

# ***Clinical and Technical Review***

**Biomarker for Diagnosis  
and Monitoring of Degenerative  
Joint Diseases**

*always your partner*

**An estimated 100 million people worldwide** suffer from joint disease (arthritis) which is mainly degenerative arthritis/osteoarthritis (OA) but also inflammatory arthritis including rheumatoid arthritis (RA) and ankylosing spondylitis (AS). Many others suffer from traumatic joint damage which frequently leads to the onset of osteoarthritis. The incidence of these conditions will likely increase further due to demographics and lifestyle changes.

The loss of articular cartilage following joint damage and particularly during joint destruction in OA is usually not preventable in the adult. Current therapies are able to stop cartilage loss in the case of the recent biologic therapies for inflammatory joint disease. Thus, it is of special importance to diagnose joint damage and arthritic joint diseases as early as possible and to initiate appropriate therapy before the disease becomes radiologically apparent, at which time much damage has usually taken place.

The analysis of biomarkers in body fluids offers opportunities to detect early skeletal damage involving joint cartilage and bone as well as early arthritis-related inflammation that occurs in association with the onset of joint damage. Biomarkers also offer us the chance to monitor or even predict the outcome or course of disease, early responses to therapy and joint damage and repair.

Our aim is to provide a brief overview of biomarkers that are available and have been successfully used for preclinical studies employing cartilage cultures and animal models and in clinical investigations involving the early detection and monitoring of joint damage and of their use in predicting progression (OA) and detecting early responses to treatment (RA and AS) in joint diseases. A short introduction to the most common types of joint disease as well as the composition and turnover of articular cartilage should help to visualize the possibilities of biomarker analysis in the study of joint metabolism in health and the differences in damage and disease.

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01/2010

# Contents

<b>1 Joint injury and disease</b> .....	<b>5</b>
1.1 Joint injury .....	5
1.2 Joint inflammation/Synovitis .....	5
1.3 Osteoarthritis or degenerative joint disease (OA) .....	5
1.4 Rheumatoid arthritis (RA) .....	5
1.5 Ankylosing spondylitis (AS) .....	6
<b>2 Composition and turnover of articular cartilage</b> .....	<b>6</b>
2.1 Type II collagen in cartilage and type I collagen in bone .....	6
2.2 Proteoglycan aggrecan in cartilage .....	6
2.3 Other cartilage components .....	7
<b>3 Biomarkers for the detection of cartilage damage, repair and joint inflammation</b> .....	<b>7</b>
3.1 Inflammation biomarkers – synovitis .....	7
3.1.1 PIIINP (N-terminal peptide of type III procollagen) .....	8
3.1.2 anti-CCP .....	8
3.1.3 YKL-40 (Chitinase 3-like protein 1) .....	8
3.1.4 Hyaluronic acid .....	8
3.1.5 Glc-Gal-PYD (Glucosyl–Galactosyl–Pyridinoline) .....	8
3.2 Type II collagen .....	9
3.2.1 Synthesis markers .....	9
3.2.1.1 PIINP, PIIANP (N-terminal propeptides of type II and IIA procollagen) .....	9
3.2.1.2 PIICP (C-terminal propeptide of type II and IIA procollagens) .....	9
3.2.2 Degradation markers .....	9
3.2.2.1 CTX-II (C-terminal telopeptide of type II collagen) .....	9
3.2.2.2 C2C (COL2-3/4CLong; type II collagen collagenase cleavage neoepitope) .....	10
3.2.2.3 C1,2C (COL2-3/4Cshort; types I and II collagens collagenase cleavage neoepitope) .....	10
3.2.2.4 COL2-1 .....	10
3.2.2.5 COL2-1NO2 .....	10
3.2.2.6 HELIX II .....	10
3.2.2.7 TIINE (type II collagen collagenase cleavage neoepitope) .....	10
3.3 Aggrecan .....	12
3.3.1 Turnover markers .....	12
3.3.1.1 Chondroitin sulfate/CS 846 .....	12
3.3.2 Degradation markers .....	12
3.3.2.1 Keratan sulfate .....	12
3.3.2.2 Glycosaminoglycans (GAGs) .....	13
3.4 Other cartilage matrix proteins .....	13
3.4.1 PYD (Pyridinoline) cross-link .....	13
3.4.2 Pentosidine .....	13
3.4.3 COMP (cartilage oligomeric matrix protein) .....	13
3.4.4 CILP (cartilage intermediate layer protein) .....	13
3.5 Enzymes, Enzyme inhibitors .....	14
3.5.1 MMPs (matrix metalloproteases) .....	14
3.5.2 ADAMTSs (disintegrins & metalloproteases with thrombospondin motifs) .....	14
3.5.3 TIMPs (tissue inhibitors of metalloproteases) .....	14
3.5.4 Cathepsin K .....	14

<b>4 Detection of arthritis</b> .....	<b>15</b>
4.1 Detection of onset .....	15
4.2 Detection of disease progression and short-term responses to therapy .....	16
4.2.1 OA progression .....	16
4.2.2 RA and AS treatment .....	17
<b>5 Preclinical studies using biomarkers</b> .....	<b>17</b>
5.1 Cartilage cultures – matrix degradation and synthesis/repair .....	17
5.2 Animal models of joint injury, arthritis and progression .....	17
<b>6 Conclusions</b> .....	<b>18</b>
<b>7 References</b> .....	<b>24</b>
<b>8 Biomarker Test descriptions</b> .....	<b>35</b>
<b>9 Cartilage Antibodies</b> .....	<b>42</b>
<b>10 Cross reactivity</b> .....	<b>43</b>

# 1 Joint injury and disease

## 1.1 Joint injury

As a result of traumatic damage to articular cartilage, ligaments and menisci, joint loading can change considerably causing pathological alterations in cartilage metabolism frequently leading to the onset of osteoarthritis. These changes can be detected within days and weeks, both experimentally in animals and in people, using biomarker analyses of joint fluids, sera and urine.

Repair of articular cartilage can lead to biomarker changes, providing opportunities to monitor this process using biomarkers of cartilage synthesis and degradation, ideally in combination. The ratio of these measurements reflects the balance between these processes, which differs between pathology and repair.

## 1.2 Joint inflammation/Synovitis

In joint inflammation, rheumatoid arthritis is (RA) and to a lesser extent in osteoarthritis (OA), the lining synovial cells, many of which are of macrophage lineage (keeping joint healthy and aseptic) and others which are fibroblastic (making joint lubricants hyaluronic acid and lubricin) are activated and proliferation occurs with increased synthesis of hyaluronic acid and proteins such as COMP and YKL-40. Inflammation also stimulates the synthesis of type II collagen in the synovium and underlying capsule. These are thus provisional biomarkers of joint inflammation, although all are also made by chondrocytes.

## 1.3 Osteoarthritis or degenerative joint disease (OA)

About 10 % of western people suffer from OA also known as degenerative joint disease. Approximately one half of the population over sixty-five suffers from OA. These are conservative estimates based on limited means of detection of OA. It is the most common cause of chronic pain in Europe [1]. The progressive loss of articular cartilage during joint degeneration leads to pain and the restriction of joint function, which in turn causes immobility and disability and promotes secondary diseases. The loss of articular cartilage in OA is slow (may take place over 20–30 years), accelerated following traumatic joint injury and usually irreversible. Current therapies are directed against symptoms (pain) and are unable to stop cartilage loss.

In general, all joints can be affected by OA and multiple joints may be attacked at the same time, although this is not always apparent. Most often, large weight-bearing joints like the hip or knee joints are affected, while shoulder and elbow joints as well as joints of the foot and hand are attacked less frequently. Onset and progression of OA, like RA and AS, is believed to be under strong genetic influences and can be caused by joint trauma or chronic overloading of the joint. Risk-factors for osteoarthritis include gender, age, obesity, joint malalignment and previous meniscal or ligamentous injuries.

## 1.4 Rheumatoid arthritis (RA)

RA is a common form of inflammatory arthritis affecting approximately 1 % of most western populations. It is the fourth most common cause of chronic pain in Europe [1]. RA is observed approximately three times more often in women than in man. It most often presents between the ages of thirty and fifty. Children are affected by a related but distinct condition (juvenile idiopathic arthritis – JIA).

RA usually attacks several joints at the same time (polyarthritis). At the outset, small joints of the hands and feet are usually affected and occasionally also some large joints e.g. knee or ankle joints.

RA is caused by a dysfunction of the immune system (autoimmune disease), triggered by external factors such as stress or infection. Inflammatory cells accumulating in synovial membranes activate synovial cells. Either or both cell types produce enzymes and proinflammatory cytokines, such as TNF alpha, IL-1, IL-6 and IL-17, causing the erosion and destruction of articular cartilage and adjacent bone. Additionally, other organs such as eyes (scleritis), heart or lungs may be harmed.

## 1.5 Ankylosing spondylitis (AS)

This inflammatory arthritis, affecting children and adults, primarily involves destruction and resultant fusion of the joints of the spine. The specialized cartilages, called intervertebral discs, which are destroyed in AS have many structural components in common with articular cartilage.

Biomarker studies have revealed that the spine is a major contributor to the urine biomarker CTX-II. Therefore, other cartilage biomarkers of spinal origin likely also contribute to what is measured in serum and urine.

Some patients also suffer from inflammatory arthritis involving the joints of the arms and may also have inflammation of the eyes (iritis). It is more common than previously thought, affecting up to 2 % of western populations. Again it is genetically determined, being linked to people exhibiting the HLA B27 haplotype.

## 2 Composition and turnover of articular cartilage and bone

The bony ends of the joint are covered by hyaline cartilage. In co-operation with the synovial fluid, articular cartilage enables nearly frictionless joint movement. Additionally, it acts as a mechanical shock absorber. Adult articular cartilage is avascular, alymphatic and possesses no innervation. In intact cartilage, chondrocytes are the only cell type. In the adult, they account for about 5-10 % of the entire cartilage volume and are responsible for matrix synthesis and turnover which involves both degradation and synthesis. The extracellular matrix of articular cartilage mainly consists of type II collagen and aggrecan. Many other proteins are to be found in smaller quantities (Figure 1).

### 2.1 Type II collagen in cartilage and type I collagen in bone

The collagen network is responsible for the tensile properties and strength of articular cartilage and other tissues. About 90 % of the collagen by mass found in articular cartilage is type II collagen which consists of three identical alpha-1(II) chains that form a triple helix. To achieve large collagen fibrils or fibers, the single collagen molecules are aligned together and crosslinked.

This triple helix can be degraded by collagenases, which are metalloproteinases, and by cathepsin K, a cysteine proteinase. Degradation also occurs in non-helical carboxy terminal regions where cross-links occur.

Antibodies can be prepared to these type II collagen specific collagenase cleavage sites, to cross-links and to these non-helical domain sites where degradation occurs. These form the basis of biomarker assays for cartilage collagen degradation, which can be used to detect the release of these degradation products within and from cartilage into culture media, and into body fluids such as synovial fluid, serum and urine.

In type I collagen, antibodies have also been prepared to amino and carboxy terminal telopeptides incorporating cross-links. These are used to detect mainly bone resorption as above. Antibodies are also available that react with the collagenase generated neoepitope in type I collagen which cross-react with the corresponding type II collagen neoepitope. They can be used to study type I cleavage in a variety of conditions including lung disease.

When these collagens are synthesized, they are secreted to extracellular sites as larger procollagen molecules from which the propeptides are removed by proteolysis. The carboxy and amino propeptide contents reflect synthesis of these two collagens. Antibodies to them are used to detect synthesis of type I and II collagens in bone and cartilage respectively.

### 2.2 Proteoglycan aggrecan in cartilage

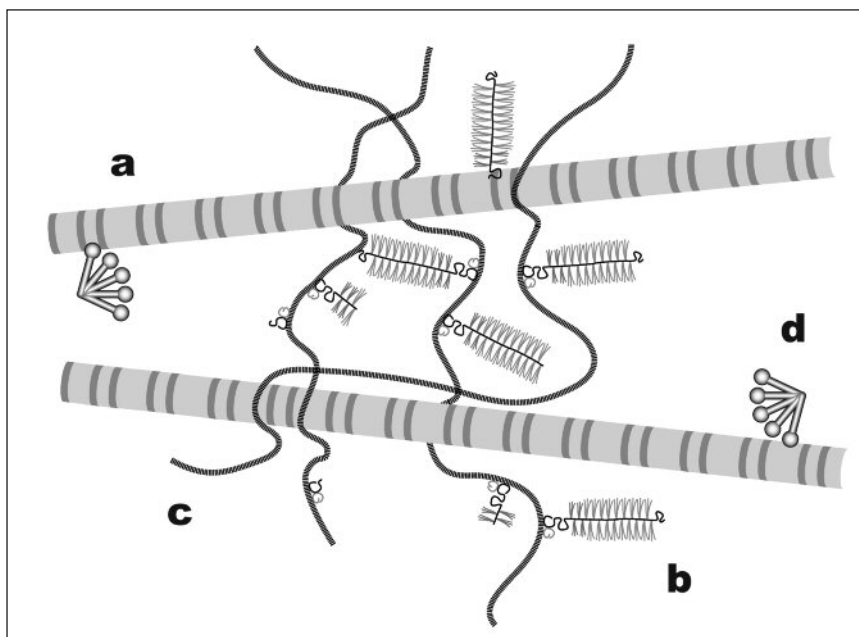
In articular cartilage, aggrecan is the largest and most frequently found proteoglycan. The main components by mass of aggrecan are glycosaminoglycans (GAGs). Many sulfated polysaccharide chains of chondroitin sulfate (CS) and keratan sulfate are linked to a central core-protein. These chains enable aggrecan to bind large quantities of water. Adult articular cartilage consists of up to 80 % water, accounting for its compressive stiffness. Aggrecan molecules are bound to hyaluronic acid by a specific amino terminal binding region (G1), this binding being stabilized by link protein. At the other end of the molecule is another globular domain (G3) that is present on newly synthesized molecules prior to their degradation in the matrix. Adjacent to this domain are CS chains that bind an antibody called CS 846. This antibody is thought to detect recently synthesized and intact molecules of aggrecan and it is used to detect the turnover of this molecule.

### 2.3 Other cartilage components

Many other molecules are found in smaller quantities in articular cartilage. They either play a role during matrix formation or regulate cell function and are collagens (e.g. type VI, IX, X and XI collagens), a variety of non-collagenous proteins (e.g. several smaller proteoglycans like biglycan, decorin and fibromodulin), numerous other proteins (e.g. fibronectin, COMP [cartilage oligomeric matrix protein], CILP [cartilage intermediate layer protein]) other smaller proteoglycans with unique core proteins PRELP (proline arginine-rich and leucine-rich protein), link protein and cross-links and hyaluronic acid. Pyridinoline Cross-links and smaller collagens like type IX collagen are also involved in linking type II collagen molecules to each other as well as with other matrix components. Additional cross-linking and thus stabilization of the collagen network is achieved by COMP which consists of five identical sub-units linked by disulfide bonding (Figure A).

Hyaluronic acid - a non-sulfated glycosaminoglycan (GAG) – is a basic component of all connective tissues. In joints, it is a main component of both the cartilage matrix and synovial fluid.

In cartilage, Hyaluronic acid binds aggrecan molecules together as well as interacting with chondrocytes.



**Figure A:**  
Composition of hyaline articular cartilage: Type II collagen (a), aggrecan (b), Hyaluronic acid (c) and COMP (d).

Modified part of Figure A from:  
AR Poole, G. Rizkalla, M. Ionescu, A. Reiner et al. Osteoarthritis in the human knee: a dynamic process of cartilage matrix degradation, synthesis and re-organization. in *Joint Destruction in Arthritis and Osteoarthritis*. Eds. WB van den Berg, PM. van der Kraan, PLEM. van Lent, Birkhauser verlag, Basel Agents and Actions Supplements 39:3-13, 1993.

## 3 Biomarkers for the detection of cartilage damage, repair and joint inflammation

Clinical evaluations provide limited information about disease onset and activity, especially with respect to structural joint damage. MRI and radiographic evaluations only detect damage or repair once it has occurred. What is needed is a means of following degradative and repair processes as they occur rather than waiting many months (RA) or years (OA, AS and cartilage repair) to observe the outcome by imaging modalities. Moreover, measures of joint inflammation are important in detecting and treating arthritis. Thus additional tools are needed. These are offered by a variety of biochemical/molecular markers of joint disease, its treatment and repair, which are described below.

### 3.1 Inflammation biomarkers – synovitis

Joint inflammation is a key feature of RA and also occurs to a lesser degree in OA. Proliferative synovial inflammation leads to the increased syntheses of different biomarkers. Although they are not synovium-specific, being also produced in cartilage and other tissues, their use has been shown to reflect synovitis.

### **3.1.1 PIIINP (N-terminal peptide of type III procollagen)**

PIIINP is the amino-terminal propeptide of type III procollagen which is cleaved off during collagen fibril formation. Increased PIIINP concentrations are found during proliferative changes in the synovial membrane [2]. In contrast to normal patients, both OA and RA patients show increased PIIINP levels with markedly higher synovial than serum concentrations [3]. In RA patients, the PIIINP levels correlate with the concentrations of systemic inflammation markers (ESR, CRP) and Hyaluronic acid, a marker of synovitis. Patients with active and progressive disease show higher initial PIIINP values, which decline with therapy [4, 5, 6].

### **3.1.2 anti-CCP**

Citrullin arises from enzymatic modification of the amino-acid arginine such as with inflammatory processes. Auto-antibodies against cyclic citrullinated peptides (anti-CCP) are detected in up to 80 % of RA patients with very early disease [7, 8], but in very few patients with juvenile idiopathic arthritis (JIA) [9]. The concentrations of anti-CCP are not correlated to the disease activity score (DAS28), but to radiographic signs of joint destruction [10, 11]. Anti-rheumatic immunosuppressive therapy (Infliximab) reduces the sanguineous anti-CCP level in RA patients [12].

### **3.1.3 YKL-40 (Chitinase 3-like protein 1)**

YKL-40, also called human glycoprotein-39, is synthesized both by synovial cells and chondrocytes [13]. It enhances the synthesis of glycosaminoglycans/proteoglycans by chondrocytes in cell culture [14]. In traumatized or degenerating cartilage, YKL-40 seems to be an integral part of the initial repair process as it activates matrix synthesis [15]. In OA as well as RA patients, increased YKL-40 concentrations reflect the degree of joint inflammation, with RA patients showing higher YKL-40 levels than OA patients [16].

More progressive disease is observed in RA patients with higher initial YKL-40 values [17, 18]. In patients with knee OA, serum levels tend to be higher late in disease [19]. Due to a correlation between the levels of YKL-40 and CRP, YKL-40 is viewed as an inflammation marker in OA patients [20, 21]. YKL-40 levels do not correlate with joint space width and are not considered by some to be suitable for the detection of the degree of inflammation [22]. Synovial YKL-40 levels are significantly higher than those in serum [23].

### **3.1.4 Hyaluronic acid**

Hyaluronic acid is a common component of most connective tissues as well as being a principal component of the synovial fluid, being secreted by the fibroblastic synovial lining cells. In patients with knee OA, serum hyaluronic acid correlates with the degree of synovial proliferation and the sizes of osteophytes but not with the femoral cartilage thickness [24]. Increased serum hyaluronic acid is observed in OA and levels are even higher in RA. Patients with higher initial values show a more rapidly progressive course of disease [5, 25, 26]. Serum hyaluronic acid can correlate with the degree of joint space narrowing [27]. RA patients with synovial inflammation show a decrease in hyaluronic acid after anti-inflammatory therapy [6].

### **3.1.5 Glc-Gal-PYD (Glucosyl-Galactosyl-Pyridinoline)**

Glucosyl-Galactosyl-Pyridinoline is the glycosylated analogue of the pyridinoline cross-link synthesized by synovial cells. Compared to normal patients, those with RA have higher urinary Glc-Gal-PYD levels. A more progressive disease course is observed for patients with higher initial values [28]. The initial Glc-Gal-PYD values correlate with the radiographic degree of joint destruction [29, 30]. In patients with knee OA, Glc-Gal-PYD levels decrease on treatment with a NSAID (Ibuprofen) [31].

## 3.2 Type II collagen

### 3.2.1 Synthesis markers

Cartilage damage following trauma or disease onset (human or experimental) rapidly leads to the increased synthesis of matrix components in order to compensate for losses. Type II collagen is synthesized by the chondrocytes as type II procollagen. During collagen formation, the amino and carboxyterminal propeptides are cleaved off and released into body fluids (Figure B).

#### 3.2.1.1 PIINP, PIIANP (N-terminal propeptides of type II and IIA procollagen)

PIINP is the amino-terminal propeptide of type II procollagen. There are two different splice variants of type II procollagen: type IIA and type IIB procollagen. Type IIA possesses an additional cysteine-rich globular sequence of 69-amino-acids missing in type IIB procollagen (Figure B). While the PIINP-assay detects a linear sequence of amino-acids that is present in both variants, the PIIANP-assay only detects the cysteine-rich globular sequence in PIIANP. Cartilage type IIB collagen is expressed in healthy adult cartilage while type IIA is synthesized during fetal development and during cartilage repair.

Compared to normal subjects, the serum PIINP levels are reduced in RA patients [32]. PIIANP concentrations of OA and RA patients are also reduced compared to normal subjects [33, 34]. Patients with knee OA that have higher initial PIIANP values exhibit a greater risk for disease progression [35, 36].

#### 3.2.1.2 PIICP (C-terminal propeptide of type II and IIA procollagens)

PIICP, also called CPII or chondrocalcin, is the carboxy-terminal propeptide of type II procollagen (Figure B). Serum PIICP concentrations reflect the rate of type II collagen formation in intact and OA cartilage with a half-life of approximately 18 hrs. [37]. The synovial concentrations of PIICP correlate with the body mass index (BMI) and are increased in OA patients compared to intact individuals [38]. In RA patients PIICP levels are reduced in early disease [39] and increased in more progressive disease. In patients with knee OA analysed over more than four years, the initial synovial PIICP levels correlate with the degree of joint space narrowing [40]. Patients with atrophic hip OA show lower serum PIICP levels than patients with hypertrophic hip OA [41].

### 3.2.2 Degradation markers

The enzymatic degradation of type II collagen leads to the generation of various cleavage neopeptides which are released from cartilage matrix into culture media and in vivo into body fluids (Figure B). The triple helix is initially cleaved by collagenases into two fragments: a  $\frac{3}{4}$  piece and a  $\frac{1}{4}$  length fragment. This promotes unwinding (denaturation) of the triple helix exposing cryptic epitopes for detection which are located on the alpha (II)-chains. These denatured collagen molecules are subject to further cleavage by a variety of proteases. Cleavage by proteinases including collagenases also occurs in non-helical telopeptide domains. The chondrocyte collagenase MMP-13 is thought to be especially active in OA whereas the collagenase MMP-1 produced by synovia is active in cartilage damage in RA.

#### 3.2.2.1 CTX-II (C-terminal telopeptide of type II collagen)

The CTX-II epitope is a part of the non-helical carboxyterminal crosslinked telopeptide and consists of six amino-acids attached to a cross-link (X) (Figure B). It was originally described by D. Eyre [109]. It is released during the degradation of type II collagen. It is mainly concentrated in calcified articular cartilage at the junction with sub-chondral bone [110]. Compared to normal subjects, urine CTX-II is increased in OA as well as RA patients [35, 42]. Patients with higher initial values have a greater risk for disease progression [36, 43-46]. Additionally, urine CTX-II content is associated with knee pain [47]. CTX-II concentrations correlate with radiographic signs of joint destruction [30]. An early rise in CTX-II predicts joint destruction in experimental arthritis [48]. Another study shows no relationship to joint space narrowing in knee OA [111]. Urine CTX-II concentrations can correlate with the success of anti-inflammatory therapy both in OA and RA patients [49], but in other clinical trials no correlations are seen with therapy [137].

### **3.2.2.2 C2C (COL2-3/4Clong; type II collagen collagenase cleavage neopeptide)**

The neopeptide C2C is the “new” carboxyterminal end of the  $\frac{3}{4}$  length fragment arising during primary cleavage of type II collagen by collagenases (Figure B). Additional cleavage leads to a smaller fragment consisting of forty-five amino-acids containing the C2C epitope [112]. C2C concentrations in articular cartilage are elevated in OA [113]. In patients with knee OA treated with biologic therapy, the serum concentrations of C2C correlate with radiographic findings: increasing C2C levels indicate disease progression, decreasing values indicate disease remission [50]. In OA patients with no radiographic signs of joint destruction (OA detected by MRI), increased urine (but not serum) C2C levels are observed [51]. C2C serum content shows a strong correlation with MRI T2 imaging [114]. C2C values also correlate with radiographic signs of joint destruction [50, 52]. High C2C levels (in combination with high C1,2C and CS846 levels) indicate a progressive course of joint destruction in RA patients [52]. Biologic therapy was able to reduce the serum C2C concentration [50, 52]. Serum C2C is predictive of disease progression [52]. In knee OA patients, disease progression is reflected by the ratio of serum C2C to CPII [54]. In AS patients, effective treatment with etanercept is correlated with a reduction in serum C2C [115].

### **3.2.2.3 C1,2C (COL2-3/4Cshort; types I and II collagens collagenase cleavage neopeptide)**

C1,2C consists of a slightly shorter neopeptide than C2C. It is common to both type II and type I collagens. C1,2C concentrations are elevated in cartilage of OA patients compared to normal [53]. In patients with knee OA, disease progression favors an increased C2C/CPII ratio [54]. In RA patients, high serum C1,2C levels (in combination with increased serum C2C levels) indicate a progressive joint destruction [50, 52].

### **3.2.2.4 COL2-1**

The COL2-1 epitope consists of nine amino-acids located in the triple helical section of type II collagen (Figure B). In both patients with knee OA and RA, the serum levels of COL2-1 are higher than in normal subjects [55, 56]. A correlation between initial urinary values and the WOMAC scores (for pain and physical function) is observed for patients with knee OA. Increasing COL2-1 levels over one year correlate with disease progression [56, 57].

### **3.2.2.5 COL2-1NO2**

COL2-1NO2 is the nitrated form of COL2-1 (Figure B). Compared to normal subjects, patients with knee OA as well as RA show increased serum COL2-1NO2 levels with significantly higher values observed in RA compared to OA patients [55, 56]. In both OA and RA, the concentration of COL2-1NO2 correlates with the serum level of CRP [55, 56]. In knee OA, a correlation between initial urinary values and the WOMAC score is observed. Increasing COL2-1NO2 levels over one year also indicate progressive joint space narrowing [56, 57].

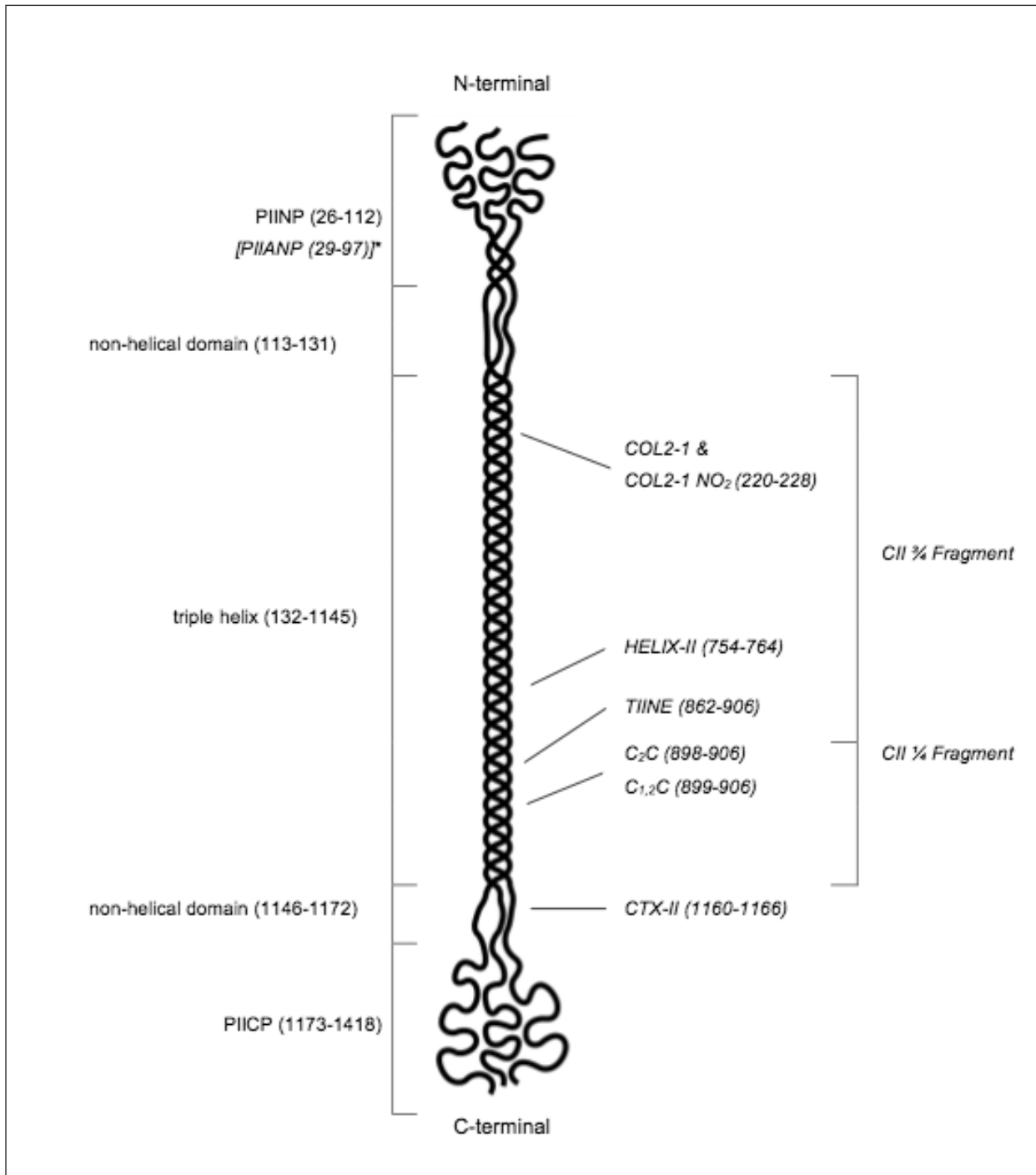
### **3.2.2.6 HELIX II**

The HELIX II epitope is reported to be located in the triple helical section of type II collagen and consists of eleven amino-acids (Figure B). However, a recent report reveals that the sequence recognized by the HELIX II antibody does not exist in type II collagen, putting into question the specificity of this assay [138].

Patients with hip as well as knee OA show increased urinary HELIX II levels compared to normal subjects. Higher values were observed for patients with a progressive course of disease. A high HELIX II level correlates with a low joint space width [43, 58].

### **3.2.2.7 TIINE (type II collagen collagenase cleavage neopeptide)**

TIINE sandwich assay involves the use of two antibodies, one with the specificity of C1,2C and the other recognizing a more N-terminal intrahelical sequence which is specific for type II collagen [116]. (Figure B). In OA patients, TIINE levels correlate with the course of joint space narrowing [59].



**Figure B:**

Schematic drawing of type II procollagen and the localization of the different epitopes. Numbering is based on the amino-acid sequence of an alpha (II) chain of human type IIB collagen (COL2A1\_HUMAN, P02458, UniProtKB, Swiss).

\* Type IIA procollagen would include an additional sequence of sixty-nine amino-acids at position 29-97, the numbering of the following sections would be shifted accordingly.

Modified from Figure 6-2 in AR. Poole, *Immunology of cartilage in osteoarthritis*. In *Osteoarthritis, Diagnosis, Medical/Surgical Management*. 2nd. ed. R. Moskowitz, D. Howell, V. Goldberg, H. Mankin, eds. WB. Saunders Co. Philadelphia, pp.135-189, 1992.

### 3.3 Aggrecan

#### 3.3.1 Turnover markers

As with type II collagen, cartilage damage and degeneration cause an increase in aggrecan synthesis and turnover (increased degradation) as well as a compensatory response. Newly synthesized aggrecan molecules are larger, being less degraded than those already present in the matrix, and similar to those found during fetal development [62].

##### 3.3.1.1 Chondroitin sulfate / CS 846

Apparently intact and more recently formed aggrecan in OA cartilages can be detected as a result of glycosylation differences in the chondroitin sulfate chains manifested as different epitopes (e.g. 3B3, 7D4 and CS846). The CS846 epitope recognized by an IgM antibody is located at the end of large aggrecan molecules found in fetal cartilage (Figure 2), as predicted by analytical analyses of aggrecan size [62] and as revealed by rotary shadowing and electron microscopy [117]. CS846 can be found in increased amounts in adult articular cartilage in OA and during cartilage growth and repair [62]. The concentration of CS846 correlates with the rate of aggrecan synthesis by chondrocytes [118].

Patients with early [119] and advanced [62] OA lesions exhibit markedly higher cartilage CS846 concentrations compared to normal. In early focal cartilage lesions in ankle and knee joints, important differences in responses to degeneration have been observed for CS846 as well as for CPII and C1,2C, which may explain why OA is more common in the ankle [120]. CS846 levels are elevated in synovial fluid following joint injury and in serum in OA [60-62]. For progressive OA, the CS846 levels correlate with the degree of joint space narrowing [52, 63]. Increased serum CS846 levels are seen in chronic slow progressive RA patients, while patients with rapid progressive RA show reduced CS846 values [64], probably due to inhibition of aggrecan synthesis by cytokines such as IL-1 and TNF alpha. In a more recent study, serum CS846 was persistently elevated in more rapid RA progressors together with C2C and C1,2C but not CPII [52].

In AS patients, effective treatment with etanercept is associated with an increase in serum CS846 [115].

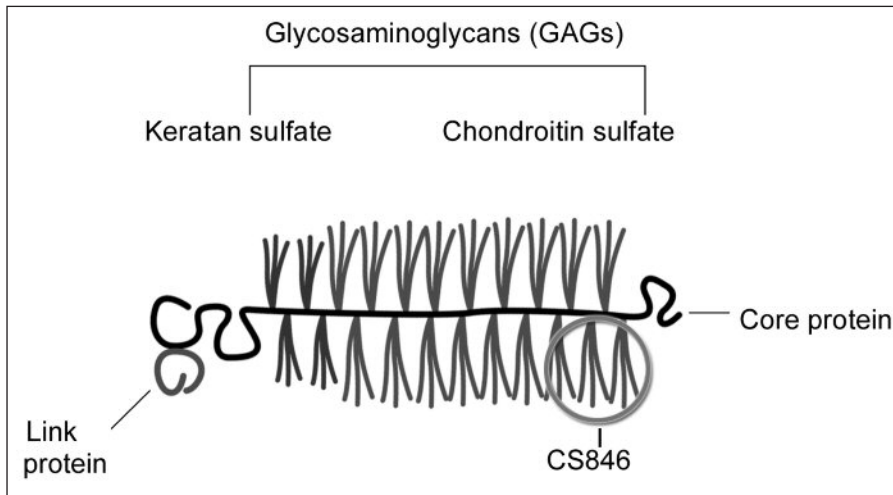
#### 3.3.2 Degradation markers

##### 3.3.2.1 Keratan sulfate

Fragments of aggrecan core protein with attached keratan sulfate chains are released during the cleavage of proteoglycans (e.g. 5D4 and ANP91) [139]. In OA and RA patients, total serum keratan sulfate detected with monovalent antibody Fab is reduced in content, [140] probably because newly synthesised molecules are deficient in this glycosaminoglycan. Keratan sulfate levels can correlate with the degree of cartilage degradation [65].

##### 3.3.2.2 Glycosaminoglycans (GAGs)

Glycosaminoglycans (keratan sulfate and chondroitin sulfate) are the main components of aggrecan and are released attached to core protein following cleavage of the latter. Synovial fluid GAG has been reported as elevated or reduced both in OA and RA patients [66]. Horses with OA as well as traumatic arthritis have higher synovial serum and urine GAG concentrations than normal horses or horses with infected joints [67]. In dogs with OA, increased synovial GAG is seen in the affected joints compared to the normal contralateral joints whereas the serum GAG levels remain unchanged [68].



**Figure C:**  
Schematic drawing of the composition of aggrecan.  
Modified from a part of Figure A in AR Poole, G. Rizkalla, M. Ionescu. A. Reiner et al. Osteoarthritis in the human knee: a dynamic process of cartilage matrix degradation, synthesis and reorganization. in *Joint Destruction in Arthritis and Osteoarthritis*. Eds. WB van den Berg, PM. van der Kraan, PLEM. van Lent, Birkhauser Verlag, Basel Agents and Actions Supplements 39:3-13, 1993.

### 3.4 Other cartilage matrix proteins

#### 3.4.1 PYD (Pyridinoline) cross-link

Pyridinoline is the most common collagen cross-link found in cartilage. Markedly increased PYD levels are observed in the synovial tissue of RA patients. Urinary PYD concentrations increase in parallel to the synovial concentrations while the serum PYD levels remain unchanged [69, 70].

#### 3.4.2 Pentosidine

Pentosidine is an advanced glycation end product (AGE) generated by non-enzymatic glycation of matrix molecules. Articular cartilage has an increased pentosidine content with age which might make the cartilage more susceptible to damage [71, 72]. Increased pentosidine is observed in inflammation. Patients with knee OA have both increased synovial and serum pentosidine levels [27, 73] but those in urine do not predict cartilage loss [141].

Increased pentosidine concentrations are observed in RA patients with those positive for rheumatoid factor having higher pentosidine levels [74]. Successful treatment of RA patients with etanercept is reflected by reduced urinary pentosidine. Additionally, pentosidine correlates with the disease activity score (DAS28) and the degree of joint swelling [75].

#### 3.4.3 COMP (cartilage oligomeric matrix protein)

COMP is not only synthesized by cartilage but also by other cell types including synovial cells and osteoblasts [76]. It is released during cartilage degradation, but high serum COMP might also indicate synovial inflammation [24, 77]. In OA patients, serum COMP is increased with active disease progression [78]. Compared to unaffected persons, those patients with hip OA have higher serum COMP with high values indicating a greater risk for radiographic joint destruction [79]. In patients with knee OA, COMP levels correlate with the degree of synovial proliferation and osteophytosis but not with the femoral cartilage thickness [24, 77].

High initial COMP values indicated a poor prognosis in OA patients [80]. RA patients have increased COMP levels during early disease stages but reduced levels afterwards [7, 81]. High initial values of COMP correlated with a worsening of Larsen-Score results and thus predict the progression of joint destruction [64, 82]. In RA patients, the success of biologic therapy (Infliximab, Etanercept) correlates with a decrease in serum COMP [83, 84].

#### 3.4.4 CILP (cartilage intermediate layer protein)

CILP is a non-collagenous protein normally found mainly in the middle zone of intact articular cartilage. In younger individuals, lower CILP values are observed [85]. The cartilage of OA patients has increased CILP [86]. Antibodies against CILP are detected in both OA and RA patients [87, 88].

## **3.5 Enzymes, Enzyme inhibitors**

### **3.5.1 MMPs (matrix metalloproteases)**

MMPs are involved in the cleavage of type II collagen and aggrecan. RA patients have higher concentrations of serum MMP-1 than OA patients [39]. Compared to normal subjects, higher levels of MMP-3 (stromelysin) are seen in RA patients [29]. The initial plasma MMP-3 concentration reflects disease progression in OA and serial values are correlated with joint space narrowing [89]. In patients with AS, MMP-3 can predict structural damage after 2 years [121]. OA patients show increased MMP-9 levels compared to normals [27]. Increased serum MMP-9 is also detected in RA patients, decreasing after antirheumatic therapy [90]. Additionally, RA patients have both increased synovial and serum MMP-13 [91].

### **3.5.2 ADAMTSs (disintegrins & metalloproteases with thrombospondin motifs)**

ADAMTSs are involved in the degradation of aggrecan (aggrecanases) but also COMP [92-94]. In OA cartilage, an increased expression of primarily ADAMTS-4 was reported [94] but others have found, using gene deletion, that ADAMTS-5 is the key aggrecanase in mice. Compared to intact tissue, markedly increased concentrations of ADAMTS-7 and ADAMTS-12 are present in the cartilage and synovial tissue of RA patients [92]. Increased concentrations of ADAMTS-12 are also observed in the cartilage and synovial tissue of OA patients [93].

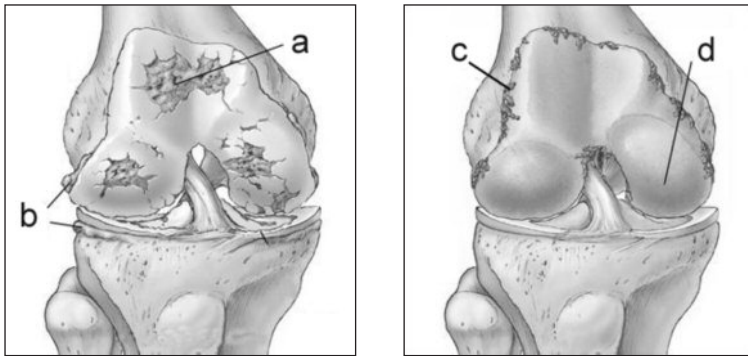
### **3.5.3 TIMPs (tissue inhibitors of metalloproteases)**

TIMPs inhibit the activity of MMPs. The synthesis of TIMPs seems to correlate with the production of certain cartilage components [95, 96]. Patients with knee OA showed increased TIMP levels compared to normal subjects [27].

### **3.5.4 Cathepsin K**

Cathepsin K is a protease synthesized by osteoclasts (from macrophage precursor cells) and activated synovial macrophages and chondrocytes, and can cleave helical collagen like a collagenase but at different sites. Both OA and RA patients showed increased Cathepsin K levels [97]. Using an immunoassay that detects a cathepsin K type II collagen cleavage site, it is evident that cleavage increases, like collagenase cleavage, in articular cartilage with ageing and furthermore in OA [113].

## 4 Detection of arthritis



**Figure D:**

*(left) knee joint with progressive osteoarthritis: cartilage erosions reveal the subchondral bone (a) and at the joint margins osteophytes can be observed (b); (right) knee joint with progressive rheumatoid arthritis: synovial inflammation with marked proliferation of the synovial membrane (c) and thinning out of the articular cartilage (d).*

Progressive osteoarthritis is characterized by extensive areas of cartilage erosion in combination with the loss or cystic changes of the subjacent bone plate. Bony spurs become visible at the joint margins (osteophytes) as an attempt to enlarge the joint's bearing surface. (Figure D, left). In contrast, the characteristic signs of progressive rheumatoid arthritis are a marked synovial inflammation with proliferation of the synovial membrane (pannus) causing a thinning out of the articular cartilage layer (Figure D, right).

At present, degenerative joint diseases are diagnosed by anamnesis, clinical symptoms and radiographic techniques. The additional analysis of laboratory parameters can be helpful to exclude joint inflammation.

Radiographic techniques are suitable to picture alterations of the bone structure as well as changes of the joint space width caused by the loss of articular cartilage (joint space narrowing). However, radiography is less appropriate for the imaging of soft tissues and cartilage.

Magnetic resonance imaging (MRI) is superior to radiography since enabling the detection of soft tissue damage in the joint as well as early changes of the bone morphology. Recently, changes of the cartilage morphology can be visualized.

Arthroscopy is also a valuable method to diagnose early degenerative changes of the articular cartilage in OA since it offers a direct examination of the joint. However, arthroscopy is normally not conducted only for diagnostic purposes and is not a practical screening consideration.

Laboratory analyses for inflammation markers (e.g. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)) can be helpful to detect joint inflammation in cases such as RA. However, inflammation markers only provide information about systemic inflammation. Thus, current laboratory analyses are not joint specific and are less helpful in the case of joint disease.

### 4.1 Detection of onset

The onset of RA is characterized by inflamed painful joint(s), but in the case of OA it can be asymptomatic for many years. When symptoms appear, radiographic examination often reveals that joint damage is advanced. Similarly, in AS, early onset is often difficult to detect. Experience with biologic treatments for RA has revealed greater effectiveness and maximization of joint protection against damage when treatment is initiated soon after onset. It is therefore extremely important to improve our ability to detect early arthritis, especially OA and AS where detection is often very difficult.

Progressive advanced OA detected radiographically is characterized by extensive areas of cartilage erosion in combination with the loss or cystic changes of the subjacent bone and the appearance of osteophytes at the joint margins. These changes may take decades to present starting as small focal lesions in articular cartilage [119]. The extensive advanced changes reflect primarily bone remodelling and only detect cartilage loss indirectly as a loss of joint space. In contrast, MRI can detect very early degeneration of articular cartilage and early subchondral bone cysts, but again these changes reflect outcomes, not the processes that are measured by biomarkers. Additionally, MRI technology is often not available, usually because of costs and accessibility.

The use of biomarkers offers new affordable opportunities. Moreover, biomarker changes reflect changes as they occur, rather than physical outcomes which might take months / years to detect with RA and often over two years in the case of OA and AS.

If RA is suspected, rheumatoid factors and anti-citrullinated molecule antibodies are of much value, although, in approximately 20 % of patients with RA, no rheumatic factor can be detected (seronegative RA).

Recent biomarker studies of the onset of early pre-radiologic symptomatic knee OA have revealed that it is possible to detect an increase over normal values in the urine biomarkers C1,2C and C2C with onset of MRI detectable articular cartilage degeneration [122]. These increases in collagenase activity were not detectable in serum. This may be because this collagenase generated fragment of type II collagen is concentrated in urine [112]. With radiologic change, these and the biomarker CTX-II are all elevated, unlike COMP, HA and NTX-1.

## **4.2 Detection of disease progression and short-term responses to therapy.**

### **4.2.1 OA progression**

The rate of progression of arthritic disease can vary between individuals and with time. Based on experiences with disease modifying biologic therapies, we now know that the more rapid the progression of RA, the more aggressive the treatment that is required. The same tenet should apply in OA. Thus, it is extremely important to develop effective measures of disease progression. Moreover, in creating an OA clinical trial using radiology, we must recognize that often as few as 20 % of (several hundred) patients with knee OA may progress in a 1.5-2.0 year period. All recent trials for potentially disease modifying OA drugs (DMOADS) have been unsuccessful, in part because the study populations have so few progressors exhibiting a radiographically measurable loss of joint space. Thus, there is a great need to try and identify progressors and enrich clinical trial populations with these patients.

Biomarker research in recent years has provided us with some insights into how this can be done. Baseline analyses using type II collagen biomarkers of degradation (CTX-II in urine and C2C in serum) and synthesis (serum type IIA N-propeptide and type II C-propeptide) have revealed that in progressors, the ratio favors degradation markers over those for synthesis [35, 54]. Interestingly, in naturally occurring OA in guinea pigs, the same association is observed [123]. Elevated baseline MMP-3 also favors progression [126]. Thus, use of such biomarkers prior to commencement of a clinical trial for knee OA offers new opportunities in establishing more useful study populations.

### **4.2.2 RA and AS treatment**

In the treatment of patients with RA, it usually takes up to 9-12 months to see a clear evidence of protection against erosive joint damage. This is a long time to wait to see if a drug is effective. By using biomarkers, again particularly of collagen turnover, recent studies in industry and academia have revealed that disease modification can be seen after initiation of treatment with biologics, weeks after initiation of treatment which is predictive, with clinical data, of what is seen radiologically 9–12 months later [50]. Similar short-term effects on biomarkers in patients with AS have also been seen, although long term evidence of reduction in disease activity was not determined [115].

## **5 Preclinical studies using biomarkers**

### **5.1 Cartilage cultures- matrix degradation and synthesis/repair**

By culturing intact cartilages and chondrocytes, one can measure with biomarker assays the degradation and synthesis of extracellular matrix components such as type II collagen and aggrecan. These can be used to investigate effects of cytokines and growth factors and chondroprotective agents in culture. Examples include collagen cleavage [53, 124, 125] and synthesis [127, 119]. Studies of cartilage degeneration following joint injury are also possible [127].

### **5.2 Animal models of joint injury, arthritis and progression**

Surgical removal of menisci and/or anterior cruciate ligament section creates joint instability resembling traumatic injury. Within weeks, these changes lead to measurable changes in cartilage biomarkers in serum and urine C2C, serum CS 846 and CPII indicative of increased cartilage degradation and proteoglycan turnover [128, 129]. In natural onset OA in Dunkin-Hartley guinea pigs, there is an increase in cartilage collagenase activity reflected by serum C2C and CPII biomarker changes [123]. Responses of rabbits with experimental OA to glucosamine treatment have been investigated with biomarkers to study cartilage damage [130]. Equine studies of joint disease using biomarkers have become very important in recent years [131]. Similarly, in models of inflammatory arthritis, such as in the rat, C2C collagenase-generated type II serum collagen fragments show increases with time and then decreases in response to treatment [132]. In synovial fluid in the rabbit, an increase in C2C as well as type IX collagen is also seen with onset of an experimental inflammatory arthritis [133].

## 6 Conclusions

Recently, much progress has been made in the development of biochemical/molecular biomarkers for the detection and study of articular cartilage injury, disease and repair, both in situ and in body fluids. New assays have been developed and assessed for their value in many preclinical and clinical studies. Furthermore, a classification scheme has been proposed to help identify the clinical value or potential of each marker (BIPED)<sup>1</sup> [98].

Biomarkers have also proved of value in veterinary medicine where they are utilized diagnostically, mainly in horses and dogs.

In clinical studies with defined patient cohorts, several biomarkers have proved of value in assisting the early diagnosis of joint injury and onset of arthritis, in predicting disease progression, and in detecting much earlier responses to treatment than are possible with imaging modalities. This is of special importance since therapy should start ideally when joint destruction has not yet become radiographically apparent. Additionally, changes in biomarkers have been shown to correlate with radiographic changes in OA.

Furthermore, baseline levels of some biomarkers are reflective of the rate of subsequent disease progression. In RA and OA, combinations of biomarkers, and in concert with clinical records, have often proved to be of more value than individual biomarker measures. All these studies have involved both community based and clinical trial populations of patients. It remains to be seen if these biomarkers have diagnostic value for individual patients. At the moment, this is a work in progress as biomarkers are now being examined head to head in clinical studies.

Short-term changes in biomarker levels predictive of a subsequent reduction in joint damage detected radiologically 6-9 months later have been observed following onset of anti-inflammatory therapy in RA patients. Consequently, biomarkers offer promising new tools for monitoring early responses to treatment and thus are proving to be of much help in the development of new therapies.

Similar to the analysis of bone turnover, variability in biomarker concentrations may be seen in relationship to age, gender, menopause and ethnicity. This has to be kept in mind when establishing cohorts and analysing biomarkers of cartilage and synovium turnover [99-101]. This is also of special importance in preclinical studies involving both inbred and outbred laboratory animals such as mice, rats, guinea pigs, rabbits and dogs. Additionally, the concentrations of some biomarkers are influenced by diurnal variations [102, 103]. Physical activity can also influence the marker level [104-106].

Furthermore, the sample type used for analyses (synovial fluid, serum, urine) is of importance. Most of the biomarkers show marked concentration differences between the different sample types, dependant upon their tissue(s) of origin, their site(s) of clearance from the circulation and whether they are present in urine [3, 23, 69, 70, 101]. Synovial fluid levels are usually higher but not in all cases, such as the C-propeptide of type II collagen [37]. Urine may reflect important changes in collagen degradation biomarkers, such as C1,2C and C2C, which are not seen in serum [129]. This is probably because the 45KD fragment recognized by these assays is more concentrated in urine [112].

In contrast to synovial levels, biomarker concentrations determined in serum and/or urine depict the entire body's joint and non-articular cartilages contributions from matrix turnover. Additionally, in patients with unilateral joint disease and experimental arthritis in animals, the joint metabolism of at least the contralateral joint changes so it might not be a suitable normal reference [107].

<sup>1</sup> 1 BIPED = Burden of disease, Investigative, Prognostic, Efficacy of intervention & Diagnostic

Furthermore, it remains to be clarified how different joints varying in size and cartilage turnover affect the serum or urine concentrations of a specific marker and what threshold level of tissue degradation or formation must be exceeded to impact serum or urine concentrations.

Markers of cartilage are not specific to diarthrodial joints such as type II collagen. Although it is the primary component of articular cartilage, it is also found in other specialized cartilages and the annulus and nucleus pulposus of intervertebral discs [108, 134]. The CTX-II biomarker has been shown to originate from the spine as well as other joints [142]. Therefore, the reliable diagnosis of injury and joint disease always requires clinical and imaging data.

To minimize variability, a body fluid sample should be collected under standardized conditions to obtain valid and reproducible results (e.g. in terms of time of day, fasting or sample type). An individual reference range should be created and patients - if possible - should be referred to their individual initial marker value. Synovial fluid samples can be reliably collected from joints with little or no inflammation (such as injured or OA) by a prior 20ml. saline lavage employing knee flexion/extension 10 times prior to sample withdrawal. For rabbits, a 2ml. saline intra-articular injection followed by lavage can be used. This procedure can permit collection of repeat samples over time. Dilution issues can be addressed where necessary by examining biomarker ratios which are independent of sample dilution.

In joint biomarker studies, it is also important to consider the additional analysis of bone turnover biomarkers, since bone turnover changes in joint injury, degeneration and inflammation, particularly in OA, RA and AS.

It can be assumed that further joint biomarkers will be introduced in the near future. Besides, enzymes and cytokines, molecules which are involved in signaling cascades like RANKL or osteoprotegerin (OPG), are possible candidates for further analyses. Additionally, genetic approaches involving peripheral blood analyses [136] should improve our knowledge and understanding of the pathogenesis of joint injury and joint disease.

Finally, the challenge remains to select a small set of biomarkers from the existing opportunities that will allow early and reliable diagnosis of injury, disease onset, progression and response to treatment, in the individual patient as well as further monitoring. These will no doubt vary according to the need.

In the case of joint repair involving tissue engineering, comparatively little work has been done with biomarkers to date. However, the same opportunities are already being realized in the study and treatment of joint disease and many lessons learned in this area can be applied in engineering joint repair.

## Biomarker according to NIH Osteoarthritis Biomarker Network

	Proliferation/Formation	Degradation
<b>Synovium</b>		
Type III collagen	PIIINP	
Noncollagenous proteins	anti-CCP	Glc-Gal-PYD
	COMP	PYD
	Hyaluronic Acid	
	Pentosidine	
	YKL-40	
Enzymes	MMPs	
	TIMPs	
<b>Cartilage</b>		
Aggrecan	Chondroitin sulfate	Keratan sulfate
		GAGs
Type II collagen	PIICP	C1, 2C
	PIINP	C2C
	PIIANP	COL2-1
		COL2-1NO2
		CTX-II
		HELIX II
		TIINE
Noncollagenous proteins	YKL-40	COMP
		CILP
Enzymes	TIMPs	MMPs
		ADAMTSs
		Cathepsin K

**Table 1: Biomarker of synovium and cartilage turnover.**

## Biomarker – clinical suitability for the diagnosis of OA and RA

	Osteoarthrose (OA)	rheumatoid Arthritis (RA)	Sample type	References
Non-collagenous proteins	anti-CCP		+ in 80 % of RA patients ‡ after antirheumatic therapy	blood (serum) [7–12]
	CILP	↑ in cartilage of OA patients		tissue [86]
	COMP	↑ inflammatory proliferation of synovial membrane AND/OR ↑ cartilage degradation ↑↑ compared to intact subjects = progressive course	↑ inflammatory proliferation of synovial membrane AND/OR ↑ cartilage degradation ↓ early stage of RA ‡ late stage of RA after antirheumatic therapy	blood (serum) [24, 77–79]
	Glc-Gal-PYD		↑ compared to intact subjects	urine [28–31]
	Hyaluronic Acid	↑ = proliferation of synovial membrane ↑↑ = progressive course correlation with radiography (JSN)	↑ = proliferation of synovial membrane ↑↑ = progressive course correlation with radiography (JSN) ‡ after antiinflammatory therapy	blood (serum) [5, 6, 24–27]
	Pentosidine	↑ compared to intact subjects	↑ compared to intact subjects	synovia, blood (serum) [27, 73–75]
	PYD	↑ compared to NSAID therapy	↑ compared to intact subjects	urine, synovia [69, 70]
	YKL-40	↑ = proliferative changes	↑ = proliferative changes ↑↑ = progressive course	synovia, blood (serum) [16–23]
Aggrecan	Chondroitin Sulfate CS846	↑ compared to intact subjects late stage of disease ↑↑ correlation with radiography (JSN)		blood (serum) [52, 60–64]
	GAGs	↑ compared to intact subjects		synovia [66]
	Keratan sulfate	+ early stage of OA correlation with cartilage damage		blood (serum) [65]

**Table 2: Overview of the biomarker – clinical suitability for the diagnosis and study of disease activity in osteoarthritis and rheumatoid arthritis**

+ = detectable  
 ↓ = level decreased  
 ↑ = level increased  
 ‡ = reduced concentration  
 ↑↑ = higher initial concentration  
 JSN = joint space narrowing

## Biomarker – clinical suitability for the diagnosis of OA and RA

		Osteoarthrose (OA)	rheumatoid Arthritis (RA)	Sample type	References
Type II collagen	C2C	↑ already if no radiographically visible correlation with radiography	↑↑ = progressive course correlation = progressive course ‡ after antirheumatic therapy	blood (serum) urine	[50-52]
	C1, 2C	↑ in cartilage of OA patients C2C:C1, 2C ratio predicts course of OA	↑↑ = progressive course	tissue, blood (serum)	[50,52-54]
	COL2-1	↑ compared to intact subjects correlation with radiography (JSN)	↑ compared to intact subjects	blood (serum) urine	[55-57]
	COL2-1NO2	↑ compared to intact subjects correlation with CRP correlation with radiography (JSN)	↑ compared to intact subjects correlation with CRP	blood (serum) urine	[55-57]
	CTX-II	↑ compared to intact subjects ↑↑ = progressive course correlation with knee pain correlation with radiography ‡ after antiinflammatory therapy	↑ compared to intact subjects ↑↑ = progressive course correlation with knee pain correlation with radiography ‡ after antiinflammatory therapy	urine	[30, 35, 36, 42-47, 49]
	HELIX II	↑ compared to intact subjects ↑↑ = progressive course correlation with radiography (JSN)	↑ compared to intact subjects	urine	[43, 58]
	PIIANP, PIINP	↓ compared to intact subjects ↑↑ = progressive course	↓ compared to intact subjects	blood (serum)	[32-36]
	PIICP	↑ compared to intact subjects correlation with radiography (JSN)	↓ early stage of RA	synovia, blood (serum)	[38-41]
	TIINE	correlation with radiography (JSN)		urine	[59]
Enzymes	ADAMTSs	↑ in cartilage & synovial tissue	↑ in cartilage & synovial tissue	tissue	[92, 93]
	Cathepsin K	↑ in OA patients	↑ in OA patients	blood (serum)	[97]
	MMPs	↑ compared to intact subjects ↑↑ = progressive course correlation with radiography (JSN)	↑ compared to intact subjects ↑↑ = progressive course correlation with radiography (JSN) ‡ after antirheumatic therapy	synovia, blood (serum, plasma)	[27, 29, 39, 89-91]
	MMPs	↑ compared to intact subjects		blood (serum)	[27]
Collagen III	MMPs	↑ = inflammatory proliferation of synovial membrane	↑ = inflammatory proliferation of synovial membrane ↑↑ = progressive course ‡ after antirheumatic therapy ‡ after antiinflammatory therapy	synovia, blood (serum)	[2-6]

**Table 2: Overview of the biomarker – clinical suitability for the diagnosis and study of disease activity in osteoarthritis and rheumatoid arthritis**

+ = detectable  
 ↑ = level increased  
 ↑↑ = higher initial concentration  
 ↓ = level decreased  
 ‡ = reduced concentration  
 JSN = joint space narrowing

### Suitability of biomarker for different sample types and species

	Sample type				Species				
	Synovia	Blood	Urine	Cell culture	human	horse	dog	rat	mouse
ADAMTSs	X	X		X	X				
anti-CCP		X			X				
C1, 2C	X	X	X	X	X	X	X	X	X
C2C	X	X	X	X	X	X	X	X	X
Cathepsin K		X			X			X	X
Chondroitin sulfate	X	X			X	X	X		
CILP		X		X	X				
COL2-1		X	X		X	X			
COL2-1NO2		X	X		X	X			
COMP	X	X			X	X	X		
CS846	X	X		X	X	X	X	X	X
CTX-II		X*	X		X	X	X	X	X
sGAG	X	X	X	X	X	X	X	X	X
Glc-Gal-PYD			X		X				
HELIX II		X	X		X	X	X	X	X
Hyaluronic Acid	X	X	X	X	X	X	X	X	
Keratan sulfate	X	X			X	X	X		
MMPs	X	X		X	X	X	X	X	
PIIANP		X		X	X				
PIICP (CPII)	X	X		X	X	X	X	X	X
PIINP		X		X	X				
PIIINP	X	X	X		X	X	X	X	
Pentosidine	X	X	X	X	X			X	
PYD	X	X	X		X	X	X	X	X
TIMPs	X	X		X	X		X	X	
TIINE	X	X	X		X		X	X	
YKL-40	X	X		X	X	X	X		

**Table 3: Suitability of different sample types for the analysis of biomarker in different species. Note that TIINE is used in serum and urine studies**

\* pre-clinical only

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## 8 Biomarker Test descriptions

### TECO Hyaluronic Acid - Hyaluronan (HA)

<b>Cat. No.:</b>	TE1017
<b>Tests:</b>	96
<b>Method:</b>	ELISA
<b>Range:</b>	25–1500 ng/ml
<b>Sensitivity:</b>	~13 ng/ml
<b>Incubation time:</b>	3 hours
<b>Sample volume:</b>	30 µl
<b>Sample type:</b>	Human or animal biological fluids - serum, urine, synovial fluid and cell culture supernatant
<b>Sample preparation:</b>	Fasting blood collection. Serum or EDTA Plasma stable for 72 hours at 2–8 °C, 6 months at -20°C, longer storage at -80°C. Maximum 3 freeze- and thaw cycles.

**Reference values:** Hyaluronic Acid Values are dependent on age and sex and influenced by food intake and physical activity.  
Values in EDTA-Plasma are 10 % lower.

Clinically Healthy Subjects n=53 between 16 and 79 years.

45.6 ± 16.5 ng/ml.

Women premenopausal 13.8 - 49.8 ng/ml

Women postmenopausal 33.2 - 83.6 ng/ml

Men 20.8 - 79.2 ng/ml

Cutoff 80 ng/ml ± 2 SD

**Species:** Human, dog, horse, rat and other animal models

#### Intended use:

Hyaluronic acid is a high molecular weight (1000-5000 kD) anionic polysaccharide composed of repeating disaccharides of glucuronic acid and N-acetylglucosamine. Hyaluronic acid is mainly produced by synovial fibroblasts and within the joint.

#### Rheumatoid arthritis and Osteoarthritis:

Hyaluronan is a component of the cartilage matrix but is most concentrated in the synovial fluid. Increased Hyaluronic acid levels are observed in OA as well as RA patients. RA patients with synovial inflammation show decreasing Hyaluronic acid concentrations after anti-inflammatory therapy.

#### Liver diseases:

Studies have shown that serum HA levels are elevated and directly correlated to liver diseases, like liver fibrosis and liver cirrhosis, as the liver is the main organ for the removal of HA from the circulation. HA is a useful tool to monitor patients with chronic hepatitis C and after liver transplantation.

#### Other areas:

Recent publications have shown that HA levels in urine are an indicator for bladder cancer. Data also suggest enhanced breakdown of HA in the lungs of patients with chronic obstructive pulmonary disease.

## IBEX C2C

### Neopeptide (at C-terminus of $\frac{3}{4}$ peptide) generated through cleavage of type-II collagen by collagenases

<b>Cat. No.:</b>	60-1001-001
<b>Tests:</b>	96
<b>Method:</b>	ELISA
<b>Range:</b>	10–1000 ng/ml
<b>Incubation time:</b>	2.5 hours
<b>Sample volume:</b>	25 $\mu$ l per replicate, triplicates recommended
<b>Sample type:</b>	Serum (also used for plasma, urine synovial fluid, cell and tissue culture and cartilage extracts)
<b>Sample preparation:</b>	Serum samples should be aliquoted, rapidly frozen and stored at -70 °C. Avoid repeated freezing/thawing of samples. Serum: stable > 1 month at -20 °C and 1 year at -80 °C. Stable for at least one freeze/ thaw cycle. Centrifuge and vortex samples gently before testing.
<b>Species:</b>	Human (also used for bovine, rabbit, dog, guinea pig, rat, horse, rhesus macaque, sheep). The use of mouse samples may lead to high background interference.
<b>Cross reaction:</b>	No cross-reaction with uncleaved triple-helical and heat-denatured human collagen types I and II and uncleaved alpha-chains of collagen types I and II. No cross-reaction with correspondingly cleaved alpha-chains of collagen type-I.

#### Intended use:

The C2C neopeptide is generated by the cleavage of type II collagen by collagenases and is found at the C terminus of the  $\frac{3}{4}$  length type II collagen collagenase cleavage product and any subsequent degradation products of this large peptide which contain the C-terminal C2C neopeptide (Billinghurst et al., 1997; Poole et al., 2004). The assay can be used to analyse degradation in articular cartilages revealing increased cleavage of type II collagen by collagenases (Dejica et al, 2008). Serum C2C is increased in rheumatoid arthritis (RA) and baseline levels are prognostic of progression (Verstappen et al, 2006). Singly, or in combination with the C1,2C and CPII assays together with clinical information, Early serum biomarker responses to therapy at 1 month are predictive of radiologic changes at 12 months (Mullan et al.2007).

There are a number of low molecular weight peptides present in human urine containing the C2C neopeptide (King et al., 2006). Nemirovskiy et al. (2007) recently characterized these peptides in detail using liquid chromatography-tandem mass spectrometry and demonstrated that a 45 aa peptide in particular was produced specifically by MMP-13 in human cartilage. MMP-13 is thought to be the predominant MMP involved in the pathology of OA. This MMP-13 specific 45 mer with the C-terminus C2C neopeptide containing peptides was found in rat urine. In a cross-sectional population-based study of human subjects with knee pain, (Cibere et al A&R, 2009) reported that higher levels of urinary C2C neopeptide were significantly associated with symptomatic radiographic and symptomatic pre-radiographic MRI detected OA compared to subjects with knee pain but no evidence of OA. Thus, C2C neopeptide was elevated in urine prior to radiographically visible damage in this population of individuals with knee pain. Thus, the C2C neopeptide in urine and serum may have clinical relevance.

As a ratio to CPII, serum C2C is indicative of progression/non-progression of knee OA (Cahue et al, 2007). This is of much potential value in establishing cohorts for clinical trials.

In patients with RA, C2C together with C1,2C and CPII provide indications of early responses to biologic therapy that are predictive of what is seen almost a year later (R.Mullan et al, 2007).

## **IBEX C1, 2C**

### **Collagen resorption**

#### **C-Terminus of <sup>3</sup>/<sub>4</sub> peptide, generated through cleavage of types I and II by collagenases**

<b>Cat. No.:</b>	60-1002-001
<b>Tests:</b>	96
<b>Method:</b>	ELISA
<b>Range:</b>	0.03–10 µg/ml
<b>Incubation time:</b>	2.5 hours
<b>Sample volume:</b>	25 µl per replicate, triplicates recommended
<b>Sample type:</b>	Serum (also used for plasma, urine, cell culture, tissue, synovial fluid, cartilage extracts, bronchoalveolar lavage)
<b>Sample preparation:</b>	Serum samples should be aliquoted, rapidly frozen and stored at -70 °C. Avoid repeated freezing/thawing of samples. Serum: stable > 1 month at -20 °C and 1 year at -80 °C. Stable for at least one freeze/ thaw cycle. Centrifuge and vortex samples gently before testing.
<b>Species:</b>	Human (also used for dog, bovine, horse, mouse, rhesus macaque, rat, guinea pig, sheep). The use of rabbit samples may lead to high background interference.
<b>Cross reaction:</b>	Negligible cross-reaction with uncleaved triple-helical and heatdenatured human collagen types I and II and intact or cleaved alpha-chains of collagen type-II.

#### **Intended use:**

C1,2C as a biomarker is used in the investigation of the progression of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, reflecting cartilage, ligament, skin, synovium/capsule, lung and bone damage or inflammation or repair, in human as well as some animal models. Increased serum C1,2C concentrations give an indication of a rapid progressive joint damage in RA.

## **IBEX CP II**

### **Collagen synthesis**

#### **C-terminal Propeptide of Collagen Type-II (PIICP)**

<b>Cat. No.:</b>	60-1003-001
<b>Tests:</b>	96
<b>Method:</b>	ELISA
<b>Range:</b>	50–2000 ng/ml
<b>Incubation time:</b>	4 hours
<b>Sample volume:</b>	25 µl per replicate, triplicates recommended
<b>Sample type:</b>	Serum (also used for cell culture, synovial fluid, cartilage extract)
<b>Sample preparation:</b>	Serum samples should be aliquoted, rapidly frozen and stored at -70 °C. Avoid repeated freezing/thawing of samples. Serum: stable > 1 month at -20 °C and 1 year at -80 °C. Stable for at least one freeze/ thaw cycle. Centrifuge and vortex samples gently before testing
<b>Species:</b>	Human (also used for horse, bovine, dog, mouse, rat, rhesus macaque, sheep, guinea pig). The use of rabbit samples may lead to high background interference.

#### **Intended use:**

CP-II as a biomarker is used in the investigation of the cartilage synthesis in rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and cartilage injury and repair in human as well as some animal models. The CP-II-concentration in cartilage correlates directly with the synthesis of the type-II collagen.

## **IBEX CS-846**

### **Chondroitin Sulfate 846 Epitope of Proteoglycan Aggrecan**

<b>Cat. No.:</b>	60-1004
<b>Tests:</b>	96
<b>Method:</b>	ELISA
<b>Range:</b>	20–1000 ng/mL
<b>Incubation time:</b>	3 hours
<b>Sample volume:</b>	10 µl per replicate, triplicates recommended
<b>Sample type:</b>	Serum (also used for synovial fluid, cell culture, cartilage extract)
<b>Sample preparation:</b>	Serum samples should be aliquoted, rapidly frozen and stored at -70 °C. Avoid repeated freezing/thawing of samples. Serum: stable > 1 month at -20 °C and 1 year at -80 °C. Stable for at least one freeze/ thaw cycle. Centrifuge and vortex samples gently before testing.
<b>Species:</b>	Human (also used for horse, bovine, dog, rabbit, guinea pig, rat, rhesus macaque, sheep). The use of mouse samples may lead to high background interference.
<b>Cross Reaction:</b>	Chondroitin sulfate, dermatan sulphate and keratan sulfate alone, either in their native or degraded state, show no reactivity.

#### **Intended use:**

CS-846, a chondroitin sulfate epitope, which is normally only present on intact aggrecan “fetal-like” proteoglycan, is used as a biomarker in the investigation of the cartilage synthesis/turnover in rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and cartilage injury and repair in human as well as some animal models.

**COMP**  
**Collagen resorption**  
**Cartilage Oligomeric Matrix Protein**

<b>Cat. No.:</b>	COMP 200
<b>Tests:</b>	96
<b>Method:</b>	ELISA
<b>Range:</b>	10–80 ng/ml
<b>Sensitivity:</b>	~5 ng/ml
<b>Incubation time:</b>	overnight plus 3 hours
<b>Sample volume:</b>	60 µl (1:50 diluted)
<b>Sample type:</b>	Serum, synovial fluid
<b>Sample preparation:</b>	Use samples immediately or store at -20 °C. Repeated freeze/thaw cycles (up to 5 times) do not affect results. Before use, thaw specimens at low temperature and mix them thoroughly. Specimens which are stored at room temperature overnight show a loss of about 30 % COMP.
<b>Reference values:</b>	Men and women (22–59 years) Serum: Range 0.99–2.54 µg/ml, (average 1.35 ± 0.4 µg/ml) Men and women Synovial: appr. 10–20 higher than in serum synovial from the knee: 14–48 µg/ml
<b>Species:</b>	Human, horse
<b>Standards:</b>	Purified human COMP

**Intended use:**

Cartilage Oligomeric Matrix Protein is an abundant cartilage glycoprotein also found in tendon and other tissues. Synthesized by chondrocytes, synovial and other skeleton cells. Intact and fragmented COMP in synovial fluid or serum is correlating to cartilage degradation in OA and RA RA disease: COMP levels can be used for monitoring the effect of anti-rheumatic drug therapy. Treatment with TNF- $\alpha$  blockers or adalimumab, suppresses COMP levels. OA disease: Changes in serum COMP are prognostic for disease progression in knee OA. A significant serum COMP increase during the first year indicates progressive disease. Baseline COMP levels do not have prognostic significance.

## **sGAG**

### **Alcian blue binding Assay for the Detection of Sulfated Glycosaminoglycans**

<b>Cat. No.:</b>	GAG 201
<b>Tests:</b>	96
<b>Method:</b>	Colorimetric
<b>Range:</b>	12.5–400 µg/ml
<b>Sensitivity:</b>	~2 µg/ml
<b>Incubation time:</b>	1.5 hours
<b>Sample volume:</b>	50 µl
<b>Sample type:</b>	serum, plasma, synovial fluid, tissue extract
<b>Sample preparation:</b>	GAGs are stable at 4 °C in the absence of cells. Cell debris and insoluble materials should be removed by centrifugation. Samples should be stored at 2-8 °C for a maximum of 5 days. Longer storage at -20 °C. Avoid freeze-thaw cycles.
<b>Species:</b>	Human

#### **Intended use:**

The most abundant heteropolysaccharides in the body are the glycosaminoglycans (GAGs). They are located primarily on the surface of cells or in the extracellular matrix. As an example, cartilage is composed to a large extent of GAGs, which are the dominant part of the proteoglycan Aggrecan.

## **YKL-40 for Rheumatology and Oncology (Quidel®)**

### **Human Cartilage Glycoprotein 39**

<b>Cat. No.:</b>	8020
<b>Tests:</b>	96
<b>Method:</b>	ELISA
<b>Range:</b>	20–300 ng/ml
<b>Sensitivity:</b>	10 ng/ml
<b>Incubation time:</b>	3 hours
<b>Sample volume:</b>	20 µl
<b>Sample type:</b>	Serum, synovial fluid, plasma, cell culture
<b>Sample preparation:</b>	Samples may be stored at 2–8 °C for a maximum of 7 days. For longer storage, freeze specimens at -20 °C or below.

**Reference values:** Women (< 60 years): 25 – 93 ng/ml;  
Men (< 60 years): 24 – 125 ng/ml

**Species:** Human, baboon, rhesus macaque, cynomolgus macaque

#### **Intended use:**

YKL-40 is a 40-kDa heparin-binding glycoprotein. Increased serum YKL-40 levels were found in patients with rheumatoid arthritis and osteoarthritis, inflammatory intestinal diseases, heavy bacterial infections and liver fibrosis.

#### **Further areas:**

- Oncology
- Asthma

Literature overview available for the different clinical application.

## **YKL-40 Control**

<b>Cat. No.:</b>	4821
	Set of 3 times 0.5 ml (3 levels)
<b>Average:</b>	45, 168, 240 ng/ml

## 9 Cartilage Antibodies

### Antibodies - Cartilage metabolism

Antibody	Quantity	Product No.	Western Blot		Immunohistochemistry	
				Dilutions		Dilutions
Ab 1320 Aggrecan NITEGE rabbit Ab	50 µL serum	50-1006	✓	1:100–1:200	✓	1:200–1:600
C1-2C Antibody (Col 2 <sup>3</sup> / <sub>4</sub> C short Ab)	100 µL serum	50-1035	(•)		✓	1:50–1:500
Col M Antibody (Col 2 <sup>3</sup> / <sub>4</sub> M Ab)	50 µL serum	50-1011	✓	1:50–1:200	✓	1:100–1:250
Type-IX Collagen Ab	50 µL serum	50-1013	✓	1:1000		

### Cross reactivity – Antibodies of cartilage metabolism

	Ab 1320	C1-2C [53]	Col M [143]	Type-IX [144]
Human	•	•	•	•
Monkey	(•)	(•)	(•)	(•)
Dog		(•)	(•)	
Rabbit	(•)	(•)	•	(•)
Rat	•	(•)	•	(•)
Mouse	•	(•)	•	•
Guinea pig	•	(•)	•	
Horse		(•)	(•)	
Bovine	•	(•)	•	•
Porcine	(•)	(•)	(•)	(•)

\* = *validated*

(•) = low cross reactivity

**Kreuzreaktionen - verschiedene Spezies / Cross reactivity - different species / Réactions croisées selon les Espèces**

Produkt Product Produit	Zellkultur Cell culture Culture cellulaire	Human Humain Homme	Elefant Elephant Éléphant	Eichhörnchen Squirrel Écureuil	Huhn Chicken Poulet	Hund Dog Chien	Kanarienvogel Rabbit Lapin	Katze Cat Chat	Cynomolgus Mäkeke Cynomolgus Macaque Macaque	Maus Mouse Souris	Meerschweinchen Guinea pig Cobaye	Pavian Baboon Babouin	Pferd Horse Cheval	Rind Bovine Bovin	Schwein Pig Porc	Ratte Rat Rat	Rhesus Affe Rhesus macaque Singe Rhesus	Truthahn Turkey Dinde	Schaf Sheep Mouton	Schimpanse Chimpanzee Chimpanzé	Ziege Goat Chèvre	Hamster Hamster Hamster	Alle Spezies All species Toutes espèces
<b>KNOCHEN METABOLISMUS / BONE METABOLISM / MÉTABOLISME DE L'OS</b>																							
BAP	X	X				X	X	X	X	N		X	X	X	X	N	X		X		X		
Cathepsin K	X																						
CICP/PICP	X	X				N	X		X	N			N		X	N			X		N		
Creatinine																							X
DKK-1	X	X				X																	
Helical Peptide	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
NTX Serum	(X)	X					X							X	X	X	X		X				
NTX Urine	X	X				X								X	X	X	X						
Osteocalcin Intact	X	X				N	X		X	N	X		X	X	X	N			X		X		
Osteocalcin Mouse	X									X				X									
OsteocalcinN-MID (1-43/49)	X	X																					
Osteocalcin Rat	X					X										X							
Osteopontin Human	X	X																					
Osteopontin Mouse	X								X														
Osteopontin Rat	X															X							
Osteoprotegerin (OPG) Human	X	X					N			N	N	X	N	N		N	X		N		N		
Osteoprotegerin (OPG) Human	X	X													X								
Pyridinoline (Pyd) Serum		X				X		X		X	X	X	X	X		X	X		X				
Pyrilinks (Pyd + Dpd)	X	X				X	X		X	X	X		X		X	X	X		X				
Pyrilinks D (Dpd)	X	X		X		X	X	N	X	X	X	X	X	X	X	X	X		X				
Sclerostin TECO	X	X								N							N						
sRank Human High Sensitive	X	X													X								
Total Dpd	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
TRAP5b Human	X	X								N	N	N		N		N	N		N		N		
TRAP5b Human	X															X							
<b>KNORPEL METABOLISMUS / CARTILAGE METABOLISM / MÉTABOLISME DU CARTILAGE</b>																							
C1-2C	X	X				X	(X)			X	(X)		X	X		(X)	X		(X)				
C2C, Serum/ Urine	X	X				X	X			(X)	X		X	(X)		X	X		(X)				
COMP		X											X										
CP II/PIICP	X	X				X	(X)			X	(X)		X	X		X	X		(X)				
CS-846	X	X				X	X			(X)	X		X	X		X	X		(X)				
Hyaluronic Acid TECO	X	X				X				X			X			X							

## Kreuzreaktionen - verschiedene Spezies / Cross reactivity - different species / Réactions croisées selon les Espèces

Produkt Product Produit	Zellkultur Cell culture Culture cellulaire	Human Humain Homme	Elefant Éléphant	Fischhähnchen Squid Ecrevisse	Huhn Chicken Poulet	Hund Dog	Chien	Kaninchen Rabbit	Lapin	Katze Cat	Chat	Cynomolgus Mäkeke Cynomolgus Macaque	Maus Mouse	Souris	Merschweinchen Guinea pig Cobaye	Favian Falcon Épave	Pferd Horse Cheval	Rind Bovine Bovin	Schwein Porcine Porc	Ratte Rat	Rat	Rhesus Ape Rhesus macaque Singe Rhesus	Truthahn Turkey Dinde	Schaf Sheep Mouton	Schimpanse Chimpanzee Chimpanzé	Ziege Goat Chèvre	Hamster Hamster	Hamster	Alle Spezies All species Toutes espèces		
<b>KNORPEL METABOLISMUS / CARTILAGE METABOLISM / MÉTABOLISME DU CARTILAGE</b>																															
sGAG		X																													
YKL-40	X	X										X			X							X									
<b>ENTZÜNDUNG / INFLAMMATORY / INFLAMMATION</b>																															
Chemerin	X	X																													
hs-CRP		X																													
Myeloperoxidase (MPO)		X																													
Progranulin		X																													
YKL-40	X	X										X			X							X									
<b>KALZIUM METABOLISMUS / CALCIUM METABOLISM / MÉTABOLISME DU CALCIUM</b>																															
Calcitonin Human		X																													
Calcitonin Rat	X											X										X									
Fetuin A Human	X	X																													
Fetuin A Rat	X											N										X									
FGF-23 Intact	X	X										N										N									
FGF-23 Intact (Kainos)		X										X										X									
FGF-23 (C-Term) 2nd Generation	X	X										X	N											N		N					
FGF-23 Mouse (C-Term)	X					X						X							X	X											
PTH 1-34 Anti-Human Antibody	X	X																													
PTH 1-34 Human High Sensitive	X	X																													
PTH 1-84 Bioactive Human	X	X						X	X												X	X			X						
PTH 1-84 Bioactive Rat	X					N						X							N	X											
PTH 1-84 Bovine	X	X				(X)			(X)	(X)						X		(X)	(X)	(X)						(X)					
PTH 1-84 Intact Dog	X					X																									
PTH 1-84 Intact Human		X																													
PTH 1-84 Intact Mouse	X					N						X							N	X											
PTH 1-84 Intact Rat	X					N						(X)					X		N	X											
PTH 1-84 Porcine	X	X				X						(X)					X	X	(X)												
PTH C-Terminal Human	X	X																													
PTH C-Terminal Rat	X																				X										
PTH Horse	X	X														X									N		N				
PTH Rat	X	X				(X)			X	X							X	X	X	X	X	X									
25 OH Vitamin D direct	X	X																													

**Kreuzreaktionen - verschiedene Spezies / Cross reactivity - different species / Réactions croisées selon les Espèces**

Produkt Product Produit	Zellkultur Cell culture Culture cellulaire	Human Humain Homme	Elefant Elephant Éléphant	Eichhörnchen Squirrel Écureuil	Huhn Chicken Poulet	Hund Dog Chien	Kanarienvogel Rabbit Lapin	Katze Cat Chat	Cynomolgus Macaque Macaque	Maus Mouse Souris	Meerschweinchen Guinea pig Cobaye	Pavian Baboon Babouin	Pferd Horse Cheval	Rind Bovine Bovin	Schwein Pig Porc	Ratte Rat Rat	Rhesus Affe Rhesus macaque Singe Rhesus	Truthahn Turkey Dinde	Schaf Sheep Mouton	Schimpanse Chimpanzee Chimpanzé	Ziege Goat Chèvre	Hamster Hamster Hamster	Alle Spezies All species Toutes espèces	
<b>MUSKEL – SKELETT / MUSCLE – SKELETON / MUSCLE - SQUELETTE</b>																								
Myostatin		X																						
BMP 7		X																						
<b>DIABETES &amp; ADIPOSITAS / DIABETES &amp; OBESITY / DIABÈTE &amp; OBÉSITÉ</b>																								
Adiponectin Human TECO	X	X																						
Adiponectin Mouse										X														
Adiponectin Rat																	X							
Chemerin	X	X																						
Fetuin A Human	X	X																						
Ghrelin					X	X	X	X		X	X		X	X	X	X			X		X	X		
GLP-1 Active (7-36) Human		X																						
GLP-1 Active (7-36) Mouse/Rat										X							X							
GLP-1 Total	X	X								X							X				X			
Intact Proinsulin TECO	X	X							(X)								(X)			(X)				
Leptin Human TECO	X	X																						
Leptin Mouse/Rat	X									X							X							
Resistin	X	X															N							
YKL-40	X	X							X			X					X							
<b>LEBERERKRANKUNG / LIVER DISEASE / MALADIES DU FOIE</b>																								
Hyaluronic Acid TECO	X	X				X				X			X			X								
M30-Apoptosense Chronic Liver Disease		X																						
<b>WACHSTUMSSTOFFWECHSEL / GROWTH METABOLISM / MÉTABOLISME DE LA CROISSANCE</b>																								
hGH / High Sensitive	X	X																						
IGFBP-1	X	X																						
IGFBP-2	X	X				X		X					X									X		
IGFBP-2 Mouse/Rat								X		X			X		X	X								
ALS (Acid Labile Subunit)		X																						
hGH		X											N											
IGFBP-3	X	X																						
IGFBP-3 Functional	X	X																						
IGFBP-3 Mouse/Rat										X							X							
IGF-I (BP blocked)		X			X	X	X	X		X		X	X	X	X		X		X	X	X			
IGF-I Mouse/Rat										X							X							
IGF-II	X	X																						
IGF-II										X	X						X							

## Kreuzreaktionen - verschiedene Spezies / Cross reactivity - different species / Réactions croisées selon les Espèces

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<b>KARDIOVASKULÄRE MARKER / CARDIOVASCULAR MARKER / MARQUEUR CARDIOVASCULAIRE</b>																																					
Big Endothelin	X	X											N										N	N													
BNP Fragment	X	X				N	N	N	N	N	N	N	N				N	N	N	N	N	N	N	N	N		N										
Endothelin	X	X					X			X							X				X	X															
NT-proANP	X	X											X										X														
NT-proCNP	X	X					X						X				X	X	X	X	X	X				X											
<b>OXIDATIVE STRESS MARKER / OXIDATIVE STRESS MARKER / MARQUEUR DU STRESS OXYDATIF</b>																																					
oLAB		X				N	N	N	N	N	N	N	N				N	N	N	N	N	N	N	N			N										
Oxidized LDL		X																																			
OxyStat		X																																			
<b>CELL PROLIFERATION &amp; CYTOTOXICITY ASSAY</b>																																					
EZ4U	X																																				
<b>APOPTOSE / APOPTOSIS / APOPTOSE</b>																																					
M30-Apoptosense		X									X				X								X					X									
M30-CytoDeath	X										X				X			X					X					X									
M65-EpiDeath	X	X									X	A*			X								A*	X				X									
<b>ANDERE PARAMETER / OTHER PARAMETERS / AUTRES PARAMÈTRES</b>																																					
ACTH		X											X										X														
Erythropoetin		X																																			
Fecal Calprotectin		X																																			
Prekallikrein Activator Assay (PKA)		X																																			
TPMT		X																																			
TSH Receptor Antibody 2 Gen. TECO		X																																			

## Kreuzreaktionen - verschiedene Spezies / Cross reactivity - different species / Réactions croisées selon les Espèces

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KOMPLEMENT TEST / COMPLEMENT TEST / TEST DE COMPLÈMENT >																								
AH50 Eq		X																						
Bb Plus		X							X			X						X						
C1-Inhibitor		X																						
C3a Plus		X							X			X						X						
C4d		X							X			X						X						
C5a		X																						
CH50 Eq		X							X															
CIC-C1q		X																						
CIC-C3d (Raji-Cell-Replacement)		X																						
iC3b		X																						
SC5b-9 Plus		X							X			X						X						

A\* = Humane Xenotransplantate / human xenograft / Hétérogrefe humaine

N = keine Kreuzreaktion / no cross reactivity / pas de réaction croisée

(x) = schwache Kreuzreaktion / low cross reactivity / faible réaction croisée  
= Nicht getestet / not tested / pas testé

x = Kreuzreaktion / cross reactivity / réaction croisée



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