

REVIEW

Biochemical markers of ongoing joint damage in rheumatoid arthritis - current and future applications, limitations and opportunities

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Abstract

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease associated with potentially debilitating joint inflammation, as well as altered skeletal bone metabolism and co-morbid conditions. Early diagnosis and aggressive treatment to control disease activity offers the highest likelihood of preserving function and preventing disability. Joint inflammation is characterized by synovitis, osteitis, and/or peri-articular osteopenia, often accompanied by development of subchondral bone erosions, as well as progressive joint space narrowing. Biochemical markers of joint cartilage and bone degradation may enable timely detection and assessment of ongoing joint damage, and their use in facilitating treatment strategies is under investigation. Early detection of joint damage may be assisted by the characterization of biochemical markers that identify patients whose joint damage is progressing rapidly and who are thus most in need of aggressive treatment, and that, alone or in combination, identify those individuals who are likely to respond best to a potential treatment, both in terms of limiting joint damage and relieving symptoms. The aims of this review are to describe currently available biochemical markers of joint metabolism in relation to the pathobiology of joint damage and systemic bone loss in RA; to assess the limitations of, and need for additional, novel biochemical markers in RA and other rheumatic diseases, and the strategies used for assay development; and to examine the feasibility of advancement of personalized health care using biochemical markers to select therapeutic agents to which a patient is most likely to respond.

Introduction

It is now widely acknowledged that early diagnosis of rheumatoid arthritis (RA) and aggressive treatment to control disease activity offer the highest likelihood of preserving function and preventing disability. RA is a chronic autoimmune disease characterized by poly-articular inflammation associated with synovitis, osteitis, and peri-articular osteopenia, often associated with erosion of subchondral bone and progressive joint space narrowing [1]. These features commonly lead to progressive joint damage, impaired function, and progressive disability [2-4]. Since roughly half of RA patients suffer disability within 10 years of diagnosis, it is critical to effectively treat the disease early to suppress inflammation and prevent destruction of bone and joint

cartilage [5,6]. Treatment is commonly determined by the extent or severity of disease activity, assessed by counting the number of swollen and tender joints, measuring patient-reported outcomes (for example, patient global quality of life assessment), and assaying acute phase responses, such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels.

While inflammation markers are clinically relevant, markers that reliably detect ongoing bone and cartilage damage are potentially more useful for timely monitoring of efficacy of treatment. Joint inflammation and damage are so far assessed by various imaging methods, including hand and feet radiographs, hand magnetic resonance imaging (MRI), and high-resolution ultrasound of specific joints [7]. Biochemical markers of bone and cartilage turnover are also receiving increasing attention in other conditions characterized by joint and/or skeletal inflammation and damage [8]. They may provide an additional and potentially more sensitive method of detection of active bone and cartilage degradation that is likely to lead to structural damage in RA [0].

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An evolving line of evidence suggests that markers associated with clinical response may not be the same biomarkers that predict risk of further joint damage, as verified by radiological progression, and thus different marker combinations are likely to be needed, with specific combinations selected for specific uses, potentially contributing to personalized health care [10-12]. Prognostic markers could be divided into at least two categories: those that predict clinical response in terms of signs and symptoms of RA, and those that predict and monitor joint damage, as detected cumulatively by various imaging modalities, and ultimately demonstrated by the clinical manifestations of deformity and dysfunction.

The aims of this review are to describe pathobiology that generates biochemical markers of joint metabolism/damage in RA, including application in assay development; to survey the current use of biochemical markers of joint damage in RA and some other relevant diseases; to discuss the limitations of some of these established biochemical markers, including the need for further research into serum and urine markers, to encourage optimal study designs and sample acquisition; to describe how biochemical markers may allow for diagnosis of patients who are experiencing joint damage with rapid degradation of bone and/or cartilage and thus are most in need of timely, aggressive treatment; and to discuss how advances in personalized health care, including mapping of a patient's specific biomarker and clinical profile, will allow treatment selection according to those that will be most likely to benefit.

Pathobiological processes associated with progression of joint damage, and biochemical markers of joint damage

The different cellular phenotypes involved in joints (osteoblasts, osteoclasts, chondrocytes, macrophages, B cells, T cells, fibroblast-like synoviocytes and macrophages) play distinct complex and inter-related roles in the pathogenesis and progression of RA joint damage [13]. Subchondral bone erosion, sclerosis and articular cartilage degradation leading to joint space narrowing are central features of joint damage in RA. Synovitis and osteitis associated with osteoclast activation and degradation of bone by matrix metalloproteinases (MMPs) and cathepsin K appear to precede erosions visualized by MRI or radiography [13-17]. Further, cytokines such as IL-1, TNF- α , IL-6, and IL-17 stimulate chondrocyte activation and expression of MMPs and aggrecanases, resulting in articular cartilage degradation. Thus, a wide range of processes contribute to the pathobiology of joint damage that eventually leads to joint failure [3,10,11,14] (Figure 1). A detailed discussion of the cellular interactions and molecular pathways involved in bone and cartilage

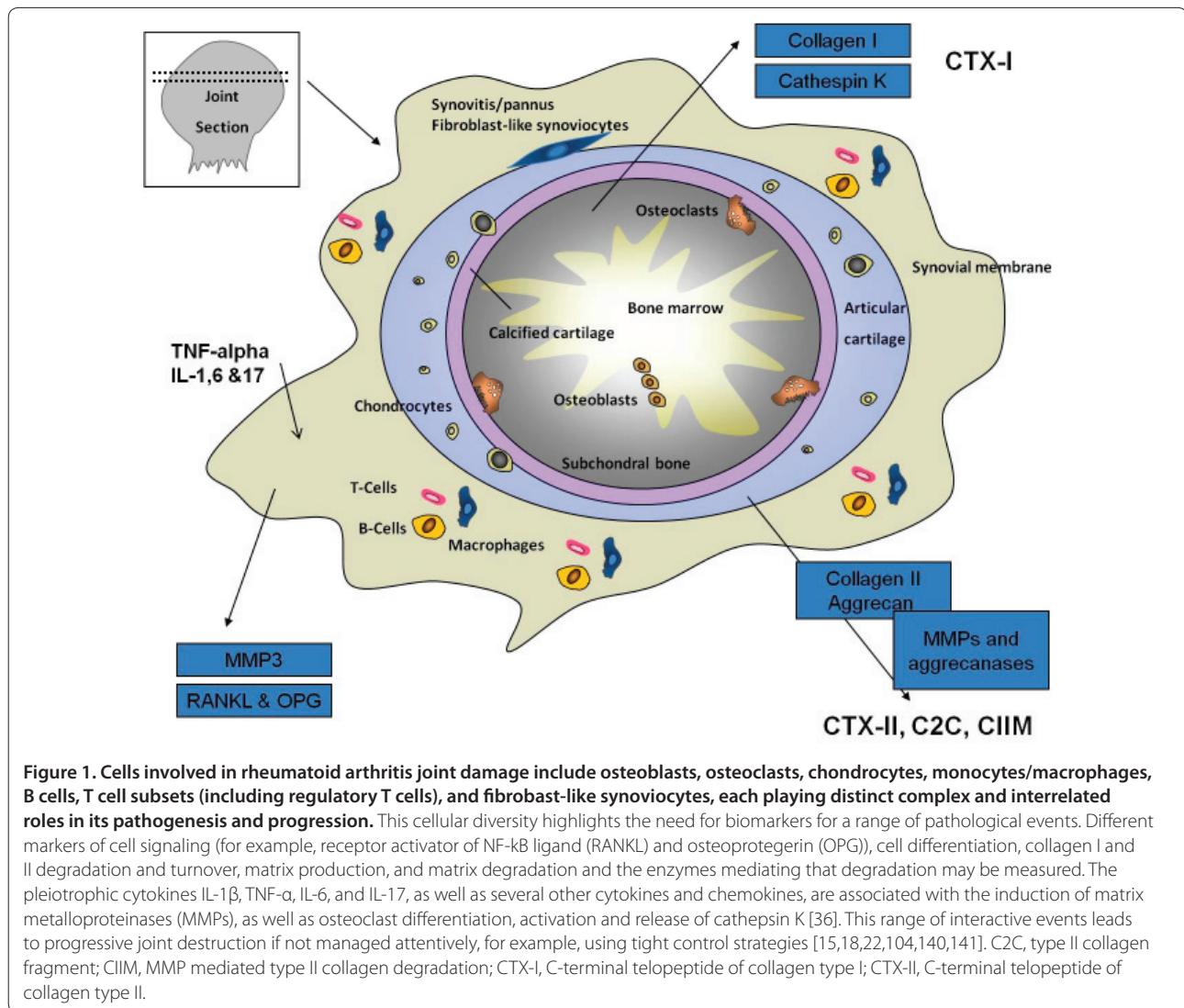
damage in RA is out of the scope of this review, and has been documented elsewhere [3,10,12-15,17,].

The generation of a tissue-specific biochemical marker is presented in Figure 2. The enzymes in the inflamed joint generate specific biochemical metabolic products from the extracellular matrix; the actual protein fragments of type II collagen and aggrecan that are the result of pathobiological actions in the joint are schematically presented in Figure 3. These specific products, which will be described, can be measured [7,18], facilitating assessment of various molecular, cellular and pathophysiological processes in the joint. Each marker may provide unique insights into the pathology of the disease by allowing quantitative information on the level of disease activity in terms of target tissue damage, on the action of cytokines driving disease progression, and on the specific mode of action and potential efficacy of therapeutic interventions. These features provide perspective for the characterization of the ongoing pathobiology using sets of biomarkers that potentially describe the type of damage occurring. A combination of specific biomarkers may thus provide more detailed and accurate information on joint pathology and ongoing structural damage than individual markers.

As described above, the biochemical markers may be useful by providing quantitative information on the pathology and unique processes associated with joint damage in RA. In addition, from a patient-management perspective, the biochemical markers may be useful for the diagnosis of patients with ongoing, active damage to, and degradation of, bone and/or cartilage, for early detection and monitoring of response to treatment, and for personalizing health care. Patients with such ongoing, active damage and degradation of bone and/or cartilage might be classified as 'rapid progressors' and are those most in need of effective treatment. They may be identified by detection of abnormal serum and/or urine levels of bone, synovium and/or cartilage degradation/turnover markers, prior to established, irreversible damage being identified using one or more imaging modalities. Early detection and monitoring of response to treatment potentially provides more rapid verification of control of joint damage than improvement in clinical symptoms or imaging changes, since a minimum of 6 months is needed to ascertain radiological progression, although newer MRI technologies may detect changes in osteitis and synovitis within several months. Health care can be personalized by identifying patients most likely to respond or not to a particular treatment, thus enabling informed selection of an appropriate therapeutic agent, as well as timely verification of its expected efficacy.

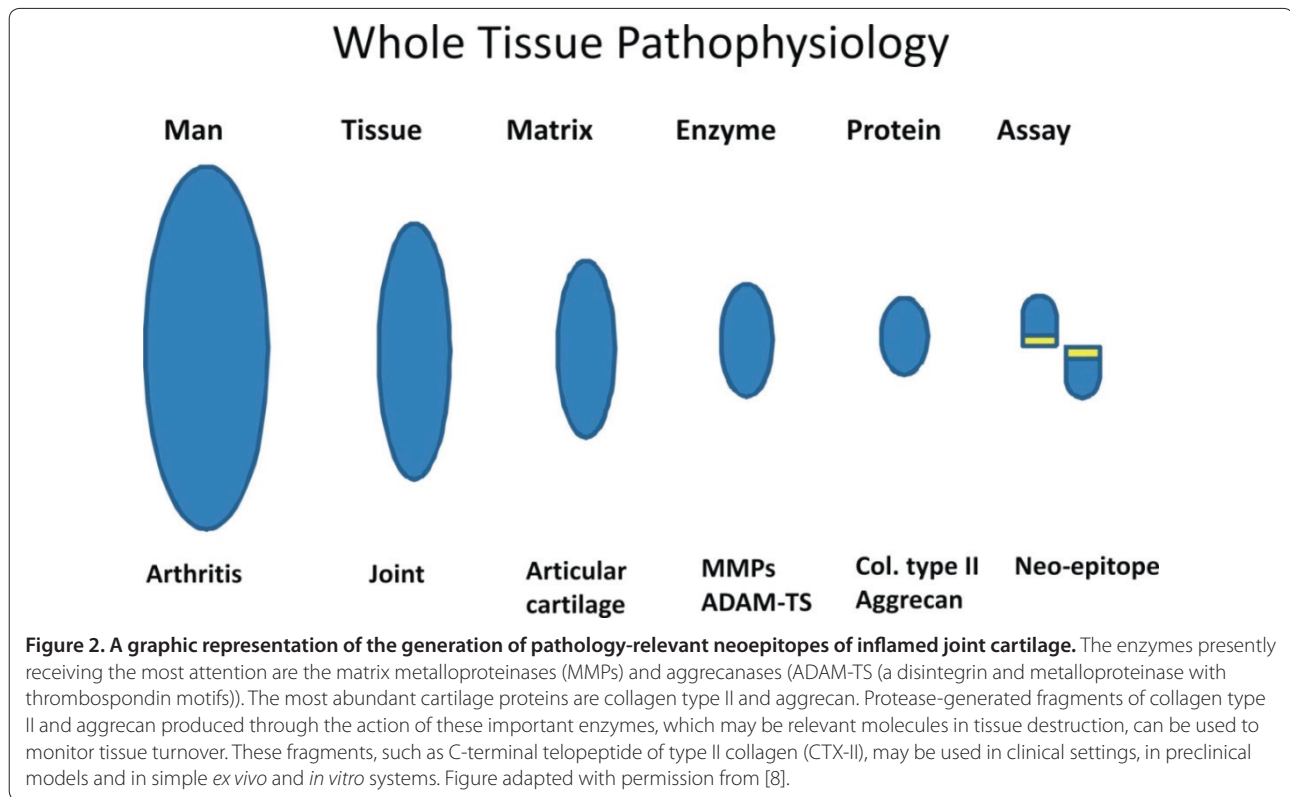
Biochemical markers as predictors of progression of structural damage

Biochemical markers of bone turnover have been used as standard practice to measure the effects of therapy in



osteoporosis (OP), a slowly progressing condition [17]. For example, early changes in CTX-I (C-terminal telopeptide of collagen type I), a marker of bone resorption, and changes in osteocalcin, a marker of bone formation, can be used to predict increases in bone mineral density [8]. In contrast to imaging techniques, biochemical markers of bone and cartilage turnover, measured in serum or urine samples collected during fasting or as second morning void specimens, show clinically relevant changes over a larger range compared with the imprecision of the assay (8% to 10%) [17]. A typical decrease of 50 to 80% or an increase of 100 to 200% is observed in the level of biochemical markers within days to weeks after initiation of treatment with anti-resorptive or anabolic drugs [17]. However, the respective change in bone mass ranges from 6 to 7% after 2 years of bisphosphonate therapy, which is a comparatively small increment relative to a precision error of 1 to 2% for bone mineral

density (BMD) measurements, as reviewed recently [17], and thus could be considered inferior to the dynamic range observed with biochemical markers. Because biochemical markers are sensitive and dynamic indicators of tissue turnover, they have the potential to provide information on treatment efficacy more rapidly than a variety of imaging methods (Figure 4) [16]. This use of biochemical markers of bone turnover has so far been validated in OP, as have markers of cartilage turnover in osteoarthritis (OA) [8,17]. In OP, a dynamic biochemical marker such as CTX-I changes within days of initiating treatment with anti-resorptives or the anti-receptor activator of NF- κ B ligand (RANKL) drug denosumab, whereas BMD improvements can only be reliably detected over 6 to 12 months. Similarly, urinary CTX-II (C-terminal telopeptide of collagen type II) levels have been shown to predict articular cartilage degradation [19] in OA. The same markers have been examined in RA



[20]. CTX-II as a marker of cartilage (collagen II) degradation and CTX-I as a marker of bone (collagen I) degradation in RA at 4 and 12 weeks have been demonstrated to predict joint damage (Tables 1, 2 and 3).

Research efforts are underway to apply these principles to proactive management of RA to enhance the detection and prevention of joint damage. X-ray imaging is the standard technique for diagnosis and measurement of efficacy of therapies aimed at inhibiting joint damage. Further efforts are ongoing to validate the use of MRI in this process, and even combine the use of biochemical markers and imaging modalities [7,21]. In RA, joint damage characterized by subchondral bone erosions and joint space narrowing, rather than BMD as in OP, is measured by various scoring methods applied to X-rays of hands and feet. However, X-ray imaging in both diseases is encumbered by rather low precision and could conceivably benefit from combination with biochemical marker analysis (Figure 4).

Need for biochemical markers to facilitate treatment decisions

Recently, three biological agents with novel mechanisms of action, rituximab, abatacept and tocilizumab, have become available for the treatment of RA, adding to the armamentarium already containing the approved TNF- α inhibitors (infliximab, etanercept, adalimumab,

certolizumab and golimumab). Clinical studies with these agents have demonstrated that they are effective in RA patients who did not respond to treatment with at least one disease-modifying antirheumatic drug (DMARD) and/or TNF inhibitor. In the absence of head-to-head trials, the use of specific biochemical markers may aid in differentiating the onset and/or the magnitude and even duration of efficacy of the different drugs, and in understanding which patient may respond best to a given intervention. The early identification of responders and non-responders to the increasing range of treatments for RA, a disease recognized to lead to loss of function and disability if not aggressively treated, will prove valuable to patients, regulators, healthcare providers and payers. The emphasis in RA management today is on early diagnosis and treatment to prevent the progressive joint deterioration predominantly driven by inflammation [22-24]. Selecting the most appropriate intervention has become increasingly complex because, for example, combinations of some therapies have proven more effective in clinical trials than single agents alone and also because different interventions may be more appropriate than others according to the stage and risk of disease progression in individual patients. In some patients, joint damage progresses slowly over time and then begins to progress in a more rapid and dynamic fashion. In those where inflammation is more severe, structural damage can occur

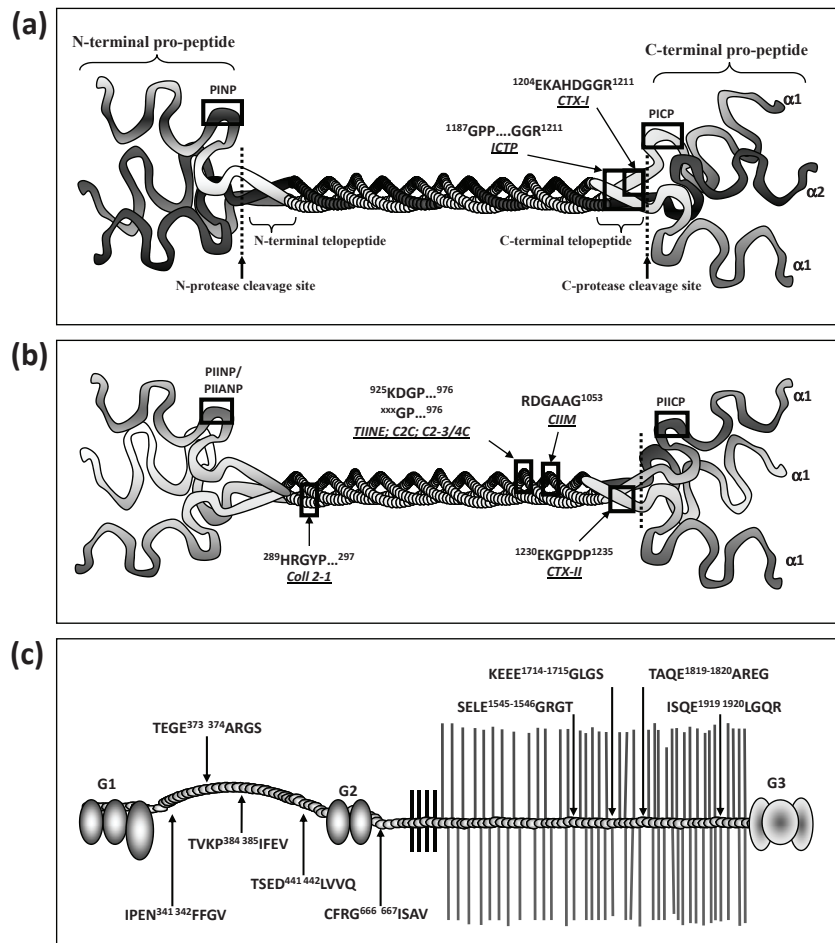
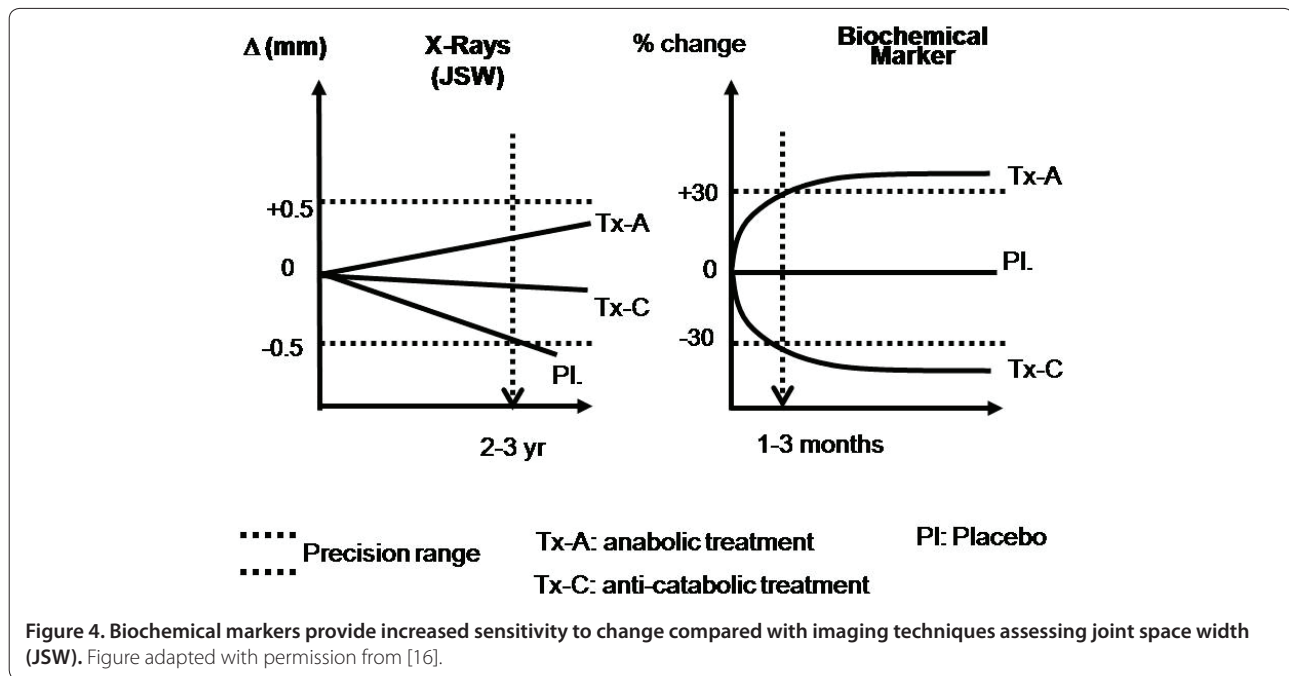


Figure 3. Protease-generated neopeptides in aggrecan and collagen type I and II. (a,b) The amino- and carboxy-terminal pro-peptides PINP (amino terminus propeptide of type I procollagen), PICP (carboxyl terminus propeptide of type I procollagen), PIINP (amino terminus propeptide of type II procollagen) and PIICP (carboxyl terminus propeptide of type II procollagen) in collagen type I (a) and collagen type II (b) are used to define protein formation, as they are released during formation of the matrix. (a) In contrast, the degradation markers ICTP (type I collagen; MMP mediated) and C-terminal telopeptide of type I collagen (CTX-I; cathepsin-K mediated) located in the carboxy-terminal telopeptide are found in body fluids after degradation of collagen type I. (b) The CTX-II (MMP mediated) degradation marker is located in the carboxy-terminal telopeptide in collagen type II. Coll 2-1, TIINE, C2C, and C2-3/4C are degradation markers located in the helix of collagen type II. (c) The aggrecan molecule is shown with the MMP cleavage sites (upward arrows) and ADAM-TS (a disintegrin and metalloproteinase with thrombospondin motifs) cleavage sites (downward arrows). CIIM is a novel MMP mediated type II collagen degradation marker [142]. Figure adapted with permission from [8].

within just a few months after disease onset. Consequently, the greatest opportunity to change the course of the disease could be through the identification of those patients who either have, or are at risk of developing, rapidly progressive disease. Using biomarkers to predict risk and response to therapy will not only aid the selection of an appropriate, effective intervention for the individual but will also protect patients with less severe disease from possible aggressive over-treatment and toxicities, and may have a significant influence on allocation of health care resources. Several biological markers and clinical indicators have been discovered to identify such patients.

Biochemical markers of joint damage

Currently, there is no single clinical or laboratory characteristic that identifies RA patients with rapidly progressing joint damage and systemic bone effects. The best-characterized predictors of risk for rapid progression are the number of swollen joints and levels of acute-phase reactants such as CRP and ESR. This is not surprising because swollen joints are a clinical manifestation of synovitis, and the acute-phase response acts as a biomarker of pro-inflammatory cytokine production. It is well documented that elevated CRP is associated with increased risk of radiological progression in RA [24,25], and correlation between synovitis and subchondral bone



erosions has been established [26-29]. While not all patients with high disease activity that manifests in high swollen joint counts and elevated CRP are immediately eligible for biologic therapy, those who also show ongoing degradation of joint structure proteins may benefit from the most intensive therapy, especially if efficacy can be detected early to manage benefit and risk considerations [25]. The focus of research into joint damage biomarkers has been the identification of proteins that might be surrogates of whole tissue metabolism and of bone and cartilage loss. One approach to identifying pathologically relevant molecules is to combine tissue-specific protein markers with the pathological expression of proteolytic enzymes. The action of enzymes on extracellular matrix components results in matrix degradation fragments, or neopeptides. The most abundant molecules in the articular cartilage extracellular matrix are collagen type II and aggrecan. These proteins are sequentially degraded when cartilage damage occurs in either RA or OA. Protease-generated fragments of collagen type II and aggrecan produced by MMPs and aggrecanases (ADAM-TS) are considered relevant molecules in cartilage degradation [8] (Figure 3). Whole joint tissue pathophysiology may be assessed by the one or more markers of cartilage degradation, but these are only a subset of a larger panel of markers that provide information on bone and inflamed synovial tissue in the joint (Table 1).

As also described in Table 1, additional cartilage degradation markers are becoming available, aimed at more accurate and precise detection of articular cartilage damage. Specific fragments of cartilage proteins have

been identified as specific markers of joint damage. Much of this work has been applied according to the US Food and Drug Administration critical path for the development of biochemical markers in translational research [8], where such markers may be applied in both preclinical and clinical research settings.

Joint turnover markers

Inflammatory joint diseases such as RA lead to alterations in the metabolism of the articular cartilage and subchondral as well as periarticular bone [30-35]. Unique markers have been developed, and others are under development, to reflect different pathobiologic processes. How these processes occur at different stages in the pathogenesis, and result in unique metabolic products of joint inflammation, is discussed in the sections below.

Cartilage turnover markers

Cartilage turnover normally occurs in a controlled fashion, with a balance between degradation and formation. However, in the inflamed joint, an imbalance is skewed towards degradation rather than formation [36]. Formation and degradation can be monitored by measuring several unique molecules generated during cartilage degradation and turnover [17]. Cartilage is predominantly composed of collagen type II (comprising 60 to 70% of the dry weight of cartilage) and proteoglycans (10% of dry weight), of which aggrecan is the most abundant [37]. The key mediators of cartilage degradation include the MMPs and the closely related aggrecanases, which are members of the ADAM-TS family [38,39].

Table 1. Biochemical markers of bone and cartilage turnover measurable in serum, urine and synovial fluid

Assay	Target molecule	Short description	Evidence of potential usefulness
KS/mAb OA-1	Aggrecan	Cartilage degradation. Sandwich ELISA using mAb to keratan sulfate and mAb OA-1 to AGase neoepitope ARGSVIL [143]. Detection of fragments in human synovial fluid [143]	[143]
CS846	Aggrecan	Cartilage turnover. mAb αHFGP-846 (IgM) recognizing chondroitin sulfate moieties on aggrecan. Manufacturer: Ibex, Canada	[144,145]
342-G2	Aggrecan	Cartilage degradation. Sandwich ELISA using mAb AF28 binding to the neoepitope 342FFGVG and monoclonal antibody F78 binding to G1/G2 for detection of MMP-generated aggrecan fragments	[45]
G1-G2	Aggrecan	Cartilage turnover. Sandwich ELISA using mAb F78 binding to G1/G2 both as capture and detector antibody for detection of intact aggrecan and all aggrecan fragments carrying G1 and/or G2	[45,146]
Serum CRP	C-reactive protein	General inflammation. CRP, an acute phase protein, the assay for which is highly sensitive to detect small changes in magnitude of inflammation	[60,126, 147-149]
COMP	Cartilage oligomeric protein	Cartilage turnover. Competition ELISA using polyclonal antibodies [58]. However, sandwich ELISA based on two monoclonal antibodies recognizing different antigenic determinants is described [150]. Manufacturer: AnaMar Medical, Sweden	[60,61, 151-155]
PICP	Carboxyl terminus propeptide of type I procollagen	Bone formation. RIA using polyclonal antibodies raised to fibroblast PICP digested with bacterial collagenase [156]. Manufacturer: Orion Diagnostic, Finland	[156]
PINP	Amino terminus propeptide of type I procollagen	Bone formation. RIA using polyclonal antibodies recognizing PINP [157]. Manufacturer: Orion Diagnostic, Finland. Electrochemiluminescence using mAbs to PINP. Manufacturer: Roche Diagnostics, Germany	[158]
CTX-I	Type I collagen	Bone resorption. A sandwich ELISA using mAb F1103 and F12, both binding to a cathepsin K-derived C-telopeptide neoepitope EKAHD-β-GGR, where D-β-G denotes an isomerized linkage between D and G [84]. Manufacturer: IDS, UK. Also available in an automated version (manufacturer: Roche Diagnostics, Germany)	[87-89]
NTX-I	Type I collagen	Bone resorption. EIA detecting a fragment of the N-telopeptide of type I collagen. Manufacturer: Inverness, US	[159]
ICTP	Type I collagen	MMP-mediated type I collagen type degradation. RIA detecting a fragment of the C-telopeptide of type I collagen. Manufacturer: Orion Diagnostic, Finland	[81,160]
PIINP	Amino terminus propeptide of type II procollagen	Cartilage formation. mAb recognizing the amino acid sequence GQPAGEQGPRGDR located in the amino-terminal propeptide of type II procollagen [46]	[46]
PIIANP	Amino terminus propeptide of type II procollagen, splice variant A	Cartilage formation. An ELISA using rabbit polyclonal antibodies raised to recombinant exon-2 of the amino-terminal propeptide of type II procollagen	[71]
CPII	C-propeptide of type II collagen	Cartilage formation. EIA using rabbit polyclonal antibodies binding to the C-propeptides of type II collagen, that is, a marker of collagen synthesis. Manufacturer: Ibex, Canada	[161]
9A4/5109	Type II collagen	Cartilage degradation. The collagenase-derived neoepitope GEGAAGP5GAEGPPGQG ⁷⁵ containing the carboxyl terminus of the long three-quarter fragment. mAb 5109 detects the first underlined sequence, mAb 9A4 the second (neoepitope)	[162]
CTX-II	Type II collagen	Cartilage degradation. Competition ELISA using mAb F4601 recognizing the C-telopeptide neoepitope EKGDPD (manufacturer: IDS, UK) and mAb 2B4 recognizing the C-telopeptide neoepitope EKGDPD	[42,163]
uTIINE	Type II collagen	Cartilage degradation. An LC-MS/MS assay using mAb 5109 (see above) to affinity purify fragments subjected to MS/MS. Detects a collagenase-derived 45-mer containing the carboxyl terminus of the long three-quarter fragment	[53,54]
HELIX-II	Type II collagen	Cartilage degradation. A competition ELISA using polyclonal rabbit antibodies recognizing the neoepitope ⁶²² ERGETGPP*GTS ⁶³² , where P* denotes hydroxyproline. However, a recent publication has highlighted unspecificities [164]	[164-166]
C2C	Type II collagen fragment	Cartilage degradation. EIA using a monoclonal antibody recognizing the carboxyl terminus of the three-quarter piece of the degraded alpha1(II) chain. Manufacturer: Ibex, Canada	[167,168]
C1,C2	Type II collagen fragment	Collagen degradation. EIA using rabbit polyclonal antibodies binding to the carboxy-terminal (COL2-3/4C(short)) neoepitope generated by cleavage of native human type II collagen by collagenases. Cross-reactivity to type I collagen	[169]
PIIINP	Amino terminus propeptide of type III procollagen	Collagen type II formation. RIA using polyclonal antibodies recognizing PIIINP. Manufacturer: Orion Diagnostic, Finland	[170]

Continued overleaf

Table 1. Continued

Assay	Target molecule	Short description	Evidence of potential usefulness
Glc-Gal-PYD	Glucosyl-galactosyl-pyridinoline	Synovial inflammation. HPLC method for determination of the non-reducible collagen cross-linker glucosyl-galactosyl-pyridinium present in synovium and absent in bone cartilage and other soft tissue	[171]
Serum HA	Hyaluronic acid	Cartilage turnover. Based on HA binding protein isolated from bovine cartilage. Manufacturer: for example, Pharmacia, Sweden	
YKL-40	Human glycoprotein 39	Cartilage turnover. RIA using polyclonal antibodies to a 40 kDa glycoprotein. A combined monoclonal capture and polyclonal (rabbit) detector sandwich assay is available. Manufacturer: Quidel Corporation, US	[172]
OC	Osteocalcin	Bone formation. Numerous assays available	
MMP-3 and MMPs	Varous cell types	Numerous assays available	[173,174]
DKK1/Sclerostin	Dkk-1	Measurement of Wnt signaling	[175,176]
TRACP 5a	Macrophages	Inflammation	[111]
TRACP 5b	Osteoclasts	Osteoclast number. mAb to TRACP 5b, which may be specific for osteoclasts but not their activity	[74,111, 177,178]
Cat K	Osteoclasts	Osteoclast number	[110]

CRP, C-reactive protein; EIA, enzyme immunoassay; HA, hemagglutinin; LC, liquid chromatography; mAb, monoclonal antibody; MMP, matrix metalloproteinase; MS/MS, tandem mass spectrometry; RIA, radioimmune assay. Modified and extended from [179].

Aggrecan is degraded by both MMPs and aggrecanases, whereas collagen type II is degraded by MMPs [40]. The action of these proteases results in the release of collagen and aggrecan peptide fragments that can be measured by ELISA-type assays both *in vitro* and *ex vivo* [17] (Figure 4). Since collagen type II is the most abundant protein in cartilage, several different degradation fragments of collagen type II have been identified as useful for monitoring the impact of joint inflammation on cartilage [17,41].

One example of a novel biochemical marker based on neopeptides [16] is CTX-II, an MMP-generated neopeptide derived from the carboxy-terminal part of type II collagen [42,43]. Measurement of CTX-II has proven useful for monitoring degradation of type II collagen in experimental models assessing cartilage degradation [17,42,43]. Cartilage degradation and formation can be efficiently studied in *ex vivo* cultured explants of bovine articular cartilage [40,44-47]. In this model, a high rate of cartilage degradation can be induced, for example, by the combination of TNF- α and oncostatin M, which induce cartilage degradation in a time- and concentration-dependent manner. The role of MMPs is demonstrated by the abrogation of cytokine-induced CTX-II release by the addition of the MMP inhibitor GM6001, but not the cysteine proteinase inhibitor E64. Further, biochemical studies showed that both MMP-9 and MMP-13 had the ability to generate CTX-II fragments [40]. In addition, immunohistochemical localization of CTX-II revealed that it is highly present in areas corresponding to

proteoglycan depletion in TNF- α - and oncostatin M-treated explants [40]. Additional analysis of CTX-II demonstrated that it was localized in the damaged areas of the articular cartilage [48-50]. In clinical studies, high levels of CTX-II have been shown to be associated with the diagnosis of OA and to predict progression of RA and OA joint damage [51]. Thus, the assay for this MMP-generated collagen type II neopeptide, CTX-II, is an example of a clinically and pathologically validated indicator of cartilage degradation, although its responsiveness to therapeutic intervention continues to undergo intensive investigation. With further characterization in prospective clinical trials, the CTX-II assay may provide an example that assays for neopeptides generated by a specific combination of enzyme and matrix molecules are potentially relevant for monitoring risk of joint damage and impact of therapy. The development of assays to assess cartilage degradation and formation is not limited to just CTX-II (Table 4). Degradation markers include urinary TIINE, serum C2C, C1C2, Coll-2-1, ICTP and HELIX-II, and synthesis markers include PIINP and PIIANP, as they are based on propeptides. COMP and YKL-40 have also been used to assess cartilage degradation, but have also been characterized to detect matrix turnover [17,19,45,47,52-74].

Bone turnover markers

Bone turnover is a continuous process that ensures calcium homeostasis and bone quality [75]. The total skeleton is completely replaced every 10 years on average,

Table 2. Biochemical markers in rheumatoid arthritis clinical trials: selected studies evaluating biochemical markers

Reference	N	Design/study if named	Therapy	Markers evaluated	Results/timeframe
[168]	47	Open, single arm	Adalimumab	MMP-1, -3; COMP	Decrease at 2 years only
[55]	49	Open, single arm	Infliximab (32)/etanercept (17)	COMP	Decrease at 3 months
[169]	68	Open, single arm	Infliximab	Osteocalcin	Increase weeks 2 to 6
				P1NP	Increase weeks 2 to 6
				BAP	No change
				CTX-I	No change
				ICTP	Decrease week 6
[170]	102	Open, single arm	Infliximab	Osteocalcin	No change
				CTX-I	Decrease weeks 14 to 42
				RANKL	Decrease week 14
				OPG	No change
[24]	144	Post hoc, substudy in DB RCT	Infliximab (two dose levels) versus MTX	CTX-I	No change
				Col2-3/4c	No change
				MMP-3	Decrease week 2
[98]	139/138	24-week DB RCT, MTX versus two dose levels ^a	Tocilizumab	Osteocalcin	Increase high dose
				CTX-I	Decrease both doses
				ICTP	Decrease both doses
				PIIANP	Decrease, dose-related
				HELIX-II	Decrease, dose-related
				MMP-3	Decrease, dose-related
[171]	132/124	DMARD monotherapy	Sulfasalazine, MTX, and adjunctive corticosteroids	MMPs, TIMP-1 COMP, glu-gal-pyr CTX-II	2 years, AUC measurements; MMP-3 + CTX-II, AUC was 81% for predictive accuracy
[172]	155	DMARD monotherapy	Sulfasalazine, MTX, and adjunctive corticosteroids	CTX-I, CTX-II	Normalization of CTX-II predicted RA intervention efficacy
[106]	48	1-year, open, single arm (with BMD) ^b	Infliximab	P1NP	No change weeks 6 and 52
				CTX-I	Decrease week 6
				ICTP	Decrease week 52
				CTX-II	No change
[109]	66	1-year, open, single arm, with X-rays at baseline and week 52 ^c	Infliximab	CTX-II	No change
[110,111]	145/157	1-year, open RCT/X-rays (SAMURAI)	Tocilizumab (anti-IL-6R)	Glc-Gal-PYD	No change
				Osteocalcin	Increase
				NTX	Decrease
				PIIANP ^d	Decrease
				MMP-3 ^d	Decrease

^aChanges with anti-IL-6R evident within 4 to 16 weeks, and at week 24 for CTX-I. ^bStable bone mineral density at month 12. ^cPatients with progressive joint damage had higher baseline levels. ^dWith hsCRP, modest correlation with progression of joint damage. AUC, area under the curve; BAP, bone alkaline phosphatase; COMP, cartilage oligomeric protein; CTX-I, C-terminal telopeptide of collagen type I; CTX-II, C-terminal telopeptide of collagen type II; DB, double blinded; DMARD, disease-modifying antirheumatic drug; hsCRP, high-sensitive CRP; ICTP, type I collagen; MMP, matrix metalloproteinase; MTX, methotrexate; NTX, N-terminal telopeptide of collagen type I; OPG, osteoprotegerin; PIIANP, amino terminus propeptide of type II procollagen, splice variant A; PINP, amino terminus propeptide of type I procollagen; RA, rheumatoid arthritis; RANKL, receptor activator of NF- κ B ligand; RCT, randomized controlled trial; TIMP, tissue inhibitor of metalloproteinases.

emphasizing the dynamic nature of this organ and reflecting changes in endocrine function as well as the effects of disease, drugs, and nutritional deficiencies [76]. Perturbation of this delicate balance leads to pathological conditions such as OP and fracture risk, that is, bone loss. Bone turnover is mediated by activated osteoclasts, which degrade the established bone matrix, and

osteoblasts, which form new bone matrix, two processes that, under normal circumstances, are tightly coupled and balanced [77]. The primary osteoclast driver is RANKL [78], although co-stimulators such as the cytokines IL-1 β , IL-6 and/or TNF- α co-stimulate osteoclasts to secrete cathepsin K into the resorption lacunae [79,80], resulting in degradation of the organic

Table 3. Biochemical markers in rheumatoid arthritis clinical trials: selected studies evaluating MRI-based measures and biochemical markers

Reference	N	Design/study if named	Image modality	Markers evaluated	Results
[180]	84	Longitudinal analysis	MRI and X-ray	sCTX-I and uCTX-II sOPG, sYKL-40, sCOMP and sMMP-3	sCTX-I and uCTX-II were significant predictors of progressive joint destruction
[181]	377	Cross-sectional analysis	MRI	CTX-II	Correlation of uCTX-II with BME
[155]	98	Cross-sectional analysis	MRI	COMP, MMP-3, CRP	COMP was elevated in those with bone erosions
[182]	72	Longitudinal analysis	MRI	IL-6, VEGF, YKL-40, CRP and ESR	Only IL-6 correlated with disease progression

BME, bone marrow edema; COMP, cartilage oligomeric protein; CRP, C-reactive protein; CTX-I, C-terminal telopeptide of collagen type I; CTX-II, C-terminal telopeptide of collagen type II; ESR, erythrocyte sedimentation rate; MMP, matrix metalloproteinase; OPG, osteoprotegerin; s, serum; u, urinary; VEGF, vascular endothelial growth factor.

Table 4. Parameters for optimal use and interpretation of markers

Biological parameters	Sampling parameters	Analyte features	Assay format	Assay parameters	Study parameters
Food intake [183]	Sample acquisition	Active enzyme	Competitive assay	Dilution recovery	Mode of action
Diurnal variation [184-188]	Sample matrix (serum, urine, plasma or synovial fluid)	Latent enzyme	Sandwich assay	Buffer robustness	Duration of study
Seasonal variation	Anticoagulant (EDTA, heparin, citrate)	Total protein	Monoclonal or polyclonal antibody	Range of quantization	Onset of action
Joint activity [189,190]	Freeze-thaw cycles	Fragment of the protein [9]	Multiplex or other technique	Sensitivity and limit of detection	Number of samples, sampling frequency (time course)
Medical condition	Shipping and storage conditions		Sample volume	Specificity and selectivity of pathology and parameter	Patient population ^a

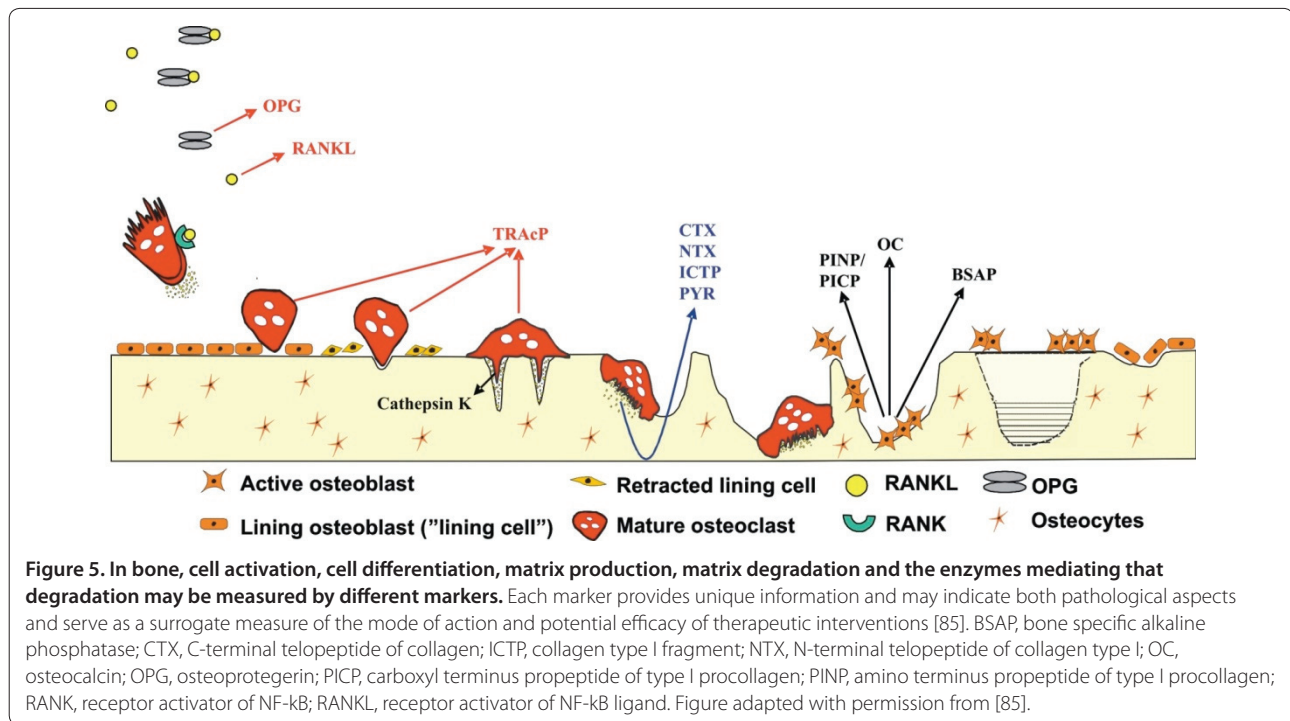
Compilation of parameters known to influence biological variation or analytic performance of a given biochemical marker. These parameters include, but are not limited to, biological variation or analytical performance of a given biochemical marker. ^aAge, gender, menopause status, ethnicity, duration of rheumatoid arthritis, prior treatments such as TNF antagonists, concomitant medications such as corticosteroids, estrogen, SERMs, and bisphosphonates, and comorbidities such as osteoporosis, diabetes, and hypertension with or without renal insufficiency.

matrix of bone. Type I collagen is the most abundant protein in bone [75], and its degradation by cathepsin K leads to the release of the CTX-I or N-terminal telopeptide of collagen type I (NTX) neoepitope [81,82] (Figure 5). CTX-I levels increase in line with elevated levels of IL-6 after the menopause, indicating increased osteoclast activity and bone resorption [83,84]. CTX-I can be measured in both urine and serum and decreases rapidly in response to anti-resorptive treatment in OP [84-86]. Decreased CTX-I levels within 4 weeks of initiating anti-resorptive therapy correlate with BMD increase at 1 year, demonstrating the efficacy of the intervention [87-89]. As a result, CTX-I is being used in a large number of studies [88-93] to monitor the efficacy of anti-resorptive therapies.

In RA a variety of factors, such as the impact of systemic inflammation, corticosteroid use, and menopause, may influence bone resorption, bone turnover and skeletal status over time. Activated osteoclasts participate in altered bone balance since absence of osteoclasts or absence of osteoclast activities will lead to attenuation of bone resorption but only modest effects on cartilage

degradation [3,94,95]. The role of cathepsin K has been extensively studied, and the data are somewhat conflicting for RA [40,96-98]. Levels of cathepsin K are increased in RA, indicating that it can be used as a marker [99,100], although cathepsin K does not appear to be the primary enzyme driving bone destruction in RA [98,101,102]. CTX-I levels correlate only to some extent with joint damage in RA, and are likely also influenced by loss of skeletal structure/osteopenia/OP, which are also prevalent in RA [22,103,104]. MMPs also play a role in inflammation-associated bone loss [105,106]. Studies showing that the MMP-derived collagen type I fragment ICTP is increased in RA may indicate that osteoclasts induce MMP-mediated matrix degradation under these circumstances [82,107,108]. Infliximab and tocilizumab treatment have been shown to reduce ICTP levels, as well as osteoclast numbers [103,109], consistent with osteoclast MMP-mediated bone degradation in RA. However, a direct link between the production of ICTP and osteoclasts has not been demonstrated yet.

As illustrated in Figure 5, a range of different markers is available for assessing bone balance in RA. The most



important markers are those of bone formation (PINP, osteocalcin, bone specific alkaline phosphatase (BSAP)) and bone resorption (for example, NTX and CTX), while assessment of osteoclast numbers by levels of the enzymes TRACP 5b and cathepsin K has more recently provided additional information complementary to bone resorption markers [74,110]. Another TRACP isoform, TRACP 5a, is a macrophage marker, and appears to correlate with inflammation [111]. Circulating levels of the formation marker PINP correlate with histomorphometric indices of bone formation [17]. Osteocalcin levels are characteristically low in RA, associated not only with systemic bone loss but also corticosteroid treatment, and levels may stabilize or increase with effective control of inflammation [112-114].

In conclusion, the inflamed joint is composed of several tissues, each of which is subject to degradation and dysregulated collagen and matrix metabolism, in contrast to a normal joint where the balance between formation and degradation is tightly controlled. Changes in biochemical markers generated as a result of dysregulated metabolism may be useful for timely detection of changes in response to treatment in order to limit joint damage and bone loss in RA.

Currently available biochemical markers of joint damage

The strategy for developing biochemical marker assays has evolved with experience in applying results from disease diagnosis and prognosis as well as from

monitoring the effects of treatments for conditions commonly associated with joint damage. The selection of particular assays depends on the objectives for study, but in most settings these include: examination of the mechanism of action to verify potential benefits in limiting joint damage; prediction of risk of joint damage; diagnosis of ongoing bone and/or cartilage degradation in joints; and monitoring for timely detection of onset of action and maintenance of effect to limit joint damage.

Currently available and commonly used biochemical marker assays are described in Table 1. This is not intended to be an exhaustive list; rather, it is intended to orient the reader to assays that have been commonly reported in clinical studies in arthritis, together with several assays that are currently being examined for improvements to meet the above objectives.

Biological marker assays for detection of tissue turnover in the human joint

Biochemical markers in ankylosing spondylitis - examining unique features of dysregulated bone and cartilage metabolism

Due to the paucity of information provided by standard clinical and laboratory parameters to guide treatment decisions, several of the biomarkers studied in RA have been analyzed in other inflammatory joint diseases, particularly spondyloarthritis (SpA), on the basis that these disorders may share aspects of pathophysiology with RA. There has been particular interest in evaluating biomarkers in AS that reflect disease activity and predict

structural progression [13,15,21,115]. For disease activity, CRP and ESR lack the sensitivity seen in RA, as these markers are elevated in only about 50% of ankylosing spondylitis (AS) patients [116]. Unlike RA, they also correlate poorly with clinical measures of disease activity, although good correlations have been noted with MRI evidence of inflammation in the spine [117,118]. In contrast to RA, they do not appear to predict progression of structural damage, although similar to RA, CRP does predict clinical response to anti-TNF therapy [119,120].

The primary biomarker reflecting tissue turnover related to inflammation in AS that has been analyzed is MMP3. Most studies have shown lower levels of MMP3 in SpA than in RA. Levels are elevated mainly in patients with concomitant peripheral joint inflammation compared to those with only axial inflammation, and levels correlate with the histopathological grade of inflammation [121,122]. As for RA, there is evidence that levels of MMP3 can predict progression of radiographic changes, although for AS this means new bone formation rather than the erosive changes documented in RA [123]. This finding is one observation that supports the concept of a link between inflammation and ankylosis in AS. Reductions in MMP3 levels following anti-TNF therapy correlated with reductions in CRP, although MMP3 levels have not been shown to predict clinical response [124].

Biomarkers reflecting cartilage turnover have been analyzed in limited cross-sectional studies of patients with AS. Elevated levels of CPII and the aggrecan 846 epitope were observed, as they were in RA [125], and normalization was seen with anti-TNF therapy [126]. One study has shown that urinary CTX-II may predict progression of structural damage in AS, as also documented for RA [127]. However, unlike RA, the collagen II degradation markers C2C and C1-2C were not elevated.

Assessment of biomarkers reflecting bone turnover in SpA have shown variable results depending on the stage and activity of disease, but most studies have reported lower levels for markers of bone resorption than in RA [128]. A major inhibitor of osteoblastogenesis, DKK-1, is markedly elevated in RA but is not predictive in AS [125], while sclerostin is increased in RA and reduced in AS [126]. These changes are consistent with the excess bone formation observed in AS and impaired bone formation in RA.

In the current context, this use of biochemical markers in AS emphasizes that biochemical markers of bone and cartilage may be applied to SpA in well-controlled settings and studies.

Major clinical findings with selected interventions and cohort studies

Bone and cartilage biomarkers have been used with various levels of success in both degenerative and

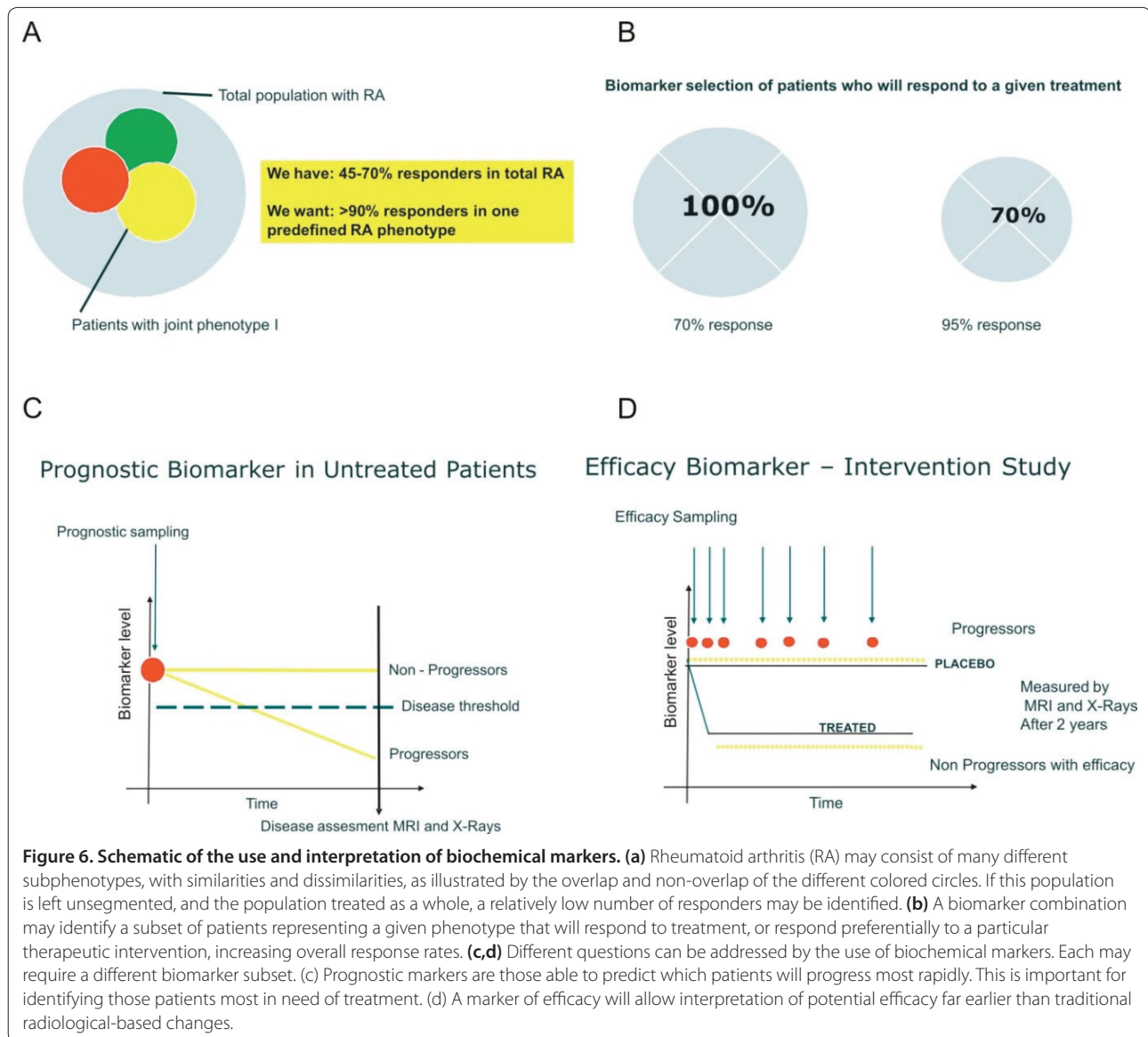
inflammatory joint disease. Table 2 shows those applicable to RA in combination with X-ray imaging and Table 3 provides the current available publications on MRI and biochemical markers in RA. These tables clearly indicate that a subset of markers have already proven useful for investigating efficacy in RA, although surprisingly few combinations of MRI and biochemical markers are currently used. These tables also do not constitute a full list of relevant studies; important information is available in other publications to complement the condensed information here [19,20,22,51,104,123,126,127,129-137].

Strategies for use of biochemical markers to enhance the benefit:risk ratio of RA therapies

The lack of consensus on the optimal biochemical marker combination in RA is understandable given the varying outcomes from diverse studies in which their predictive value has been assessed. However, these differing outcomes are likely due, at least in part, to differences in patient populations, such as varying duration of RA, and confounders, such as current and prior treatments, concomitant corticosteroids and other medications, as well as comorbid conditions (Table 4). Typically, studies with biologics with novel and unique mechanisms of action often recruit patients who have failed to respond to one or more therapies and were receiving a variety of concomitant medications. Thus, even though patient populations at first glance may seem somewhat similar, important differences exist and these need to be carefully considered when interpreting results.

Based on our current knowledge on RA, different marker combinations may be useful at different disease stages for identifying severity and risk of progression of joint damage. This concept is illustrated in Figure 6a, and elaborated in Figure 6b-d. However, the use of biochemical marker profiles to identify individual patients who will respond to a particular intervention, or are more likely to experience rapid progression of joint damage, still remains a major challenge.

The pathology of RA appears to consist of a variety of different phenotypes. If RA is left un-segmented and the population treated as a whole, the proportion of patients experiencing remission is relatively low in most clinical trials. As illustrated in Figure 6b, if a biomarker combination can identify a subset of patients representing a given phenotype who will respond to treatment, or demonstrate a superior response to a specific therapeutic intervention, then response rates in this patient subset will be far greater than those in the unstratified population. This is an important socio-economic opportunity. By targeting the optimal treatment to patients who will derive the most benefit, the most favorable benefit:risk ratio will be obtained.



The optimal biomarker combinations for specific purposes and questions need to be carefully investigated, as illustrated in the figures in this paper. Combinations may depend on the duration and stage of disease in addition to the disease activity and associated effects on bone and cartilage tissues. It is now recognized that anti-TNF therapies may limit joint damage, even in clinical non-responders, and responders to DMARD treatment may continue to experience ongoing joint damage, albeit at a slower rate [23,138]. Thus, a specific combination of biochemical markers may not enable discrimination between clinical responders and non-responders for both radiological progression and patient assessment schemes as outlined by the American College of Rheumatology responder criteria or Disease Activity Score systems. This feature of current therapies remains a further challenge

for the use of 'optimal' combinations of biochemical markers and highlights the potential usefulness of biochemical markers of active joint damage.

Lastly, as illustrated in Figure 6c,d and as discussed for the Burden of Disease, Investigative, Prognosis, Efficacy of Intervention and Diagnostic (BIPED) categorization of biochemical markers [139], different questions can be addressed by using these tools. As illustrated in Figure 6c, prognostic markers are those able to predict who will progress most rapidly. This is an important part in identification of those in most need of treatment. The prognostic marker may also allow for identification of particular patient phenotypes that will respond to treatment (Figure 6a,b). A marker of efficacy as illustrated in Figure 6d is a measurement at baseline or a temporal measurement compared to baseline, allowing the

interpretation of potential efficacy ahead of traditional radiological-based techniques, such as illustrated in Figure 2. In particular, in the field of certain bone diseases, CTX-I is a surrogate marker of efficacy, aiding the prediction of a patient's response to treatment before standard radiological assessment is possible [16].

Biomarker classification

Not all biochemical markers provide the same information. Some may be diagnostic, whereas others may aid prognosis, and others indicate the potential efficacy of interventions. Thus, one biomarker that may fail in one function or scenario may provide important information in another. This highlights the need for a framework to understand terminologies in the development and use of biochemical markers. The recently proposed BIPED classification, developed by the Osteoarthritis Biomarkers Network, which is funded by the US National Institutes of Health, has further highlighted the need for understanding biomarkers and their use [139]. The BIPED classification provides specific biomarker definitions with the goal of improving the development and analysis of OA biomarkers and of communicating advances within a common framework. Briefly, the five definitions for OA are burden of disease, investigative, prognostic, efficacy of intervention, and diagnostic. Burden of disease markers assess the severity or extent of disease, for example, severity within a single joint and/or the number of joints affected. Investigative is an investigative marker with insufficient information to allow inclusion into one of the existing biomarker categories. The investigative category includes markers for which a relationship to various normal and abnormal parameters of cartilage extracellular matrix turnover has not yet been established in human subjects. The key feature of a prognostic marker is the ability to predict the future onset of OA among persons without OA at baseline or the progression of OA among those with the disease. An efficacy of intervention biomarker provides information about the efficacy of treatment among persons with OA or those at high risk for development of OA. Diagnostic markers are defined by the ability to classify individuals as either having or not having a disease.

This very simple yet elegant classification could be used in other disease indications, such as RA, to foster optimal use, and avoid miscommunication of the benefits of selected biochemical markers.

Confounders that influence the application and interpretation of biochemical marker assay results

As many factors affect the measurement and interpretation of changes in levels of biochemical markers, a critical review of sample acquisition, storage and assay parameters must be undertaken to optimally assess the

reliability of biochemical marker analysis. Some of these considerations are highlighted in Table 4, and the reader is referred to the referenced papers for an in-depth discussion of the individual assays and guidance for appropriate, evidence-based interpretation of their results. Multiple biological or analyte-related factors, assay specifications, study parameters and the context in which the results are interpreted are often underestimated and ignored in the study design phase but can have tremendous impact on the final interpretation of the results.

Technical performance strategies for reproducible and reliable biochemical marker analysis include, but are not limited to, the following parameters. The analytical method must be validated by the laboratory for each biomarker used in a clinical study before the laboratory begins analyzing samples from the study. Although manufacturers' kit inserts provide useful assay parameters, it is mandatory that each laboratory verifies it can reproduce these parameters. The validation should be performed on the same sample matrix (serum, plasma, urine or synovial fluid) as collected in the clinical study. Results obtained from serum are not necessarily the same as those from plasma, for example. The analytical validation should include calibration curves, with at least six non-zero standards, intra- and inter-precisions and accuracy, the range of quantification and sensitivity (lower and upper limits of quantification, limit of detection, specificity and selectivity, recovery, stability and dilution linearity. Theoretically, to estimate intra- and inter-run accuracy and stability, five different validation samples should be analyzed in duplicate or more in at least six different runs. One of the major problems with assays (especially microtiter plate-based assays) is reagent-lot variation, indicating a lack of assay robustness. Quality control (QC) samples with pre-defined validated ranges must be analyzed together with the calibrators and the study sample in each run. These QC samples must be prepared in the same matrix as the study samples and, whenever possible, must cover the range of the standards curve (lower, middle and upper limits). The run must be accepted (or rejected) based on the QC acceptance criteria (typically, a 4-6-X rule, where X is a selected percent deviation from nominal value), but also on the results of the calibration standards (back-calculated value within 20% of nominal). Lastly, whenever possible, batches of samples collected during the first visits of the patients, when changes in biomarker levels in response to drug treatment could be theoretically detected, should be assayed together in the same run. This should further minimize inter-assay variation.

These examples serve to highlight that biochemical marker analysis includes a range of parameters that need to be carefully considered and accounted for in optimal

assay performance, which eventually will impact the results of the clinical trials.

Conclusion

RA is often characterized by progressive joint damage that, if not arrested by treatment, often leads to substantial limitation of function and progressive disability. It is evident that the nature of progressive joint damage varies considerably, with some RA patients experiencing more rapid progression than others, based on underlying pathobiology, levels of response to treatment, duration and stage of disease, as well as comorbidities and concomitant medications. Patients with rapidly progressing joint damage may particularly benefit from early aggressive treatment with a biologic agent. Consequently, the identification of patients with ongoing joint damage and assurance that treatment is limiting cartilage degradation and improving bone balance is important in preventing irreversible joint damage. Biological markers and clinical measures can be used to help identify this group of patients, including elevated CRP levels and the number of swollen and tender joints. Additional application of biochemical markers, which are able to sensitively detect ongoing joint damage, may facilitate the appropriate use of targeted therapy in RA and help reduce the progression of joint damage in these patients.

Abbreviations

ADAM-TS, a disintegrin and metalloproteinase with thrombospondin motifs; AS, ankylosing spondylitis; BIPED, Burden of Disease, Investigative, Prognostic, Efficacy of Intervention, Diagnostic classification; BMD, bone mineral density; COMP, cartilage oligomeric protein; CRP, C-reactive protein; CTX-I, C-terminal telopeptide of collagen type I; CTX-II, C-terminal telopeptide of collagen type II; DMARD, disease-modifying antirheumatic drug; ELISA, enzyme-linked immunosorbent assay; ESR, erythrocyte sedimentation rate; IL, interleukin; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; NTX, N-terminal telopeptide of collagen type I; OA, osteoarthritis; OP, osteoporosis; OPG, osteoprotegerin; QC, quality control; RA, rheumatoid arthritis; RANKL, receptor activator of NF- κ B ligand; s, serum; SpA, spondyloarthritis; TNF, tumor necrosis factor; u, urinary; VEGF, vascular endothelial growth factor.

Competing interests

MAK and CC are stockholders of Nordic Bioscience. All others are full time employees of their respective institutions which warrant full disclosure.

Author contributions

MAK and TW wrote the first draft of the manuscript and outlined the paper with AP. KH wrote sections on bone biology. WPM contributed with sections on AS and general pathophysiology. HG provided valuable imaging advice and discussion. PV and TS wrote assay characteristics and challenges sections. GS provided discussion on pathophysiology and helped outline the manuscript. CC and PQ compiled the current biochemical markers section. ACB-J participated in all aspects of this process. All authors approved the final version of the manuscript.

Acknowledgements

We gratefully acknowledge funding from the Danish Research Foundation (Den Danske Forskningsfond) supporting this work.

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Published: 28 April 2011

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doi:10.1186/ar3280

Cite this article as: Karsdal MA, et al.: Biochemical markers of ongoing joint damage in rheumatoid arthritis - current and future applications, limitations and opportunities. *Arthritis Research & Therapy* 2011, **13**:215.