Cartilage markers

Supporting early identification of cartilage damage by providing dynamic assessment of cartilage destruction

Commitment to innovation

info@idsplc.com www.idsplc.com

Cartilage is a tough but flexible connective tissue found in many areas of the body such as joints between bones, between vertebrae in the spine, ears and nose. Cartilage is made up of type II collagen, proteoglycans, non-collagenous proteins and water. The non-collagenous proteins bind to the collagen to form a mesh which attracts water. This combination of components gives the cartilage its strength but also its flexibility.

There are 3 main types of cartilage:

- **Hyaline cartilage** – springy and tough, it is found between ribs, between the joints (articular cartilage) and forms the septum of the nose
- **Elastic cartilage** – more flexible than hyaline cartilage and found in the ear and larynx
- **Fibrocartilage** – inflexible form which helps to reduce friction between bones and distribute weight, and is found in the knee and vertebrae

Cartilage can become damaged in several ways:

- **Direct effect** – heavy impact such as a fall or car accident, high impact sports such as rugby or American football
- **Wear and tear** – due to ageing individuals or increased stress placed on joints e.g. obesity
- **Immobility** – long periods of inactivity can affect joints which typically need regular movement to maintain health
- **Disease** – cartilage can be affected by several diseases such as osteoarthritis, costochondritis, herniation, achondroplasia and or tumours (benign or cancerous)

Turnover of cartilage is a conservative process due, in part, to the lack of blood vessels to supply nutrients to the matrix. Typically, the rate of matrix degradation does not exceed the rate of replacement with chondrocytes both digesting and synthesising matrix proteins. As a result, growth and repair of cartilage should it become damaged is also a slow process. Healing of damaged cartilage requires action by chondrocytes located within the cartilage tissue and potentially other signals from surrounding tissues and cells[1]. In joint diseases, due to the slow nature of synthesis, this is often exceeded by the rate of degradation causing the tissue to become weak resulting in the structural damages associated with osteoarthritis (OA) and rheumatoid arthritis (RA).

Progression of cartilage destruction and therefore progression of disease is assessed using radiological methods, by measurement of joint space width (JSW); however, these radiological methods have certain limitations. As this assessment needs to be performed in a clinical setting with access to appropriate equipment and undertaken by qualified radiologists it is not a procedure that can be used as a widespread screening tool. Therefore, by the time a patient is sent for such assessment it is highly likely that joint damage has already occurred. Considering the slow nature of cartilage synthesis, following initiation of treatment, some time would be required to wait (1 – 2 years[2]) before effect of the therapy might be readily measured radiologically. With access to rapid screening methods for determination of cartilage degradation and / or more speedy feedback about the effectiveness of a given therapy, improvements in patient management for such conditions could be observed.

Cartilage degradation markers

It has been suggested that measurement of markers of cartilage degradation may provide identification of cartilage damage at an earlier stage, giving a more dynamic assessment of damage, allowing rapid feedback of efficacy of treatment. As such, the use of biomarkers of cartilage degradation for diagnosis and monitoring of joint disease, such as OA and RA, have been investigated for several years. The different biomarkers under investigation can be classified into 3 main groups[1]:

Cartilage matrix markers – such as collagen type II pro-peptides (PIICP/PIINP), collagen type II telopeptides (CTX-II) and cartilage oligomeric protein (COMP) etc.

Synovium and inflammation markers – such as matrix metalloproteinases (MMP) and inflammation markers (CRP) etc.

Bone degradation markers – collagen type I pro-peptides (PINP, PICP), collagen type I telopeptides (CTX-I) and bone alkaline phosphatase (BAP)

Studies have indicated clear elevations of biomarkers of cartilage degradation in the presence of certain diseases such as OA and RA. Furthermore, measurements of CTX-II in patients with OA have been found to correlate with radiological measurements of structural damage, pain and physical function[3]. In both OA and RA, measurements of CTX-II have also been shown to correlate with radiological progression of the disease[4-7].

Arthritis

Arthritis is a general term used for conditions affecting the joints and surrounding tissues. Inflammatory joint disease refers to conditions leading to inflammation and tissue degeneration in skeletal joints and is caused by:

- Osteoarthritis (OA): Localised condition of cartilage degradation in specific joints
- Rheumatoid arthritis (RA): systemic autoimmune reaction causing chronic inflammation in synovial tissue and subsequently cartilage tissues in joints

Osteoarthritis (OA)

This localised condition develops in joints that are injured by repeated overuse. This overuse causes the cartilage to be worn away leading to joint pain, decreased flexibility and localised swelling. Typical assessments to lead to diagnosis would involve physical examination and radiological measurement and / or MRI scan, which often display low sensitivity and specificity. To balance this, several biomarkers have been investigated for use in disease diagnosis and assessment of disease severity, risk of onset and progression[8].

The Osteoarthritis Biomarkers Network (OBN) was assembled to investigate new and existing OA biomarkers and develop a classification scheme for biomarkers to be used in clinical trials and OA studies[8]. This proposed scheme created 5 categories to support researchers investigating biomarkers of OA. These categories are referred to by the acronym BIPED[8] which was later updated to BIPEDS[9];

- **Burden of disease** – assesses disease severity in individuals with OA
- **Investigative** – a marker with insufficient information available for inclusion of the marker into another group
- **Prognostic** – ability to predict onset of OA or progression of OA for those already affected
- **Efficacy of intervention** – provides information concerning the efficacy of treatment for those with or at risk of developing OA
- **Diagnostic** – distinguishes between individuals with and without osteoarthritis
- **(Safety** – used in preclinical and clinical applications to monitor the health of the joint tissues, the whole joint organ, or the skeleton in general)[9]

Several biomarkers have been identified as part of the BIPED classification scheme in Table 1:

Tissue	Molecule	Marker of		BIPEDS classification
		Synthesis	Degradation	
Bone	Type I collagen	-	CTX-I	Prognostic
	Non-collagenous	Osteocalcin	-	Prognostic
Cartilage	Type II collagen	-	CTX-II	Burden of disease Prognostic Efficacy of intervention Diagnostic
	Non-aggrecan and non-collagenous proteins	-	COMP	Burden of disease Diagnostic

Table 1. A selection of bone and cartilage markers available from IDS involved in OA. Adapted from Rousseau 2007[10]

Rheumatoid arthritis (RA)

This chronic disease affects joints, connective tissues, muscles, tendon and fibrous tissues. It results from the immune system triggering abnormal inflammation in the synovium causing pain, swelling and stiffness of the affected joint(s). In severe cases, this can affect and damage the bone, cartilage and other tissues of the joint. Being a systemic condition, it can occur in more than joint and can affect any joint in the body. It is however, a transient disease that can last for an undefined period of time or remain asymptomatic between 'flare-ups'.

Diagnosis is initially difficult in early stages as symptoms may resemble many other diseases, with no single blood test or physical investigation able to confirm diagnosis. However, the physical examination includes assessment of swelling, redness and heat in affected joints together with joint reflexes and muscle strength. Whereas serological work-up would involve assessment of inflammation markers C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), together with rheumatoid factor and anti-cyclic citrullinated peptide (anti- CCP) antibodies.

Elevated levels of both CTX-I and CTX-II are associated with radiographic progression in early RA patients1[11-13]. They are also considered to be more predictive of joint damage, when compared with assessments of CRP and ESR[11]. The biomarker COMP has also been found to support assessment of RA; in a study by Andersson *et al*[14] they observed that increasing levels during a 3-month period following diagnosis were predictive of joint damage at 1, 2 and 5 year follow up.

Uses of markers

Many potential biomarkers of arthritis show clear elevation in the presence of the disease. However, the levels of these markers often overlap with healthy controls and only few correlate to radiologically confirmed joint damage, making their use in a clinical setting problematic. However, it may be that different biomarkers are appropriate in specific disease states, without a single biomarker being suitable for all.

In a review by van Spil *et al*[15] in 2010, a total of 84 publications were considered and the performance of 26 identified biomarkers were compared for their suitability for a given tissue (knee or hip) were assessed using the BIPED classification to derive a specific score. Of the markers identified, 15 were markers of collagen metabolism and 8 of these related specifically to collagen type II and 5 for collagen type I. Of these, CTX-II and COMP were scored often: 66 and 57 times respectively with other biomarkers only receiving 1 or 2 scores per BIPED category.

Although there is a lack of consistent evidence for several commercial biomarkers, it appears that CTX-II and COMP are most frequently used in studies when compared with the others. It is however, clear that further investigations into the clinical utility of biomarkers for OA / RA and other such joint diseases is required. The further research is needed in several areas:

- Identification of turnover states: due to the nature of OA and RA it is likely that different therapies would be more appropriate. Identification of individuals with different turnover states would allow a more personalised treatment regime
- Treatment monitoring: measurement of dynamic markers would allow rapid feedback concerning individual response to treatment allowing modification of the therapy, should it be required, to ensure positive outcomes
- Outcome prediction: identification of prognostic markers could allow improved patient management by selecting appropriate therapies and monitoring intervals to ensure response for individuals recognised as having better or worse outcomes

Cartilage Product Portfolio

Product name	Size	Code
Urine CartiLaps® (CTX-II) EIA	96 wells	AC-10F1
Urine Pre-Clinical CartiLaps® EIA	96Wells	AC-09F1
Human COMP ELISA	96 wells	AC-23F1
Serum Pre-Clinical CartiLaps® ELISA	96 wells	AC-08F1

For more details on our products visit www.idsplc.com

Assay summary

Product name / code	Urine CartiLaps® (CTX-II) EIA AC-10F1	Human COMP ELISA AC-23F1
Intended use	Quantification of degradation products of C-terminal telopeptides of type II collagen (CTX-II) in urine	Quantitative determination of Cartilage Oligomeric Matrix Protein (COMP) in human serum
Analyte	CTX-II	COMP
Sample type / volume	Human urine / 40 µL	Human serum / 20 µL
Detection limit	0.2 µg/L	0.2 U/L
Certification	IVD	IVD

Product name / code	Serum Pre-Clinical CartiLaps® (CTX-II) ELISA / AC-08F1	Urine Pre-Clinical CartiLaps® (CTX-II) ELISA / AC-09F1
Intended use	Quantitative determination of degradation products of C- terminal telopeptides of type II collagen (CTX-II) in animal serum	Quantitative determination of degradation products of C-terminal telopeptides of type II collagen (CTX-II) in non-human urine and cell culture supernatant
Analyte	CTX-II	CTX-II
Sample type / volume	Animal serum / 25 µL	Non-human urine and cell culture supernatants / 10 µL
Detection limit	3.7 pg/mL	0.75 µg/L
Certification	RUO	RUO

Features and benefits

- Unique assays for detection of CTX-II and COMP
- Utilises monoclonal antibodies allowing increased confidence in consistency of product over time without changes in specificity etc. due to different bleeds
- Ready to use reagents reduces hands-on time for assay preparation and streamlines testing
- Long shelf life provides a cost-effective solution by reducing wastage due to expired kits
- Suitable for inclusion on automated plate systems to simplify scale-up of testing volume
- Supported by comprehensive panel of pre-clinical and clinical markers for assessment of cartilage metabolism

References

1. Christgau S, Cloos PAC. Cartilage degradation products as markers for evaluation of patients with rheumatic disease. Clinical and Applied Immunology Reviews 4 (2004) 277–294.
2. Ravaud P, Giraudeau B, Auleley G-R, Drape J-L, Rousselin B, Paolozzi L, et al. Variability in knee radiographing: implication for definition of radiological progression in medial knee osteoarthritis. Ann Rheum Dis 1998;57:624–9.
3. Mouritzen U, Christgau S, Lehmann H-J, Tankó L B, Christiansen C. Cartilage turnover assessed with a newly developed assay measuring collagen type II degradation products: influence of age, sex, menopause, hormone replacement therapy, and body mass index. Ann Rheum Dis 2003;62:332–336
4. Garnero P, Piperno M, Gineyts E, Christgau S, Delmas PD, Vignon E. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. Ann Rheum Dis 2001;60:619–26.
5. Garnero P, Gineyts E, Christgau S, Finck B, Delmas PD. Baseline levels of urinary glucosyl-galactosyl pyridinoline and type II collagen C-telopeptide are associated with progression of joint destruction in patients with early rheumatoid arthritis. Arthritis Rheum 2002;46:21–30.
6. Christgau S, Garnero P, Fledelins C, Moniz C, Ensig M, Gineyts E, et al. Collagen type II degradation products in urine as an index of cartilage degradation. Bone 2001;29:209–15.
7. Garnero P, Ayral X, Rousseau J-C, Christgau S, Sandell L, Delmas PD, et al. Uncoupling of type II collagen metabolism predicts progression of joint damage in patients with knee osteoarthritis. Arthritis Rheum 2002;46:2613–24.
8. Bauer DC1, Hunter DJ, Abramson SB, Attur M, Corr M, Felson D, Heinegård D, Jordan JM, Kepler TB, Lane NE, Saxne T, Tyree B, Kraus VB; Osteoarthritis Biomarkers Network. Classification of osteoarthritis biomarkers: a proposed approach. Osteoarthritis Cartilage. 2006 Aug;14(8):723-7
9. Kraus VB, Burnett B, Coindreau J, Cottrell S, Eyre D, Gendreau M, Gardiner J, Garnero P, Hardin J, Henrotin Y, Heinegård D, Ko A, Lohmander LS, Matthews G, Menetski J, Moskowitz R, Persiani S, Poole AR, Rousseau JC, Todman M; OARSI FDA Osteoarthritis Biomarkers Working Group. Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. Osteoarthritis Cartilage. 2011 May;19(5):515-42
10. Rousseau JC, Delmas PD. Biological markers in osteoarthritis. Nat Clin Pract Rheumatol. 2007 Jun;3(6):346-56
11. Garnero P, Landewé R, Boers M, Verhoeven A, Van Der Linden S, Christgau S, Van Der Heijde D, Boonen A, Geusens P. Association of baseline levels of markers of bone and cartilage degradation with long-term progression of joint damage in patients with early rheumatoid arthritis: the COBRA study. Arthritis Rheum. 2002 Nov;46(11):2847-56
12. van Tuyl LH, Voskuyl AE, Boers M, Geusens P, Landewé RB, Dijkmans BA, Lems WF. Baseline RANKL:OPG ratio and markers of bone and cartilage degradation predict annual radiological progression over 11 years in rheumatoid arthritis. Ann Rheum Dis. 2010 Sep;69(9):1623-8
13. Syversen SW, Haavardsholm EA, Bøyesen P, Goll GL, Okkenhaug C, Gaarder PI, van der Heijde D, Kvien TK. Biomarkers in early rheumatoid arthritis: longitudinal associations with inflammation and joint destruction measured by magnetic resonance imaging and conventional radiographs. Ann Rheum Dis. 2010 May;69(5):845-50
14. Andersson ML, Svensson B, Petersson IF, Hafström I, Albertsson K, Forslind K, Heinegård D, Saxne T. Early increase in serum-COMP is associated with joint damage progression over the first five years in patients with rheumatoid arthritis. BMC Musculoskelet Disord. 2013 Aug 2;14:229

Connect with us

Global Headquarters
Immunodiagnostic Systems Ltd, 10 Didcot Way,
Boldon Business Park, Boldon, Tyne & Wear NE35 9PD United Kingdom

+44 (0) 191 519 6155 | info@idsplc.com | www.idsplc.com