	[22–25]
Coll2–1 NO_2	Cartilage degradation, inflammation	Nitrated form of Coll2–1.	Oxidative degradation of articular cartilage. Associates with hsCRP and synovial inflammation.	[24,26]
C10C	Cartilage degradation, Inflammation	Type X collagen.	Chondrocyte hypertrophy [27], located in areas of re-initiation of endochondral bone formation.	[27,28]
COMP	Cartilage degradation	COMP.	Conflicting clinical associations.	[29,30]
ARGS-aggrecan	Cartilage turnover	Cleavage of aggrecan by ADAMTS-4 and −5.	PD marker and reflects early cartilage remodeling. Higher in patients undergoing TKR.	[31–34]
CTX-I	Bone resorption	Cathepsin K cleavage on β-isomerized type I collagen α1(I) C-terminal telopeptide.	Elevated in OA. Limited association to radiographic progression.	[35–37]
u-αCTX-I	Bone resorption	Native α-isomerized CTX-I.	Turnover of young bone. Localized to high turnover areas in subchondral bone, correlated with JSN progression and osteophytes.	[38,39]
N-MID	Bone formation	N-terminal middle fragment of osteocalcin.	No clear clinical associations.	[40,41]
HA	Inflammation, cartilage degradation	Circulating HA.	Proposed marker of radiographic OA progression.	[42–44]
hsCRP	Inflammation	CRP.	Low-grade systemic inflammation, pain association.	[45–47]
CRPM	Inflammation	CRP metabolite of MMP-1 and −8.	Tissue-specific marker of early disease and inflammatory subgroups.	[47,48]
C1M	Inflammation	Type I collagen degradation by MMP-2, −9, −13.	Soft-tissue specific association with inflammation, CRPM, and hsCRP.	[49,50]
C3M	Inflammation	Degradation of type III collagen by MMP-9.	Synovial inflammation. High in inflammatory OA, correlates with hsCRP.	[50–53]

αCTX-I, C-terminal cross-linked, α-isomerized telopeptide of type I collagen; ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs; ARGS-aggrecan, N-terminal neoepitope of aggrecanase-mediated degradation of aggrecan; C1M, MMP-mediated degradation fragment of type I collagen; C2M, MMP-mediated degradation fragment of type II collagen; C3M, MMP-mediated degradation fragment of type III collagen; C10C, C-terminal epitope of type X collagen; Coll2–1, N-terminal epitope on type II collagen ¾ degradation fragment; Coll2-1NO_2, nitrated form of Coll2–1; COMP, cartilage oligomeric matrix protein; CRP, C-reactive protein; CRPM, MMP-mediated degradation fragment of CRP; CTX-I, C-terminal cross-linked, β-isomerized telopeptide of type I collagen; CTX-II, C-terminal cross-linked telopeptide of type II collagen; HA, hyaluronan; hsCRP, high-sensitivity CRP; JSN, joint space narrowing; KL, Kellgren-Lawrence grade; MMP, matrix metalloproteinase; N-MID, N-terminal middle fragment of osteocalcin; PD, pharmacodynamic; PRO-C2, type IIB N-terminal propeptide of type II collagen; TKR, total knee replacement; WORMS, Whole-Organ Magnetic Resonance Imaging Score. For technical specifications of the molecular markers, we refer to Angelini et al., 2022 [10]. To ensure comparability, studies of clinical associations of a biomarker to OA were only considered for the same sample (serum/urine) and assay type as described for the biomarker in this review.

utilized in several clinical cohorts as a marker of cartilage degradation including OAI-FNIH in which uCTX-II was measured in 600 OA patients with different phenotypes on symptoms and structure. Baseline uCTX-II was one of the most prognostic markers for pain and structural progression of OA [9]. uCTX-II was also measured in the Cohort Hip and Cohort Knee (CHECK) study where it was proposed to reflect cartilage degradation as well as bone metabolism [21]. In a study on 478 postmenopausal women from the Os des Femmes de Lyon (OFELY) cohort, increased baseline levels of uCTX-II were associated with a higher risk of total joint replacement (TJR) of hips and knees [59]. In 129 radiographic KOA patients from the Prediction of Osteoarthritis Progression (POP) cohort, uCTX-II was predictive of OA progression and severity [38].

CTX-II and C2M are both cartilage degradation markers but differ in tissue distribution. CTX-II has been immunolocalized to erosion sites and at the interface between calcified cartilage and bone in human KOA cartilages, whereas C2M has been detected in lesions, surface erosions, and calcified cartilage [18]. This may indicate a reflection of different pathobiological processes of OA and highlights the value of utilizing several cartilage degradation markers, even if they are derived from the same protein [18]. While uCTX-II is considered a marker of cartilage degradation, it has also been associated with the marker of bone resorption, urinary C-terminal cross-linked, β-isomerized telopeptide of type I collagen (uCTX-I) and the bone formation marker, serum osteocalcin [21].

2.4. Coll2–1

Through unwinding of the triple helical region, the ¼ and ¾ type II collagen fragments have increased susceptibility for proteolytic activity [13]. Upon denaturation of the helical section of type II collagen, the epitope ^{289}HRGYPGLDG^{297} (accession no. P02458–1) becomes detectable at the N-terminal of the ¾ fragment [22,23]. The competitive Coll2–1 ELISA (Artialis SA, Liège, Belgium) targets this epitope in its linear state and is

thus a surrogate marker of type II collagen degradation [24]. This epitope is only found in the α1 chain of type II collagen and the α3 chain of type XI collagen. However, type XI collagen only makes up 1% wt/wt relative to α1(II) chains and is embedded in type II collagen fibrils [24]. Immunostainings for the marker have shown that Coll2–1 is located in the fibrillated zone of the ECM and is absent from surrounding unfibrillated zones [25]. As type II and XI collagens are specific to cartilage, and the fact that type XI collagen is turned over with type II collagen, it has been hypothesized that the Coll2–1 fragments are released from types II and XI collagens simultaneously and reflect cartilage ECM degradation [24]. The Coll2–1 marker has been used for studying the burden of disease, prognostics, and diagnosis of OA [60]. Coll2–1 was measured in a study of 67 KOA and 19 RA patients and 242 healthy controls [24]. Serum levels of Coll2–1 were increased in both disease groups compared to age-matched controls and increased urinary levels of Coll2–1 over a one-year period were associated with radiographic progression of OA over three years [24,25]. Recently, a study demonstrated that Coll2–1 levels were not confounded by sampling-specific conditions, circadian rhythm or seasonality, and that variations in Coll2–1 rather reflected cartilage degradation in OA [60].

2.5. Coll2-1NO$_2$

While Coll2–1 is a marker of type II collagen degradation, its nitrated form Coll2-1NO$_2$ is associated with oxidative stress and inflammation [23]. Coll2–1 contains a tyrosine (^{292}Tyr) residue susceptible to nitration by peroxynitrite anion, yielding the nitrated epitope form, ^{289}HRGY(NO$_2$)PGLDG^{297}. Chondrocytes produce high levels of reactive nitrogen and oxygen species such as nitric oxide during pathological conditions [24]. Higher concentrations of nitrite (NO_2^-) have been found in serum of OA and RA patients compared to age-matched controls [61]. As oxidative stress is involved in cartilage ECM damage and is required for the nitration of Coll2–1, Coll2-1NO$_2$ has been suggested to reflect oxidative-related degradation and the extent of oxidative stress in articular cartilage [24,26]. The competitive Coll2-1NO$_2$ ELISA (Artialis SA, Liège, Belgium) targets the nitrated form of Coll2–1 [24]. Coll2-1NO$_2$ can discriminate OA and RA patients from healthy controls and can be used as a marker of prognosis and burden of disease [24]. Unlike Coll2–1, Coll2-1NO$_2$ correlates with high-sensitivity C-reactive protein (hsCRP) in RA and OA, with higher levels of Coll2-1NO$_2$ in RA patients, suggesting an association with synovial inflammation [24,26]. In a study of 121 KOA patients, the markers were quantified along with Whole-Organ Magnetic Resonance Imaging Score (WORMS) scores, of which included knee features such as subarticular cysts, bone attritions, osteophytes, bone marrow lesions, and articular cartilage integrity [23]. While Coll2–1 correlated with subarticular cyst and bone attrition scores, Coll2-1NO$_2$ correlated with osteophytes scores and WORMS total score. Serum Coll2-1NO$_2$ also correlated with pain worsening over a one-year period [23]. As such, Coll2–1 and Coll2-1NO$_2$ reflect different pathobiological processes of OA and provide complimentary information on the catabolism of type II collagen [62].

2.6. Type X collagen and C10C

Type X collagen maintains joint tissue stiffness and aids in the normal distribution of proteoglycans within the growth plate matrix [63,64]. It is expressed by hypertrophic chondrocytes at sites of endochondral bone formation during skeletal development and growth as well as under pathological conditions including OA [65]. Type X collagen is a network-forming, cartilage-specific collagen encoded by the *COL10A1* gene. It constitutes 1% of collagen in healthy cartilage and 45% of the total collagen produced by hypertrophic chondrocytes [66,67]. Type X collagen is a homotrimer of α1(X) chains with a C-terminal, globular, non-collagenous domain (NC1), and an N-terminal non-helical domain (NC2) [66]. Through the NC1 domain, type X collagen assembles into a hexagonal lattice structure which is thought to be important for modification of the hypertrophic, cartilage matrix during endochondral bone formation [65]. Gene expression of type X collagen has been detected in OA tissues in areas of re-initiation of endochondral bone formation, such as development of osteophytes [28].

The competitive C10C ELISA (Nordic Bioscience, Herlev, Denmark) targets the epitope ^{671}SFSGFLVAPM^{680} (accession no. Q03692), located at the C-terminal end of the NC1 domain. C10C determines the level of free type X collagen and reflects its turnover. A study of 271 OA patients found that serum levels of C10C were elevated in patients with KL grade 2 compared to subjects without OA. C10C was also higher in OA patients with increased levels of hsCRP and correlated with C2M levels, indicating an association with inflammation and cartilage degradation [27].

2.7. COMP

Glycoproteins play a role in the protection of cartilage surfaces and inhibit synovial cell overgrowth, and are involved in tissue remodeling and degradation of articular cartilage [68,69]. One such glycoprotein is the non-collagenous cartilage oligomeric matrix protein (COMP) [70]. Encoded by the *COMP* gene, it is expressed in healthy cartilage and forms a crucial part of the ECM [71,72]. COMP is expressed by articular chondrocytes and cell types such as platelets, vascular smooth muscle cells, and fibroblasts [4,73]. Through its interaction with ECM proteins including aggrecan and collagens I, II, IX, XII, and XIV, it is vital for matrix assembly. COMP forms a large homopentamer with multiple domains, including type 2 epidermal growth factor (EGF)-like and thrombospondin (TSP)-like domains [71]. The pentameric form binds up to five collagen molecules which facilitates collagen–collagen interactions and fibrillation by keeping them in close proximity [73]. A number of MMPs and A disintegrin and metalloproteinase with thrombospondin motifs' (ADAMTS') cleave COMP at the EGF-like domain such as MMP-3, −12, and −13 together with ADAMTS-4, −5, and −7 [19,71]. Evidence suggests that MMP-12 is preferred for cleavage of COMP [19].

COMP is a well-established cartilage degradation marker in OA and can be quantified by the sandwich COMP ELISA (IDS, Boldon, United Kingdom), however with conflicting reports [29]. Serum levels of COMP were measured in a 20-year

longitudinal cohort of 593 middle-aged women with no KOA at baseline. The women with the highest baseline COMP levels had a 48% increased risk of developing painful, radiographic KOA, however not significantly after adjusting for age and BMI [30]. COMP was also measured in serum and SF of 121 subjects with acute anterior cruciate ligament rupture from the Knee Anterior Cruciate Ligament, Nonsurgical versus Surgical Treatment (KANON) trial together with 25 healthy controls. The study found no significant differences in serum COMP between healthy and injured subjects. However, COMP levels were two-fold higher in SF within six weeks of injury and remained increased five years later [74].

2.8. ARGS-aggrecan

Articular cartilage has limited capacity for repair or regeneration and has a finite chondrocyte population to maintain the ECM. Proteoglycans allow the cartilage to withstand compressive loading and protect chondrocytes from mechanical trauma [75,76]. Proteoglycans make up 10% of healthy articular cartilage tissue and the main type is aggrecan. Expressed primarily by chondrocytes, aggrecans provide a hydrated gel-structure through interaction with hyaluronan (HA) and link protein [5,77]. Aggrecans form large aggregates in the ECM, with each consisting of a central filament of HA with up to 100 non-covalently bound aggrecans [75,77]. Aggrecan is a multi-domain protein encoded by the *ACAN* gene and the core protein consists of three globular domains, G1–3 [77]. While G1 and G2 are separated by an interglobular domain, the region between G2 and G3 is substituted with chondroitin sulfate and keratin sulfate glycosaminoglycan chains [75]. The negatively charged anionic groups on the glycosaminoglycan chains attract cations, causing a large osmotic pressure which draws water into the cartilage. This causes expansion of the ECM which provides compressive strength [77]. The interglobular domain of aggrecan is sensitive to proteolytic activity and cleavages in this region are detrimental to the compressive strength due to the loss of glycosaminoglycan chains [78]. MMP-3, −7, −8, and −12 and aggrecanases (ADAMTS-4 and −5) are the major proteases responsible for aggrecan degradation and ADAMTS-5 is important for the initiation of ECM degradation in OA [19,78–80].

Aggrecan is subjected to cleavage by aggrecanases at six known sites, which exposes otherwise-inaccessible ECM proteins to proteolytic activity [19,81]. The most detrimental cleavage site is located between ^{392}Glu and ^{393}Arg (accession no. P16112–1) within the interglobular domain, yielding the N-terminal ^{393}ARGSVILT-C neoepitope [31,32]. The neoepitope is targeted by the sandwich ARGS-aggrecan ECLIA on the Meso Scale Discovery platform [32]. ARGS-aggrecan has been proposed as a marker of early cartilage remodeling in OA and has been used as a PD marker in clinical trials [33]. In a study of 20 OA patients undergoing total knee replacement (TKR), 20 non-surgical OA patients, and 20 age- and sex-matched controls, researchers found increased levels of serum ARGS-aggrecan in the surgical OA group compared to non-surgical OA and controls [34]. ARGS-aggrecan was evaluated in serum in 19 KOA patients and 20 young, healthy controls in a model for exercise-induced ECM turnover [82]. Only minute increases in ARGS-aggrecan were observed in the OA patients in response to moderate intensity running and cycling, which normalized within 24 hours. As the sensitivity to physical activity was low, it was deemed a reproducible and stable biochemical marker of OA [82].

3. Bone formation and resorption

The integrity and remodeling of bone are important aspects of OA pathology and the development of osteophytes and subchondral bone sclerosis are considered hallmarks of OA [83,84]. The subchondral bone plays a pivotal part in the initiation and progression of OA in which pathological bone turnover leads to its deterioration and hypomineralization [83]. Over time, changes occur to the subchondral bone including increased vascularization, sclerosis, bone-marrow lesions, and microfractures. In fact, turnover of subchondral bone is up to 20 times higher in OA compared to that of normal bone [38]. The subchondral area is thus of special interest for the development of bone formation and resorption markers in OA [4].

3.1. Type I collagen and CTX-I

Type I collagen is the most abundant protein of the body and is the main constituent of bone [13]. 90% of the bone matrix consists of collagenous proteins, predominantly type I collagen, and non-collagenous proteins such as osteocalcin. The bone ECM provides mechanical support and is essential for bone homeostasis [85]. The mineral content of the bone ECM determines bone stiffness whereas type I collagens contribute to load bearing and tensile strength [15,86]. Alterations to type I collagen fibrils can affect the biomechanical properties of bone and as such, type I collagen-derived markers have been utilized extensively in the osteoporosis and OA fields [13,86]. Type I collagen is transcribed from the *COL1A1* and *COL1A2* genes, mainly by fibroblasts and osteoblasts, that form a fibrillar heterotrimer of two α1(I) chains and one α2(I) chain with N- and C-terminal telopeptides [87]. The α1(I) and α2(I) chains are subjected to post-translational modifications such as isomerization after maturation [86]. Type I collagens assemble into large collagen fibrils, stabilized by covalent cross-linking of lysine residues [88].

Type I collagen is subjected to proteolytic activity by MMPs such as MMP-2, − 9, and −13, and cathepsin K, reflecting different biological processes [13,49]. Cathepsin K is pivotal for osteoclastic resorption of bone and cleaves type I collagen at multiple sites including the C-terminal telopeptide on the α1(I) chain [89]. Cathepsin K cleavage between ^{1214}Arg and ^{1215}Tyr generates the C-terminal neoepitope N-EKAHDGGR^{1214} (accession no. P02452) with an aspartyl-glycine motif prone to age-related β-isomerization [35,89]. This occurs through an attack by nitrogen from the peptide backbone of ^{1212}Gly onto the side-chain carbonyl group on ^{1211}Asp, producing β-Asp rather than α-Asp [86]. The sandwich Elecsys β-CrossLaps® (CTX-I) ECLIA (Roche Diagnostics Ltd., Switzerland) targets N-EKAH(β)DGGR^{1214}, reflecting resorptive osteoclast activity on mature type I collagen. C-terminal cross-linked, β-isomerized telopeptide of type I collagen (CTX-I) is

a sensitive marker of bone turnover but circadian variability due to increased bone resorption during night/early morning and fasting sensitivity is well-established [89,90]. As such, samples should be retrieved in a fasting state within narrow timeframes [89]. CTX-I was developed as a urine marker (uCTX-I) but has since become available in serum (sCTX-I) [35,91]. sCTX-I has been proposed as the recommended molecular marker of bone resorption and is reportedly most stable in plasma [91]. sCTX-I has been used to evaluate the response to anti-resorptive treatments of osteoporosis, including assessment of pamidronate in 21 patients with thyroid-hormone-induced bone loss [92]. sCTX-I correlated with bone resorption parameters, including osteoclast surfaces and eroded volume, in a study of 371 postmenopausal osteoporosis patients [93]. uCTX-I was measured in 71 progressive KOA patients, 59 osteoporosis patients, and 50 controls from the Chingford study at baseline and after one and two years [36]. uCTX-I was elevated at all time points for both disease groups compared to controls. However, a study of 1241 KOA patients found no association between uCTX-I and radiographic progression of OA [37].

3.2. αCTX-I

The urinary α-CrossLaps® (u-αCTX-I) EIA ELISA (IDS, Boldon, United Kingdom) targets the α-isomerized form of CTX-I and measures the turnover of newly synthesized type I collagen [38,39]. u-αCTX-I reflects turnover of young bone as the age-related β-isomerization has not yet occurred and has been described as a marker of high turnover areas specific for newly synthesized bone [38]. Measuring the urinary α/β ratio of CTX-I has proven useful for measuring bone age with a lower ratio reflecting older bone and has been suggested to be of clinical importance for assessment of metabolic bone diseases [35]. This was reflected in a study of 427 postmenopausal women receiving either bisphosphonates, hormonal replacement therapy or raloxifene [94]. uCTX-II and u-αCTX-I were also measured in 129 radiographic KOA patients from the POP cohort [38]. While uCTX-II localized to the bone–cartilage interface and damaged articular cartilage, u-αCTX-I was found in high bone turnover areas in subchondral bone. u-αCTX-I correlated strongly with progression of joint space narrowing (JSN) and osteophytes, indicating an association with the dynamic biology of subchondral bone remodeling, whereas uCTX-II associated with static JSN severity [38]. This emphasizes the need for a panel of molecular markers in OA as they reflect different aspects of joint tissue turnover [4].

3.3. N-MID

The most abundant non-collagenous protein of the bone ECM is osteocalcin, also known as γ-carboxyglutamic acid-containing protein. Transcribed from the *BGLAP* gene, osteocalcin is produced by mature, bone-forming osteoblasts as a pre-prohormone. Cleavage of the signal peptide between ^{23}Ala and ^{24}Lys (accession no. P02818) results in pro-osteocalcin. Prior to its secretion, osteocalcin is γ-carboxylated on three glutamic acid residues, namely ^{68}Glu, ^{72}Glu, and ^{75}Glu. The carboxylations promote the affinity for the primary mineral component of bone ECM, hydroxyapatite. Most of osteocalcin is incorporated into the bone ECM while a small fraction is released into circulation during bone formation [95]. During bone resorption, the low pH value inside the resorptive compartments facilitates decarboxylation of osteocalcin, reducing its affinity for bone, and triggers the release of under-carboxylated osteocalcin [96]. Osteocalcin is also subjected to proteolytic activity by cathepsin K and MMPs during bone resorption, resulting in smaller degradation fragments [97]. Both γ- and under-carboxylated osteocalcins are detected in serum, and the under-carboxylated form is believed to function in an endocrine manner [95,96].

Circulating osteocalcin has been associated with changes in the rate of bone turnover in metabolic diseases such as hyperthyroidism and osteoporosis but exhibits instability in serum due to a highly reactive, short C-terminal sequence. This prompted the development of an assay targeting a large N-terminal middle fragment of osteocalcin (N-MID). The sandwich Elecsys N-MID Osteocalcin ECLIA (Roche Diagnostics Ltd., Switzerland) targets osteocalcin, with epitopes located between ^{52}Tyr-^{100}Val and ^{52}Tyr-^{94}Arg [40]. As the assay quantifies both γ- and under-carboxylated osteocalcin, it may reflect tissue turnover [98]. However, bone-forming osteoblasts contribute to the majority of circulating osteocalcin and the N-MID assay has limited ability to detect smaller degradation fragments of osteocalcin. As such, N-MID is widely considered a biochemical marker of bone formation [97]. Serum N-MID was measured in a study of 67 postmenopausal women receiving the bisphosphonate alendronate for the prevention of osteoporosis, which is a disease that causes weakening of bones [99]. A negative correlation was found between two-year response in spinal bone marrow density and change from baseline at 12 months of N-MID. The researchers showed that short-term changes in the marker could monitor alendronate treatment and predict long-term response in bone mass [99]. N-MID was also measured in 151 radiographic KOA patients from the Boston Osteoarthritis of the Knee Study (BOKS) [41]. No clinically meaningful association between N-MID and the presence of bone marrow lesions was found.

4. Connective tissue inflammation

OA was originally considered an arthritic disease void of inflammation. However, inflammation has been highlighted during the past decade and is now strongly implicated in OA pathogenesis [4,100]. Recently, inflammatory endotypes have also been demonstrated in OA [10,101,102]. Inflammation precedes the development of significant radiographic changes and a clear association has been found between synovitis and future development of cartilage degradation in OA stages prior to radiographic manifestations [4,100]. During synovitis, immuno-infiltration is primarily mediated by synovial macrophages that produce pro-inflammatory cytokines, MMPs, and complement systems [100,103,104]. IL-1β, −6, and tumor necrosis factor (TNF) are involved in cartilage degradation and initiate the inflammatory cascade in OA [45,105]. Induction of the innate immune response within the damaged joint is a central feature of OA and can arise from signaling

through damage-associated molecular patterns (DAMPs). These include degradation fragments of the ECM that further promote inflammation and cartilage degradation [100].

4.1. HA

HA is a ubiquitously expressed glycosaminoglycan that is a crucial component of the joint architecture and mechanics [106]. HA resides in the ECM where it is involved in structural organization of cartilage [107]. HA is an unbranched bipolymer of a disaccharide consisting of D-glucuronic acid and *N*-acetyl-D-glucosamine [108]. It is synthesized by HA synthases and is found in varying sizes, ranging from high to low molecular weight fragments with differing roles in inflammation [107,109]. High molecular weight HA is a hydroviscous substance that exhibits extremely hydrophilic properties. It forms a voluminous, expanded aqueous solution that provides a protective structure when surrounding chondrocytes in cartilage [106]. HA is crucial for load-bearing properties of aggrecans in the articular cartilage ECM as their anchorage to cells in the pericellular region occurs through HAs [75,77]. Besides the biomechanical properties of high molecular weight HA, it is also immuno-suppressive [109]. Altered turnover of HA and elevation of oxidative stress decrease the concentration and molecular weight of HA. Low molecular weight HA fragments (<400 kDa) have pro-inflammatory properties such as upregulation of cytokines by chondrocytes and fibroblasts, and macrophage activation [105,109]. Low molecular weight HA contributes to increased ECM degradation by induction of MMP-9, −10, and −13, as well as inflammation and pain by induction of cytokines, including IL-1β and TNF-α [105]. Increased production and release of HA from arthritic joints are thought to reflect local inflammation of the synovial lining and, to a lesser degree, cartilage degradation [110].

HA is a marker of inflammation, with elevated levels demonstrated in RA and OA [42,109]. HA can be quantified by the sandwich HA ELISA (Corgenix, Colorado, USA) that uses HA-binding protein purified from bovine nasal cartilage to capture HA [42]. Serum HA and WORMS of the knee have been quantified in a study of 62 KOA patients to study the relation between the biochemical marker and structural progression of OA [43]. Elevated baseline levels of HA predicted worsening of total WORMS and could identify patients at higher risk of disease progression over a one-year period [43]. Serum HA was measured in 55 erosive and 33 non-erosive hand OA patients. Erosive OA is severe a subset of hand OA that is defined by cartilage damage, bone erosion, and a strong inflammatory component. Serum levels of HA were found to be elevated in patients with erosive hand OA compared to non-erosive [44]. It correlated with late disease phases in all hand OA patients and was proposed as a marker for radiographic progression of hand OA over a two-year period.

4.2. hsCRP

CRP is a clinical marker of systemic inflammation and is widely utilized as a diagnostic tool in acute inflammatory diseases [50]. It is an acute-phase inflammatory protein of the innate immune system with levels rapidly increasing at sites of inflammation or infection. Transcribed from the *CRP* gene, the protein is primarily expressed by hepatocytes [111]. CRP is a homopentameric, non-glycosylated protein and is comprised of five non-covalently bound globular subunits [112]. CRP molecules are synthesized as monomers that assemble into the native pentameric form. Stimulation of CRP synthesis is mainly induced by hepatic IL-6 and, to a lesser degree, IL-1β and TNF-α [113]. CRP levels increase in response to tissue damage, which activates the acute-phase inflammatory response. Increasing severity of tissue damage and inflammation lead to increasing CRP levels, and higher levels of CRP correlate with poorer disease prognosis [112]. CRP is also itself a regulator of inflammatory processes, depending on the isoform, and accumulates at sites of inflammation. The native, pentameric form activates the classical complement pathway, induces phagocytosis, and inhibits production of nitric oxide species, whereas the monomeric form promotes monocyte chemotaxis, recruitment of leukocytes, and nitric oxide production [113].

The pentameric form of CRP is utilized as a clinical marker [112]. High-sensitivity CRP (hsCRP) can be measured by the sandwich CardioPhase® hsCRP immunoassay (Siemens Healthcare, Ballerup, Denmark) and has been utilized extensively as a sensitive marker of low-grade, systemic inflammation. However, factors such as age, BMI, diabetes, smoking status, and alcohol consumption influence the levels of hsCRP [46]. It has previously been suggested that local inflammation, which is a prominent feature of OA, may be observed systemically and as a result hsCRP is also measured in the context of OA [45]. In a literature review of the relationship between serum hsCRP and hip and KOA, researchers found that hsCRP was moderately higher in OA patients compared to controls [45]. hsCRP was associated with pain and decreased physical function but not with radiographic OA, suggesting that low-grade inflammation may be of higher importance for symptoms rather than radiographic changes in OA [45]. hsCRP was also measured in a study of 770 hip or KOA patients undergoing TJR. After adjusting for confounders, severity of pain and not the extent of OA was associated with hsCRP [46].

4.3. CRPM

Due to its upregulation in all inflammatory conditions, CRP is a nonspecific, systemic marker of inflammation. CRP accumulates at sites of tissue damage and inflammation and is susceptible to local enzymatic modification during pathological tissue turnover. Modified CRP may therefore be a more pathology- and tissue-specific reflection of chronic inflammation than that of native CRP [47]. CRP is subjected to proteolysis by proteases such as MMPs, cathepsins, and ADAMTS', resulting in the release of CRP metabolites. Cleavage by MMP-1 and −8 between ^{24}Arg and ^{25}Lys generates the MMP-exclusive N-terminal neoepitope ^{25}KAFVFP-C (accession no. P02741–1) that is targeted by the competitive CRPM ELISA (Nordic Bioscience, Herlev, Denmark) [47]. CRPM and CRP were measured in serum in a study of 40 AS patients and 40 age- and sex-matched controls [47]. While native CRP was not higher in the patients compared to

controls, CRPM was elevated by 25% and patients with high levels of CRPM had odds ratios of 6.3 of having AS. Thus, CRPM exhibited more discriminatory diagnostic potential than that of CRP for AS [47]. In a study of 704 RA patients from the Tocilizumab Safety and the Prevention of Structural Joint Damage (LITHE) cohort, serum CRPM decreased in a dose- and time-dependent manner to anti-IL-6 treatment, thereby showing PD potential [114]. Serum CRPM was also measured in 781 patients with radiographic and painful KOA from the clinical intervention trial testing the efficacy of the bone-resorption inhibitor, oral salmon calcitonin (SMC) and 658 patients with RA [48]. Overall, CRPM levels were lower in OA compared to RA patients, however with 31% of OA patients exhibiting comparable levels to that of RA. CRPM was also predictive of the development of contralateral KOA over a two-year period in a case-control subset of 152 subject with no OA at baseline. CRPM was suggested as a marker of early identification of OA with an inflammatory phenotypic profile [48].

4.4. C1M

Inflammation and increased proteolytic activity in OA result in increased turnover of type I and III collagens of the ECM [115]. MMP-mediated degradation of type I collagen gives rise to the C-terminal neoepitope N-KDGVRG^{764} as a result of cleavage between ^{764}Gly and ^{765}Leu (accession no. P02452) on the α1(I) chain. The neoepitope is assessed by the competitive C1M ELISA (Nordic Bioscience, Herlev, Denmark) measured on IDS-i10 (IDS, Boldon, United Kingdom) [49]. Contrary to CTX-I, C1M is a soft tissue-specific metabolite generated by MMP-2, −9, and −13, and is not a result of bone matrix turnover by cathepsin K [49,116]. In fact, the neoepitope of C1M is destroyed by cathepsin K, the main protease in osteoclasts (bone-resorptive cells), but is rather found in soft tissues including the interstitial matrix and synovium [49]. C1M and CTX-I are thus examples of degradation markers derived from the same protein that are interpreted in very different pathobiological contexts. C1M was originally developed for the assessment of liver fibrosis but has since been associated with inflammation in RA and OA [49]. Serum levels of hsCRP, CRPM, C1M and metabolites of MMP-mediated degradation of type III collagen (C3M) were measured in 342 symptomatic KOA patients from the C4Pain and HTF-Synovitis studies [50]. C1M and C3M were elevated in subgroups with high levels of CRPM and hsCRP, and C1M associated with peripheral inflammation whereas C3M was associated with focal inflammation. Serum C1M and hsCRP have also been measured in 261 RA patients from the placebo arms of the phase III clinical trials of fostamatinib, OSKIRA 1–3 [115]. High levels of C1M and hsCRP could each predict radiographic progression and were both potential candidates for enrichment of radiographic progressors for clinical trials of RA [115]. C1M and IL-6 were quantified in a study of 104 patients with end-stage KOA, defined as being scheduled for TKR surgery, to explore associations with synovitis and pain [117]. C1M indicated a positive (not statistically significant) association with neuropathic pain and synovitis in the periligamentous subregion.

4.5. Type III collagen and C3M

While type I and II collagens are the main components of bone and cartilage, respectively, type III collagen is an important constituent of interstitial matrices in the skin, spleen, vessels, lungs, and the liver [15]. It is one of the main structural proteins of entheses and the synovial membrane, and its expression increases during synovial thickening [51,52]. It is mostly secreted by fibroblasts and is involved in numerous pathologies with inflammatory components such as alcoholic hepatitis, cirrhosis, and lung injury [118,119]. Transcribed from the *COL3A1* gene, the fibrillar collagen is a homotrimer of α1 (III) chains and is often embedded in mixed fibrils with type I collagen [15]. Type III collagen is synthesized as a procollagen with N- and C-terminal propeptides. Upon cleavage of the propeptides by N- and C-proteinases, mature collagen is integrated into the ECM [53]. As observed during inflammation, increased proteolytic activity results in the release of degradation fragments of type III collagen [52]. Type III collagen is therefore a protein of interest to reflect tissue-specific inflammatory processes. MMP-9-mediated cleavage between ^{609}Gly and ^{610}Lys (accession no. P02461–1) generates the N-terminal neoepitope ^{610}KNGETGPQGP-C. The competitive C3M ELISA (Nordic Bioscience, Herlev, Denmark) measured on IDS-i10 (IDS, Boldon, United Kingdom) targets the neoepitope [53]. The marker has been reported to reflect soft-tissue turnover associated with inflammation and serum levels of C3M are elevated in inflammatory OA subgroups [50–52]. As type III collagen is the main ECM protein of the synovium, increased tissue turnover reflected by C3M has been associated with inflammation of the synovial membrane in OA. C3M may therefore serve as a more tissue- and pathology-specific marker of inflammation as opposed to hsCRP [13,51].

Serum C3M was measured in 146 KOA patients from the New York Inflammation cohort to investigate the association between patient-reported outcomes (PROs) and C3M in patients with high or low levels of serum CRPM [120]. This included the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), which is the most widely used questionnaire for assessment of pain and functioning in hip and KOA [121]. C3M positively correlated with worsening of WOMAC pain and function in patients with low levels of CRPM. In patients with elevated CRPM, a negative association was demonstrated which was in line with previous findings [120,122]. Serum C2M and C3M were measured in 103 AS patients, 47 RA patients, and 56 healthy controls [51]. C3M was higher in both disease groups compared to controls. High levels of C2M and C3M could predict 80% of the disease progressors of AS over a two-year period and 61% of the non-progressors. This supported the predictive utility of cartilage and connective tissue turnover biochemical markers [51].

5. Other biochemical markers of osteoarthritis

This review focused on a panel of validated biochemical markers and assays measured in serum or urine for the discovery of OA endotypes in IMI-APPROACH [2,10]. U-αCTX-I, sCTX-I, and N-MID were the most important for the structural damage endotype, whereas hsCRP and C1M were the most predictive

of the inflammatory endotype [10]. However, an array of other markers not covered by this review, as well as other specimens and assays of the same markers are also utilized in OA. COMP was measured in serum with the sandwich COMP ELISA from IDS in IMI-APPROACH but can also be quantified in both serum and SF with the sandwich COMP BioVendor ELISA (BioVendor, Karásek, Czech Republic). In contrast to the IDS assay, the antibody targeting sites of the BioVendor assay are published and are located within the coiled-coil and the EGF-like domains of COMP [74].

CPII is a marker of cartilage formation but contrary to PRO-C2, which is derived from the N-terminal propeptide of type II collagen, is derived from the C-terminal propeptide (Table 2). Serum CPII has been found to be elevated in RA and lower in OA compared to controls [123]. While uCTX-II is derived from the ¼ degradation fragment of type II collagen, CIINE is generated by MMP-13 cleavage on the ¾ fragment and urinary levels of CIINE have been found to be elevated in OA compared to healthy controls [20,124]. As an alternative to C2M, the cartilage turnover marker C2C has been measured in urine and shown to predict the risk of KOA over a 12-year period compared to subjects without radiographic KOA [125]. C2C is generated through MMP-13-mediated cleavage on the C-terminus of the ¾ degradation fragment of type II collagen [126]. In serum, C2C correlated with increased collagen network disruption in arthritic joints with KL grades 1–4 [127].

PINP is the recommended bone formation marker by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [134]. Derived from the N-terminal propeptide of type II collagen, serum PINP has been found to predict KOA progression and progressive osteophytosis (formation of bone spurs) [128]. In contrast to CTX-I, which is derived from the C-terminal telopeptide of type I collagen, NTX-I is derived from the N-terminal telopeptide, and both CTX-I and NTX-I result from osteoclastic hydrolysis by cathepsin K [129]. Both markers are extensively used to assess bone resorption as a response to anti-resorptive treatments in clinical studies and have both been found to be elevated in urine in women with progressive OA (defined either as presence of osteophytes or JSN) compared to healthy controls [36]. Both serum and urine CTX-I are associated with higher levels of variability than that of NTX-I. However, lower variability of a marker is not necessarily better, and the clinical usefulness should be considered [129]. In fact, sCTX-I has been suggested to increase more than urinary NTX-I as a result of bone resorption [135].

Several inflammatory markers are measured in OA. Serum levels of IL-6 are able to predict development of radiographic KOA over a 10-year period and have been associated with cartilage loss in older adults [130,136]. Toll-like receptor 4 (TLR-4) senses DAMPs, including ECM degradation fragments, and has been connected to RA and OA [131]. IL-1β has been measured in SF of 69 KOA patients from the POP study and was associated with radiographic severity of OA, reflected by X-ray osteophyte scores [132].

Recently, a study aimed to identify serological markers for prediction of radiographic and pain progression over 48 months in OA [133]. A panel of 15 proteomic markers was measured in serum and was able to distinguish between progressors and non-progressors at a higher rate than the current 'best-in-class' marker, uCTX-II. Vitamin D binding protein (VTDB), cartilage acidic protein 1 (CRAC1), and complement C1r subcomponent (C1R) were the most important markers for the predictions. The 15 proteomic markers reached an area under the receiver operating characteristic curve (AUC) of 73% whereas uCTX-II alone reached 58%. The study highlighted the utility of a panel of serological markers for the identification of subjects at higher risk of KOA progression, defined by both structural and pain outcomes [133].

6. Conclusion

OA is a highly heterogeneous disease and patients are still to this day treated with a 'one-size-fits-all' approach, likely contributing to the lack of clinically approved DMOADs. The OA patient population may demonstrate similar clinical manifestations but have differing underlying pathobiological drivers of the disease. These can be uncovered with a set of molecular

Table 2. Examples of other biochemical markers routinely measured in osteoarthritis (OA).

Marker	Type	Molecular origin	Association with OA	Ref.
CPII	Cartilage formation	Type IIB C-terminal propeptide of type II collagen.	Low serum levels in OA compared to controls.	[123]
CIINE	Cartilage degradation	MMP-13 degradation of type II collagen ¾ fragment.	Elevated urinary levels in OA compared to controls.	[124]
C2C	Cartilage turnover	MMP-13 degradation of C-terminal type II collagen ¾ fragment.	12-year KOA risk prediction in urine. Correlates with increases collagen network disruption in KL 1–4 arthritic joints in serum.	[125–127]
PINP	Bone formation	N-terminal propeptide of type II collagen.	Serum levels predict KOA progression and progressive osteophytosis.	[128]
NTX-I	Bone resorption	Cathepsin K cleavage of type I collagen N-terminal telopeptide.	Elevated urinary levels in women with progressive OA compared to controls.	[36,129]
IL-6	Inflammation	Interleukin-6.	10-year radiographic KOA risk prediction in serum.	[130]
TLR-4	Inflammation	Toll-like receptor 4.	Increased expression in cartilage throughout OA progression.	[131]
IL-1β	Inflammation	Interleukin-1β.	Synovial fluid levels associated with radiographic OA severity.	[132]
VTDB	NA	Vitamin D binding protein.	Predictor of KOA progression in serum.	[133]
CRAC1	NA	Cartilage acidic protein 1.	Predictor of KOA progression in serum.	[133]
C1R	NA	Complement C1r subcomponent.	Predictor of KOA progression in serum.	[133]

C1R, complement C1r subcomponent; C2C, C-terminal neoepitope on type II collagen ¾ degradation fragment; CIINE, epitope on type II collagen ¾ degradation fragment; CPII, type IIB C-terminal propeptide of type II collagen; CRAC1, cartilage acidic protein 1; IL-6, interleukin-6; KL, Kellgren-Lawrence grade; KOA, knee osteoarthritis; NA, not applicable; NTX-I, N-terminal telopeptide of type I collagen; PINP, N-terminal propeptide of type II collagen; TLR-4, toll-like receptor 4; VTDB, vitamin D binding protein.

markers that reflect cartilage degradation and formation, bone formation and resorption, and connective tissue inflammation, all of which are essential pathologies of OA. A panel of 16 validated markers were quantified in IMI-APPROACH that revealed patients with endotypes driven by structural damage, inflammation, and low tissue turnover that differed on structural and pain outcomes. Reflected by tissue- and disease-specific biochemical markers, discovery of such patient subgroups may facilitate the identification of the right patient population for treatment with the right therapeutic agent at the right time for future clinical trials in OA. This may bring us closer to better, personalized treatment options for OA patients that improve how they function, feel, and survive.

7. Expert opinion

Traditionally, markers of cartilage turnover have been the focal point of biomarker discovery and development in OA. Biochemical markers capable of accurately predicting structural progression and cartilage loss, captured in X-rays and magnetic resonance imaging, have been much sought after in the field and have been extensively researched. Recently, the OA field has changed from a primary focus on structural outcomes to an increased focus on PROs [8,137]. With the new development of the Food and Drug Administration (FDA) guidelines focusing on the need to show benefits on how patients feel, function, and survive, it is a prerequisite to demonstrate effects on PROs [138]. As such, DMOADs currently in development are unlikely to be clinically approved without improvement on structural outcomes as well as PROs [1].

As the field and the FDA guidelines are evolving, molecular markers from the whole arthritic joint, encompassing the innervated areas of the synovium and soft tissue and not just the cartilage, that are related to pain and function are needed. Such markers may predict disease progression and may be modulated to predict efficacy on PROs. Understanding the diagnostic and prognostic link between PROs of pain and serological markers and elucidating which treatable endotypes that may respond to a given therapy will pave the way for new drug development in OA. These considerations are in the back-drop of the phase II clinical trial FORWARD, which clearly demonstrated long-lasting effects of the cartilage anabolic drug, sprifermin on cartilage thickness but failed to show significant improvement on PROs [139]. We need to develop strategies to predict TJRs and worsening of PROs to effectively counteract this in OA.

Molecular markers have the potential to identify clinically relevant, treatable endotypes that, when matched with the right treatment option, may demonstrate efficacy in a future clinical intervention trial. When enriching for the right patient subpopulation based on their molecular endotype at screening for a given clinical intervention trial, it is important to consider the longitudinal stability of such biomarker-based endotypes within the clinical trial period. As tissue remodeling may change over time in the individual OA patients, this may also be reflected by their endotypic profile. To our knowledge, this has yet to investigated in the OA field. Preliminary results from our research show that a considerable proportion of OA patients keep the same endotype within a typical clinical trial period. While some transiently change endotype over time, they return to their original endotype, illustrating the robustness of biomarker-based endotyping.

While a clinical phenotype relates to the observable presentation of a disease, an endotype is specific for the molecular pathogenesis of the disease [140]. It is conceivable that molecular endotypes may overlap and a clinical phenotype may encompass several molecular endotypes [141]. As such, the clinical manifestation of endotypes of OA may not differ. In other words, many OA patients will ultimately experience debilitating pain and need TKR but the underlying molecular path to the clinical manifestations may differ. Molecular endotyping can inform how to target the different OA patients by uncovering their underlying, pathological drivers of the disease. Some OA patients may also show properties of several molecular endotypes with one endotype being predominant at a given point in time, such as exhibiting structural damage of bone tissue while simultaneously having an inflammatory component that may dominate at a later point in time. These considerations may be relevant for decision-making on enrollment of patients based on their molecular endotype at screening for a clinical intervention trial. In some cases, it may be most relevant to enroll and treat patients based on the underlying disease-driving endotype that is dominant at the time of screening, while it in other cases may be more relevant to rather deselect patients based on endotypic traits that are not targeted by the disease-modifying treatment in question.

The development path for true disease-modifying therapies in OA is still debated between sponsors and agencies [138]. One approach may be a FDA Subpart H-accelerated approval on PROs of pain, followed by assessment of rates and/or postponement of TJRs over a longer study period [138]. In light of the large Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) in which an IL-1β-inhibiting, anti-inflammatory drug prevented incidents of TJRs in subjects with elevated levels of hsCRP and previous history of myocardial infarction with a median follow-up time of 3.7 years, this seems more feasible than ever [142]. However, such clinical trial designs with longer study periods require large investments.

An increasing amount of attention has recently been directed toward the clinical effects of weight-loss therapies on PROs of pain in OA. Recently, several clinical trials of KOA patients suffering with obesity have commenced, including research studies testing the efficacy of semaglutide (ClinicalTrials.gov ID: NCT05064735) or physiotherapist-guided dietary and exercise programs (ClinicalTrials.gov ID: NCT04733053) on weight-loss and PROs. Obesity is associated with low-grade, chronic inflammation and an endotype driven by metabolic syndrome has previously been described [6]. This may indicate a clinically relevant and treatable endotype that, when paired with an optimal treatment option, may exhibit superior efficacy on PROs, including WOMAC pain. This clearly illustrates the future era of disease-modifying drug development in OA. Targeting treatable endotypes that first demonstrate efficacy on PROs that with the right clinical development path and strategy may translate into postponement of TJRs, all for the benefit of the OA patients.

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A-C Bay-Jensen, C S Thudium, and M A Karsdal are full-time employees and shareholders of Nordic Bioscience, a privately owned biotechnology company developing biomarkers for fibro-inflammatory diseases. C Ladel is a consultant and provides consultancy to Regenosine, TrialSpark, Charité, Curnova, and RheumaNederland. M Uebelhoer is a full-time employee of Artialis SA. J Larkin is the founder of SynOA Therapeutics. A Mobasheri is a senior advisor to the World Health Organization Collaborating Center for Public Health Aspects of Musculoskeletal Health and Aging and 'Collaborateur Scientifique de l'Université deLiège' at the Université de Liège, Belgium. They have also provided consultancy for Genacol, Sterifarma, Sanofi (Brazil), Pfizer Consumer Health, GSK Consumer Health, and Aché (Aché Laboratórios Farmacêuticos).

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Mobasheri A, Kapoor M, Ali SA, et al. The future of deep phenotyping in osteoarthritis: how can high throughput omics technologies advance our understanding of the cellular and molecular taxonomy of the disease? Osteoarthr Cartil Open. 2021;3(4):100144. doi: 10.1016/j.ocarto.2021.100144
2. van Helvoort EM, van Spil WE, Jansen MP, et al. Cohort profile: the applied public-private research enabling OsteoArthritis clinical headway (IMI-APPROACH) study: a 2-year, European, cohort study to describe, validate and predict phenotypes of osteoarthritis using clinical, imaging and biochemical mark. BMJ Open. 2020;10(7): e035101. doi: 10.1136/bmjopen-2019-035101
   • **Cohort profile of IMI-APPROACH.**
3. Skou ST, Roos EM, Laursen MB, et al. Total knee replacement and non-surgical treatment of knee osteoarthritis: 2-year outcome from two parallel randomized controlled trials. Osteoarthr Cartil. 2018;26 (9):1170–1180. doi: 10.1016/j.joca.2018.04.014
4. Bay-Jensen AC, Thudium CS, Gualillo O, et al. Biochemical marker discovery, testing and evaluation for facilitating OA drug discovery and development. Drug Discov Today. 2018;23(2):349–358. doi: 10.1016/j.drudis.2017.10.008
5. Bay-Jensen AC, Mobasheri A, Thudium CS, et al. Blood and urine biomarkers in osteoarthritis – an update on cartilage associated type II collagen and aggrecan markers. Curr Opin Rheumatol. 2022;34(1):54–60. doi: 10.1097/BOR.0000000000000845
6. Luo Y, Samuels J, Krasnokutsky S, et al. A low cartilage formation and repair endotype predicts radiographic progression of symptomatic knee osteoarthritis. J Orthop Traumatol. 2021;22 (10):123–131. doi: 10.1186/s10195-021-00572-0
   • **Low cartilage repair OA endotype.**
7. Agustí A. Phenotypes and disease characterization in chronic obstructive pulmonary disease: toward the extinction of phenotypes? Ann Am Thorac Soc. 2013;10(SUPPL):125–130. doi: 10.1513/AnnalsATS.201303-055AW
8. Karsdal MA, Tambiah J, Hochberg MC, et al. Reflections from the 2021 OARSI clinical trial symposium: considerations for understanding biomarker assessments in osteoarthritis drug development - should future studies focus on disease activity, rather than status? Osteoarthr Cartil Open. 2022;4(3):100262. doi: 10.1016/j.ocarto.2022.100262
9. Kraus VB, Collins JE, Hargrove D, et al. Predictive validity of biochemical biomarkers in knee osteoarthritis: data from the FNIH OA biomarkers consortium Virginia. Ann Rheum Dis. 2017;76 (1):186–195. doi: 10.1136/annrheumdis-2016-209252
10. Angelini F, Widera P, Mobasheri A, et al. Osteoarthritis endotype discovery via clustering of biochemical marker data. Ann Rheum Dis. 2022;1–10. doi: 10.1016/j.joca.2022.02.143
    •• **Structural damage, inflammatory, and low tissue turnover endotypes discovered in IMI-APPROACH at baseline.**
11. Werdyani S, Liu M, Zhang H, et al. Endotypes of primary osteoarthritis identified by plasma metabolomics analysis. Rheumatology. 2021;60(6):2735–2744. doi: 10.1093/rheumatology/keaa693
    • **Endotypes of muscle weakness, arginine deficit, and low inflammation discovered with metabolomics.**
12. Luo Y, He Y, Reker D, et al. A novel high sensitivity type II collagen blood-based biomarker, PRO-C2, for assessment of cartilage formation. Int J Mol Sci. 2018;19(11):3485. doi: 10.3390/ijms19113485
13. Bay-Jensen AC, Engstroem A, Sharma N, et al. Blood and urinary collagen markers in osteoarthritis: markers of tissue turnover and disease activity. Expert Rev Mol Diagn. 2020;20(1):57–68. doi: 10.1080/14737159.2020.1704257
14. Karsdal MA, Henriksen K, Bay-Jensen AC. Biochemical markers in osteoarthritis with lessons learned from osteoporosis. Clin Exp Rheumatol. 2019;37(5):73–87.
15. Gelse K, Pöschl E, Aigner T. Collagens—structure, function, and biosynthesis. Adv Drug Deliv Rev. 2003;55(12):1531–1546. doi: 10.1016/j.addr.2003.08.002
16. Frazer A, Bunning RAD, Thavarajah M, et al. Studies on type II collagen and aggrecan production in human articular chondrocytes in vitro and effects of transforming growth factor-β and interleukin-1β. Osteoarthr Cartil. 1994;2(4):235–245. doi: 10.1016/S1063-4584(05)80075-5
17. Li S, Prockop DJ, Helminen H, et al. Transgenic mice with targeted inactivation of the Col2a1 gene for collagen II develop a skeleton with membranous and periosteal bone but no endochondral bone. Genes Dev. 1995;9:2821–2830. doi: 10.1101/gad.9.22.2821
18. Bay-Jensen AC, Liu Q, Byrjalsen I, et al. Enzyme-linked immunosorbent assay (ELISAs) for metalloproteinase derived type II collagen neoepitope, CIIM-Increased serum CIIM in subjects with severe radiographic osteoarthritis. Clin Biochem. 2011;44(5–6):423–429. doi: 10.1016/j.clinbiochem.2011.01.001
19. Zhen EY, Brittain IJ, Laska DA, et al. Characterization of metalloprotease cleavage products of human articular cartilage. Arthritis Rheum. 2008;58(8):2420–2431. doi: 10.1002/art.23654
20. Christgau S, Garnero P, Fledelius C, et al. Collagen type II C-telopeptide fragments as an index of cartilage degradation. Bone. 2001;29(3):209–215. doi: 10.1016/S8756-3282(01)00504-X
21. van Spil WE, Drossaers-Bakker KW, Lafeber FPJG. Associations of CTX-II with biochemical markers of bone turnover raise questions on its tissue origin: data from CHECK, a cohort study of early osteoarthritis. Ann Rheum Dis. 2013;72(1):29–36. doi: 10.1136/annrheumdis-2011-201177
22. Elsaid KA, Chichester CO. Review: collagen markers in early arthritic diseases. Clin Chim Acta. 2006;365(1–2):68–77. doi: 10.1016/j.cca.2005.09.020

23. Hick AC, Malaise M, Loeuille D, et al. Cartilage biomarkers Coll2-1 and Coll2-1NO2 are associated with knee OA MRI features and are helpful in identifying patients at risk of disease worsening. Cartilage. 2021;13(Suppl 1):1637S–1647S. doi: 10.1177/19476035211021892
24. Deberg M, Labasse A, Christgau S, et al. New serum biochemical markers (Coll 2-1 and Coll 2-1 NO2) for studying oxidative-related type II collagen network degradation in patients with osteoarthritis and rheumatoid arthritis. Osteoarthr Cartil. 2005;13(3):258–265. doi: 10.1016/j.joca.2004.12.002
25. Deberg MA, Labasse AH, Collette J, et al. One-year increase of Coll 2-1, a new marker of type II collagen degradation, in urine is highly predictive of radiological OA progression. Osteoarthr Cartil. 2005;13(12):1059–1065. doi: 10.1016/j.joca.2005.06.014
26. Deberg M, Dubuc JE, Labasse A, et al. One-year follow-up of Coll2-1, Coll2-1NO2 and myeloperoxydase serum levels in osteoarthritis patients after hip or knee replacement. Ann Rheum Dis. 2008;67(2):168–174. doi: 10.1136/ard.2007.073452
27. He Y, Siebuhr AS, Brandt-Hansen NU, et al. Type X collagen levels are elevated in serum from human osteoarthritis patients and associated with biomarkers of cartilage degradation and inflammation. BMC Musculoskelet Disord. 2014;15(1):309. doi: 10.1186/1471-2474-15-309
28. Hoyland JA, Thomas JT, Donn R, et al. Distribution of type X collagen mRNA in normal and osteoarthritic human cartilage. Bone Miner. 1991;15(2):151–164. doi: 10.1016/0169-6009(91)90005-K
29. Bay-Jensen AC, Reker D, Kjelgaard-Petersen CF, et al. Osteoarthritis year in review 2015: soluble biomarkers and the BIPED criteria. Osteoarthr Cartil. 2016;24(1):9–20. doi: 10.1016/j.joca.2015.10.014
30. Kluzek S, Bay-Jensen AC, Judge A, et al. Serum cartilage oligomeric matrix protein and development of radiographic and painful knee osteoarthritis. A community-based cohort of middle-aged women. Biomarkers. 2015;20(8):557–564. doi: 10.3109/1354750X.2015.1105498
31. Pratta MA, Su JL, Leesnitzer MA, et al. Development and characterization of a highly specific and sensitive sandwich ELISA for detection of aggrecanase-generated aggrecan fragments. Osteoarthr Cartil. 2006;14(7):702–713. doi: 10.1016/j.joca.2006.01.012
32. Larsson S, Lohmander LS, Struglics A. An ARGS-aggrecan assay for analysis in blood and synovial fluid. Osteoarthr Cartil. 2014;22(2):242–249. doi: 10.1016/j.joca.2013.12.010
33. van der Aar E, Deckx H, Dupont S, et al. Safety, pharmacokinetics, and pharmacodynamics of the ADAMTS-5 inhibitor GLPG1972/S201086 in healthy volunteers and participants with osteoarthritis of the knee or hip. Clin Pharmacol Drug Dev. 2022;11(1):112–122. doi: 10.1002/cpdd.1042
34. Germaschewski FM, Matheny CJ, Larkin J, et al. Quantitation of ARGS aggrecan fragments in synovial fluid, serum and urine from osteoarthritis patients. Osteoarthr Cartil. 2014;22(5):690–697. doi: 10.1016/j.joca.2014.02.930
35. Fledelius C, Johnsen AH, Cloos PAC, et al. Characterization of urinary degradation products derived from type I collagen. Identification of a β-isomerized ASP-GLY sequence within the C-terminal telopeptide (α1) region. J Biol Chem. 1997;272(15):9755–9763. doi: 10.1074/jbc.272.15.9755
36. Bettica P, Cline G, Hart DJ, et al. Evidence for increased bone resorption in patients with progressive knee osteoarthritis: longitudinal results from the chingford study. Arthritis Rheum. 2002;46(12):3178–3184. doi: 10.1002/art.10630
37. Bihlet AR, Byrjalsen I, Bay-Jensen AC, et al. Associations between biomarkers of bone and cartilage turnover, gender, pain categories and radiographic severity in knee osteoarthritis. Arthritis Res Ther. 2019;21(1):203. doi: 10.1186/s13075-019-1987-7
38. Huebner JL, Bay-Jensen AC, Huffman KM, et al. Alpha C-telopeptide of type I collagen is associated with subchondral bone turnover and predicts progression of joint space narrowing and osteophytes in osteoarthritis. Arthritis Rheumat. 2014;66(9):2440–2449. doi: 10.1002/art.38739
39. Cloos PAC, Lyubimova N, Solberg H, et al. An immunoassay for measuring fragments of newly synthesized collagen type I produced during metastatic invasion of bone. Clin Lab. 2004;50(5–6):279–289.
40. Rosenquist C, Qvist P, Bjarnason N, et al. Measurement of a more stable region of osteocalcin in serum by ELISA with two monoclonal antibodies. Clin Chem. 1995;41(10):1439–1445. doi: 10.1093/clinchem/41.10.1439
41. Hunter DJ, LaValley M, Li J, et al. Biochemical markers of bone turnover and their association with bone marrow lesions. Arthritis Res Ther. 2008;10(4):R102. doi: 10.1186/ar2494
42. Chichibu K, Matsuura T, Shichijo S, et al. Assay of serum hyaluronic acid in clinical application. Clin Chim Acta. 1989;181(3):317–323. doi: 10.1016/0009-8981(89)90237-4
43. Bruyere O, Collette J, Kothari M, et al. Osteoarthritis, magnetic resonance imaging, and biochemical markers: a one year prospective study. Ann Rheum Dis. 2006;65(8):1050–1054. doi: 10.1136/ard.2005.045914
44. Filková M, Senolt L, Braun M, et al. Serum hyaluronic acid as a potential marker with a predictive value for further radiographic progression of hand osteoarthritis. Osteoarthr Cartil. 2009;17(12):1615–1619. doi: 10.1016/j.joca.2009.06.002
45. Jin X, Beguerie JR, Zhang W, et al. Circulating C reactive protein in osteoarthritis: a systematic review and meta-analysis. Ann Rheum Dis. 2015;74(4):703–710. doi: 10.1136/annrheumdis-2013-204494
46. Stürmer T, Brenner H, Koenig W, et al. Severity and extent of osteoarthritis and low grade systemic inflammation as assessed by high sensitivity C reactive protein. Ann Rheum Dis. 2004;63(2):200–205. doi: 10.1136/ard.2003.007674
47. Skjøt-Arkil H, Schett G, Zhang C, et al. Investigation of two novel biochemical markers of inflammation, matrix metalloproteinase and cathepsin generated fragments of C-reactive protein, in patients with ankylosing spondylitis. Clin Exp Rheumatol. 2012;30(3):371–379.
48. Bay-Jensen AC, Bihlet A, Byrjalsen I, et al. Serum C-reactive protein metabolite (CRPM) is associated with incidence of contralateral knee osteoarthritis. Sci Rep. 2021;11(1):6583. doi: 10.1038/s41598-021-86064-x
49. Leeming DJ, He Y, Veidal SS, et al. A novel marker for assessment of liver matrix remodeling: an enzyme-linked immunosorbent assay (ELISA) detecting a MMP generated type I collagen neo-epitope (C1M). Biomarkers. 2011;16(7):616–628. doi: 10.3109/1354750X.2011.620628
50. Siebuhr AS, Petersen KK, Arendt-Nielsen L, et al. Identification and characterisation of osteoarthritis patients with inflammation derived tissue turnover. Osteoarthr Cartil. 2014;22(1):44–50. doi: 10.1016/j.joca.2013.10.020
51. Bay-Jensen AC, Wichuk S, Byrjalsen I, et al. Circulating protein fragments of cartilage and connective tissue degradation are diagnostic and prognostic markers of rheumatoid arthritis and ankylosing spondylitis. PLoS One. 2013;8(1):e54504. doi: 10.1371/journal.pone.0054504
52. Arendt-Nielsen L, Eskehave TN, Egsgaard LL, et al. Association between experimental pain biomarkers and serologic markers in patients with different degrees of painful knee osteoarthritis. Arthritis Rheumat. 2014;66(12):3317–3326. doi: 10.1002/art.38856
53. Barascuk N, Veidal SS, Larsen L, et al. A novel assay for extracellular matrix remodeling associated with liver fibrosis: an enzyme-linked immunosorbent assay (ELISA) for a MMP-9 proteolytically revealed neo-epitope of type III collagen. Clin Biochem. 2010;43(10):899–904. doi: 10.1016/j.clinbiochem.2010.03.012
54. Zhang W, Robertson WB, Zhao J, et al. Emerging trend in the pharmacotherapy of osteoarthritis. Front Endocrinol. 2019;10:431. doi: 10.3389/fendo.2019.00431
55. Bay-Jensen AC, Manginelli AA, Karsdal M, et al. Low levels of type II collagen formation (PRO-C2) are associated with response to sprifermin: a pre-defined, exploratory biomarker analysis from the FORWARD study. Osteoarthr Cartil. 2022;30(1):92–99. doi: 10.1016/j.joca.2021.10.008

•• **Stratification of OA patients by PRO-C2 reveals response to sprifermin.**

56. Wagner N, Rapp AE, Braun S, et al. Generation of matrix degradation products using an in vitro MMP cleavage assay. Int J Mol Sci. 2022;23(11):6245. doi: 10.3390/ijms23116245
57. Mort JS, Beaudry F, Théroux K, et al. Early cathepsin K degradation of type II collagen in vitro and in vivo in articular cartilage. Osteoarthr Cartil. 2016;24(8):1461–1469. doi: 10.1016/j.joca.2016.03.016
58. Tabassi N C-B, Desmarais S, Bay-Jensen AC, et al. The type II collagen fragments helix-II and CTX-II reveal different enzymatic pathways of human cartilage collagen degradation. Osteoarthr Cartil. 2008;16(10):1183–1191. doi: 10.1016/j.joca.2008.02.008
59. Garnero P, Sornay-Rendu E, Chapurlat R. The cartilage degradation marker, urinary CTX-II, is associated with the risk of incident total joint replacement in postmenopausal women. A 18 year evaluation of the OFELY prospective cohort. Osteoarthr Cartil. 2020;28 (4):468–474. doi: 10.1016/j.joca.2019.12.012
60. Hick AC, Fonck M, Costes B, et al. Serum levels of Coll2-1, a specific biomarker of cartilage degradation, are not affected by sampling conditions, circadian rhythm, and seasonality. Cartilage. 2021;13 (Suppl 1):540S–549S. doi: 10.1177/1947603519878489
61. Farrell AJ, Blake DR, Palmer RMJ, et al. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann Rheum Dis. 1992;51 (11):1219–1222. doi: 10.1136/ard.51.11.1219
62. Ramonda R, Lorenzin M, Modesti V, et al. Serological markers of erosive hand osteoarthritis. Eur J Intern Med. 2013;24(1):11–15. doi: 10.1016/j.ejim.2012.10.002
63. Kwan KM, Pang MKM, Zhou S, et al. Abnormal compartmentalization of cartilage matrix components in mice lacking collagen X: implications for function. J Cell Bio. 1997;136(2):459–471. doi: 10.1083/jcb.136.2.459
64. Luo Y, Sinkeviciute D, He Y, et al. The minor collagens in articular cartilage. Protein Cell. 2017;8(8):560–572. doi: 10.1007/s13238-017-0377-7
65. Kwan APL, Cummings CE, Chapman JA, et al. Macromolecular organization of chicken type X collagen in vitro. J Cell Bio. 1991;114(3):597–604. doi: 10.1083/jcb.114.3.597
66. Shen G. The role of type X collagen in facilitating and regulating endochondral ossification of articular cartilage. Orthod Craniofacial Res. 2005;8(1):11–17. doi: 10.1111/j.1601-6343.2004.00308.x
67. Eyre DR. The collagens of articular cartilage. Semin Arthritis Rheum. 1991;21(3 Supp. 2):2–11. doi: 10.1016/0049-0172(91)90035-X
68. Živanović S, Rackov LP, Vojvodić D, et al. Human cartilage glycoprotein 39-biomarker of joint damage in knee osteoarthritis. Int Orthop. 2009;33(4):1165–1170. doi: 10.1007/s00264-009-0747-8
69. Rhee DK, Marcelino J, Baker M, et al. The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. J Clin Invest. 2005;115(3):622–631. doi: 10.1172/JCI200522263
70. Hedbom E, Antonsson P, Hjerpe A, et al. Cartilage matrix proteins: An acidic oligomeric protein (COMP) detected only in cartilage. J Biol Chem. 1992;267(9):6132–6136. doi: 10.1016/S0021-9258(18)42671-3
71. Acharya C, Yik JHN, Kishore A, et al. Cartilage oligomeric matrix protein and its binding partners in the cartilage extracellular matrix: interaction, regulation and role in chondrogenesis. Matrix Biol. 2014;37:102–111. doi: 10.1016/j.matbio.2014.06.001
72. Maly K, Sastre EA, Farrell E, et al. COMP and TSP-4: functional roles in articular cartilage and relevance in osteoarthritis. Int J Mol Sci. 2021;22(5):2242. doi: 10.3390/ijms22052242
73. Verma P, Dalal K. Serum cartilage oligomeric matrix protein (COMP) in knee osteoarthritis: A novel diagnostic and prognostic biomarker. J Orthop Res. 2013;31(7):999–1006. doi: 10.1002/jor.22324
74. Struglics A, Larsson S, Pramhed A, et al. Changes in synovial fluid and serum concentrations of cartilage oligomeric matrix protein over 5 years after anterior cruciate ligament rupture: an exploratory analysis in the KANON trial. Osteoarthr Cartil. 2018;26 (10):1351–1358. doi: 10.1016/j.joca.2018.06.005
75. Roughley PJ. The structure and function of cartilage proteoglycans. Eur Cells Mater. 2006;12:92–101. doi: 10.22203/eCM.v012a11
76. Zhang W, Ouyang H, Dass CR, et al. Current research on pharmacologic and regenerative therapies for osteoarthritis. Bone Res. 2016;4(December 2015). doi: 10.1038/boneres.2015.40
77. Kiani C, Chen L, Wu YJ, et al. Structure and function of aggrecan. Cell Res. 2002;12(1):19–32. doi: 10.1038/sj.cr.7290106
78. Durigova M, Nagase H, Mort JS, et al. Mmps are less efficient than ADAMTS5 in cleaving aggrecan core protein. Matrix Biol. 2011;30 (2):145–153. doi: 10.1016/j.matbio.2010.10.007
79. Tortorella M, Pratta M, Liu RQ, et al. The thrombospondin motif of aggrecanase-1 (ADAMTS-4) is critical for aggrecan substrate recognition and cleavage. J Biol Chem. 2000;275(33):25791–25797. doi: 10.1074/jbc.M001065200
80. Tortorella MD, Liu RQ, Burn T, et al. Characterization of human aggrecanase 2 (ADAM-TS5): substrate specificity studies and comparison with aggrecanase 1 (ADAM-TS4). Matrix Biol. 2002;21 (6):499–511. doi: 10.1016/S0945-053X(02)00069-0
81. Durigova M, Roughley PJ, Mort JS. Mechanism of proteoglycan aggregate degradation in cartilage stimulated with oncostatin M. Osteoarthr Cartil. 2008;16(1):98–104. doi: 10.1016/j.joca.2007.05.002
82. Bjerre-Bastos JJ, Nielsen HB, Andersen JR, et al. Evaluation of serum ARGS neoepitope as an osteoarthritis biomarker using a standardized model for exercise-induced cartilage extra cellular matrix turnover. Osteoarthr Cartil Open. 2020;2(2):100060. doi: 10.1016/j.ocarto.2020.100060
83. Guangyi L, Y J, G J, et al. Subchondral bone in osteoarthritis: insight into risk factors and microstructural changes. Arthritis Res Ther. 2013;15(6):223. doi: 10.1186/ar4405
84. Zhu X, Chan YT, Yung PSH, et al. Subchondral Bone Remodeling: A Therapeutic Target for Osteoarthritis. Front Cell Dev Biol. 2021;8:607764. doi: 10.3389/fcell.2020.607764
85. Florencio-Silva R, Sasso GR da S, Sasso-Cerri E, et al. Biology of bone tissue: structure, function, and factors that influence bone cells. Biomed Res Int. 2015;2015:421746. doi: 10.1155/2015/421746
86. Viguet-Carrin S, Garnero P, Delmas PD. The role of collagen in bone strength. Osteoporos Int. 2006;17(3):319–336. doi: 10.1007/s00198-005-2035-9
87. Ghosh AK. Factors involved in the regulation of type I collagen gene expression: implication in fibrosis. Exp Biol Med (Maywood). 2002 May 29;227(5):301–314.
88. Yamauchi M, Sricholpech M. Lysine post-translational modifications of collagen. Scott I, editor. Essays Biochem. 2012 May 25;52:113–133. doi: 10.1042/bse0520113
89. Leeming DJ, Alexandersen P, Karsdal MA, et al. An update on biomarkers of bone turnover and their utility in biomedical research and clinical practice. Eur J Clin Pharmacol. 2006;62 (10):781–792. doi: 10.1007/s00228-006-0174-3
90. Schlemmer A, Hassager C. Acute fasting diminishes the circadian rhythm of biochemical markers of bone resorption. Eur J Endocrinol. 1999;140(4):332–337. doi: 10.1530/eje.0.1400332
91. Chubb SAP. Measurement of C-terminal telopeptide of type I collagen (CTX) in serum. Clin Biochem. 2012;45(12):928–935. doi: 10.1016/j.clinbiochem.2012.03.035
92. Rosen HN, Moses AC, Garber J, et al. Serum CTX: a new marker of bone resorption that shows treatment effect more often than other markers because of low coefficient of variability and large changes with bisphosphonate therapy. Calcif Tissue Int. 2000;66(2):100–103. doi: 10.1007/PL00005830
93. Chavassieux P, Portero-Muzy N, Roux JP, et al. Are biochemical markers of bone turnover representative of bone histomorphometry in 370 postmenopausal women? J Clin Endocrinol Metab. 2015;100(12):4662–4668. doi: 10.1210/jc.2015-2957
94. Byrjalsen I, Leeming DJ, Qvist P, et al. Bone turnover and bone collagen maturation in osteoporosis: effects of antiresorptive therapies. Osteoporos Int. 2008;19(3):339–348. doi: 10.1007/s00198-007-0462-5
95. Al RO, Chow J, Lacombe J, et al. Proprotein convertase furin regulates osteocalcin and bone endocrine function. J Clin Invest. 2017;127(11):4104–4117. doi: 10.1172/JCI93437

96. Moser SC, van der Eerden BCJ. Osteocalcin — a versatile bone-derived hormone. Front Endocrinol. 2019;9:794. doi: 10.3389/fendo.2018.00794
97. Gundberg CM, Lian JB, Booth SL. Vitamin K-Dependent Carboxylation of Osteocalcin: Friend or Foe? Adv Nutr. 2012;3(2):149–157. doi: 10.3945/an.112.001834
98. Rehder DS, Gundberg CM, Booth SL, et al. Gamma-carboxylation and Fragmentation of osteocalcin in human serum defined by Mass spectrometry. Mol Cell Proteomics. 2015;14(6):1546–1555. doi: 10.1074/mcp.M114.047621
99. Ravn P, Clemmesen B, Christiansen C. Biochemical markers can predict the response in bone mass during alendronate treatment in early postmenopausal women. Bone. 1999;24(3):237–244. doi: 10.1016/S8756-3282(98)00183-5
100. Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. Ther Adv Musculoskelet Dis. 2013;5(2):77–94. doi: 10.1177/1759720X12467868
101. Attur M, Belitskaya-Lévy I, Oh C, et al. Increased interleukin-1β gene expression in peripheral blood leukocytes is associated with increased pain and predicts risk for progression of symptomatic knee osteoarthritis. Arthritis Rheum. 2011;63(7):1908–1917. doi: 10.1002/art.30360
102. Hosnijeh FS, Siebuhr AS, Uitterlinden AG, et al. Association between biomarkers of tissue inflammation and progression of osteoarthritis: evidence from the Rotterdam study cohort. Arthritis Res Ther. 2016;18(1):81. doi: 10.1186/s13075-016-0976-3
103. Kumavat R, Kumar V, Malhotra R, et al. Biomarkers of joint damage in osteoarthritis: Current status and future directions. Mediators Inflamm. 2021;2021:5574582. doi: 10.1155/2021/5574582
104. Goldring MB, Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol. 2011;23(5):471–478. doi: 10.1097/BOR.0b013e328349c2b1
105. Nicholls MA, Fierlinger A, Niazi F, et al. The disease-modifying effects of Hyaluronan in the Osteoarthritic disease State. Clin Med Insights Arthritis Musculoskelet Disord. 2017;10:1–10. doi: 10.1177/1179544117723611
106. Garantziotis S, Savani RC. Hyaluronan biology: a complex balancing act of structure, function, location and context. Matrix Biol. 2019;78–79:1–10. doi: 10.1016/j.matbio.2019.02.002
107. Knudson W, Ishizuka S, Terabe K, et al. The pericellular hyaluronan of articular chondrocytes. Matrix Biol. 2019;78–79:32–46. doi: 10.1016/j.matbio.2018.02.005
108. Hargittai I, Hargittai M. Molecular structure of hyaluronan: an introduction. Struct Chem. 2008;19(5):697–717. doi: 10.1007/s11224-008-9370-3
109. Petrey AC, de la Motte CA. Hyaluronan, a crucial regulator of inflammation. Front Immunol. 2014;5:101. doi: 10.3389/fimmu.2014.00101
110. Elliott AL, Kraus VB, Luta G, et al. Serum hyaluronan levels and radiographic knee and hip osteoarthritis in African Americans and caucasians in the Johnston County osteoarthritis project. Arthritis Rheum. 2005;52(1):105–111. doi: 10.1002/art.20724
111. Banait T, Wanjari A, Danade V, et al. Role of high-sensitivity C-reactive protein (hs-CRP) in non-communicable diseases: a review. Cureus. 2022;14(10):e30225. doi: 10.7759/cureus.30225
112. Rajab IM, Hart PC, Potempa LA. How C-Reactive protein structural isoforms with distinctive bioactivities affect disease progression. Front Immunol. 2020;11:2126. doi: 10.3389/fimmu.2020.02126
113. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol. 2018;9:754. doi: 10.3389/fimmu.2018.00754
114. Bay-Jensen AC, Platt A, Byrjalsen I, et al. Effect of tocilizumab combined with methotrexate on circulating biomarkers of synovium, cartilage, and bone in the LITHE study. Semin Arthritis Rheum. 2014;43(4):470–478. doi: 10.1016/j.semarthrit.2013.07.008
115. Bay-Jensen AC, Platt A, Jenkins MA, et al. Tissue metabolite of type I collagen, C1M, and CRP predicts structural progression of rheumatoid arthritis. BMC Rheumatol. 2019;3(1):3. doi: 10.1186/s41927-019-0052-0
116. Dragsbæk K, Neergaard JS, Hansen HB, et al. Matrix metalloproteinase mediated type I collagen degradation - an Independent risk factor for mortality in women. EBioMedicine. 2015;2(7):723–729. doi: 10.1016/j.ebiom.2015.04.017
117. Radojčić MR, Thudium CS, Henriksen K, et al. Biomarker of extracellular matrix remodelling C1M and proinflammatory cytokine interleukin 6 are related to synovitis and pain in end-stage knee osteoarthritis patients. Pain. 2017;158(7):1254–1263. doi: 10.1097/j.pain.0000000000000908
118. Meduri GU, Tolley EA, Chinn A, et al. Procollagen types I and III aminoterminal propeptide levels during acute respiratory distress syndrome and in response to methylprednisolone treatment. Am J Respir Crit Care Med. 1998;158(5):1432–1441. doi: 10.1164/ajrccm.158.5.9801107
119. Teare JP, Greenfield SM, Thompson RPH, et al. Comparison of serum procollagen III peptide concentrations and PGA index for assessment of hepatic fibrosis. Lancet. 1993;342(8876):895–898. doi: 10.1016/0140-6736(93)91946-J
120. Bay-Jensen AC, Attur M, Samuels J, et al. Pathological tissue formation and degradation biomarkers correlate with patient reported pain outcomes: an explorative study. Osteoarthr Cartil Open. 2023;5(3):100379. doi: 10.1016/j.ocarto.2023.100379
121. Terwee CB, Roorda LD, Knol DL, et al. Linking measurement error to minimal important change of patient-reported outcomes. J Clin Epidemiol. 2009;62(10):1062–1067. doi: 10.1016/j.jclinepi.2008.10.011
122. Yang X, Thudium CS, Bay-Jensen AC, et al. Association between markers of synovial inflammation, matrix turnover and symptoms in knee osteoarthritis: a cross-sectional study. Cells. 2021;10(7):1826. doi: 10.3390/cells10071826
123. Nelson F, Dahlberg L, Laverty S, et al. Evidence for altered synthesis of type II collagen in patients with osteoarthritis. J Clin Invest. 1998;102(12):2115–2125. doi: 10.1172/JCI4853
124. Takahashi T, Naito S, Onoda J, et al. Development of a novel immunoassay for the measurement of type II collagen neoepitope generated by collagenase cleavage. Clin Chim Acta. 2012;413(19–20):1591–1599. doi: 10.1016/j.cca.2012.03.022
125. Kuhi L, Tamm AE, Tamm AO, et al. Risk assessment of the progression of early knee osteoarthritis by collagen neoepitope C2C: a longitudinal study of an estonian middle-aged cohort. Diagnostics. 2021;11(7):1236. doi: 10.3390/diagnostics11071236
126. Robin Poole A, Ionescu M, Fitzcharles MA, et al. The assessment of cartilage degradation in vivo: development of an immunoassay for the measurement in body fluids of type II collagen cleaved by collagenases. J Immunol Methods. 2004;294(1):145–153. doi: 10.1016/j.jim.2004.09.005
127. King KB, Lindsey CT, Dunn TC, et al. A study of the relationship between molecular biomarkers of joint degeneration and the magnetic resonance-measured characteristics of cartilage in 16 symptomatic knees. Magn Reson Imaging. 2004;22(8):1117–1123. doi: 10.1016/j.mri.2004.08.001
128. Kumm J, Tamm A, Lintrop M, et al. Diagnostic and prognostic value of bone biomarkers in progressive knee osteoarthritis: a 6-year follow-up study in middle-aged subjects. Osteoarthr Cartil. 2013;21(6):815–822. doi: 10.1016/j.joca.2013.03.008
129. Herrmann M, Seibel M. The amino- and carboxyterminal cross-linked telopeptides of collagen type I, NTX-I and CTX-I: a comparative review. Clin Chim Acta. 2008;393(2):57–75. doi: 10.1016/j.cca.2008.03.020
130. Livshits G, Zhai G, Hart DJ, et al. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: the chingford study. Arthritis Rheum. 2009;60(7):2037–2045. doi: 10.1002/art.24598
131. Gómez R, Villalvilla A, Largo R, et al. TLR4 signalling in osteoarthritis — finding targets for candidate DMOADs. Nat Rev Rheumatol. 2015;11(3):159–170. doi: 10.1038/nrrheum.2014.209
132. Denoble AE, Huffman KM, Stabler TV, et al. Uric acid is a danger signal of increasing risk for osteoarthritis through inflammasome activation. Proc Natl Acad Sci U S A. 2011;108(5):2088–2093. doi: 10.1073/pnas.1012743108

133. Zhou K, Li YJ, Soderblom EJ, et al. A "best-in-class" systemic biomarker predictor of clinically relevant knee osteoarthritis structural and pain progression. Sci Adv. 2023;9(4):1–14. doi: 10.1126/sciadv.abq5095
•• **Panel of 15 proteomic markers was able to distinguish between progressors and non-progressors of OA with AUC of 73%.**
134. Gillett MJ, Vasikaran SD, Inderjeeth CA. The role of PINP in diagnosis and management of metabolic bone disease. Clin Biochem Rev. 2021;42(1):3–10. doi: 10.33176/AACB-20-0001
135. Vasikaran S, Eastell R, Bruyère O, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int. 2011;22 (2):391–420. doi: 10.1007/s00198-010-1501-1
136. Stannus O, Jones G, Cicuttini F, et al. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarthr Cartil. 2010;18 (11):1441–1447. doi: 10.1016/j.joca.2010.08.016
137. Karsdal M, Tambiah J, Felson D, et al. Reflections from the OARSI 2022 clinical trials symposium: the pain of OA—deconstruction of pain and patient-reported outcome measures for the benefit of patients and clinical trial design. Osteoarthr Cartil. 2023;31 (10):1293–1302. doi: 10.1016/j.joca.2023.06.006
•• **Considerations on increased focus on PROs in future clinical trial designs.**
138. Kraus VB, Simon LS, Katz JN, et al. Proposed study designs for approval based on a surrogate endpoint and a post-marketing confirmatory study under FDA's accelerated approval regulations for disease modifying osteoarthritis drugs. Osteoarthr Cartil. 2019;27(4):571–579. doi: 10.1016/j.joca.2018.11.002
139. Hochberg MC, Guermazi A, Guehring H, et al. Effect of intra-articular sprifermin vs placebo on femorotibial joint cartilage thickness in patients with osteoarthritis: the FORWARD randomized clinical trial. JAMA. 2019;322(14):1360–1370. doi: 10.1001/jama.2019.14735
140. Mobasheri A, Van Spil WE, Budd E, et al. Molecular taxonomy of osteoarthritis for patient stratification, disease management and drug development: biochemical markers associated with emerging clinical phenotypes and molecular endotypes. Curr Opin Rheumatol. 2019;31(1):80–89. doi: 10.1097/BOR.0000000000000567
141. Mobasheri A, Saarakkala S, Finnilä M, et al. Recent advances in understanding the phenotypes of osteoarthritis. F1000Res. 2019 Dec 12;8:2091.
142. Schieker M, Conaghan PG, Mindeholm L, et al. Effects of interleukin-1β inhibition on incident hip and knee replacement: exploratory analyses from a randomized, double-blind, placebo-controlled trial. Ann Intern Med. 2020;173(7):509–515. doi: 10.7326/M20-0527