Review of Soluble Biomarkers of Osteoarthritis: Lessons From Animal Models

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Abstract

Objective. Osteoarthritis (OA) is one of the leading causes of disability within the adult population. Currently, its diagnosis is mainly based on clinical examination and standard radiography. To date, there is no way to detect the disease at a molecular level, before the appearance of structural changes and symptoms. So an attractive alternative for monitoring OA is the measurement of biochemical markers in blood, urine, or synovial fluid, which could reflect metabolic changes in joint tissue and therefore disease onset and progression. Animal models are relevant to investigate the early stage of OA and metabolic changes occurring in joint tissues. The goal of this narrative review is to summarize the scientific data available in the literature on soluble biomarkers in animal models of OA. **Design.** A literature search was conducted using the PubMed/Medline and Scopus databases between February 1995 and December 2015. All original articles, systematic and narrative reviews published in French or in English were considered. **Results.** We summarized the data of 69 studies and proposed a classification scheme for OA biomarkers in animal studies, largely inspired by the BIPEDS classification. **Conclusions.** Studies about biomarkers and animal models indicate that some markers could be valuable to monitor OA progression and assess therapeutic response in some animal models.

Keywords

osteoarthritis, diagnosis, animal models, biomarkers, diagnostics, BIPED classification

Introduction

Osteoarthritis (OA), a disease of the entire joint, is characterized by, synovial inflammation, ligament hyperlaxity, and abnormal subchondral bone remodeling. Currently, its diagnosis is mainly based on radiographic criteria (e.g., joint space width) and clinical symptoms (e.g., pain and loss of function).^{1,2} However, a diagnosis of the disease at a preradiographic stage, when the first molecular changes occur in joint tissues, would be helpful for a better management of the disease. In this purpose, imaging techniques such as ultrasound and magnetic resonance imaging (MRI) have been developed, but their use is limited by their cost and availability. An alternative is the measurement of soluble biomarkers in biological fluids, not only to early diagnose but also to predict the progression of the disease and to assess the effectiveness of treatments.² Furthermore, soluble biomarkers can be used as “drug development tools” at preclinical stages and throughout clinical development in phase I to IV trials.³ At the preclinical phases, the 3 major applications of biomarkers are their use in toxicology studies, in the selection of appropriate animal models and lead

compounds and in the identification of the mechanisms of action of an intervention.

In 2006, the BIPED classification was created with the aim of providing a common framework for communication in the field.¹ The acronym “BIPED” stands for Burden of disease, Investigate, Prognostic, Efficacy of intervention, and Diagnostic. The “BIPED” classification was subsequently revised to “BIPEDS,” including “Safety.” Many reviews on biochemical markers using the BIPEDS classification have been published.^{2,4–6} In those reviews, biomarkers have been classified based on data coming from human clinical trials. These soluble biomarkers have never been

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classified based on information coming from animal studies, although animal models are valuable to validate biomarkers.

Currently, several animal models of OA in rabbits, dogs, rats, mice, guinea pigs, horses are conventionally used, mainly to test therapeutic modalities. Three general categories exist for *in vivo* OA models: spontaneously occurring OA (including genetically modified animals), mechanically induced accelerated OA by surgery- (e.g., anterior cruciate ligament transection [ACLT]), trauma-, or fracture-induced posttraumatic OA,^{7,8} and chemically induced OA by intra-articular injection of chondrotoxic (e.g., iodoacetic acid), pro-inflammatory (e.g., interleukin-1 β [IL-1 β]), or catabolic (e.g., matrix metalloproteinase [MMP]) substances.⁹⁻¹¹

The goal of this narrative review was to synthesize scientific literature on soluble biomarkers measured in biological fluids in different animal models of OA and to classify these biomarkers according the BIPEDS classification.

Methods

The literature search was performed using the PubMed/Medline and Scopus databases between February 1995 and December 2015. Searches were performed using the search terms “biomarkers,” “osteoarthritis,” and “animal model.” The following word association was used in Medline: [“Biomarkers” OR “Markers”] AND [“Osteoarthritis”] AND [“Animal model”]. In the Scopus database, the code TITLE-ABS-KEY, a combined field that searches article titles, abstracts, and keywords was used. The following words association was used: biomarkers and osteoarthritis and animal model. All original papers, systematic and narrative reviews published in French or in English between February 1995 and December 2015 were included.

The first author (C.B.L.) selected the articles on the basis of the title and abstract and then assessed whether the study met the inclusion criteria. Then the full article was retrieved. The flow diagram for selection of studies is presented in **Figure 1**. Data are shown according to the species. By this way, the knowledge on biomarkers in a particular model is summarized. This approach was considered as useful for researchers using that particular model. Thereafter, we propose a classification of these biomarkers according their ability to predict the progression of the disease, to monitor the effect of a treatment or to assess the severity of OA lesions. The classification was performed based on the following definitions:

- Burden of disease (B): Biochemical marker concentrations correlated statistically and significantly with parameters of disease severity (e.g., histological, macroscopic, imaging score)
- Investigative (I): Biochemical marker concentrations that have not met criteria for another category yet

Figure 1. Flow diagram for selection of study.

- Prognostic (P): Biochemical marker concentrations correlated significantly with the progression of disease severity parameters
- Efficacy of intervention (E): Biochemical marker concentrations differed statistically and significantly between animals with or without treatment, or before and after treatment with animals
- Diagnostic (D): Biomarker concentrations were statistically significantly different between animals that developed the disease and those that did not
- Safety (S): Biomarker able to reflect tissue and/or organ toxicity of an agent or intervention

Results

Rabbits

In rabbits, a variety of surgical procedures on menisci and ligaments were described to induce knee joint laxity, and subsequently abnormal mechanical strain. These surgical procedures included cruciate ligament transection, with or without medial and collateral ligament section, total or partial meniscectomy, and creation of bucket-handled tears of the meniscus.

Prostaglandin (PG) E2 and C-reactive protein (CRP) serum levels were significantly increased 6 weeks after ACLT.¹² In contrast, no significant differences between the pre-ACLT and the post-ACLT values of serum keratan sulfate (KS), hyaluronic acid (HA), and chondroitin sulfate (CS) 846 epitope were observed.¹³ However, a significant correlation between histological score and post-ACLT HA concentration was found for the medial femoral condyle. In

contrast, thrombin-cleaved osteopontin (OPN) levels in synovial fluid progressively and significantly increased after posterior cruciate ligament transection (PCLT) to reach a maximum after 4 weeks.¹⁴ A positive significant correlation was found between thrombin-cleaved OPN synovial fluid levels and the macroscopic and histological scores.¹⁴ In a longitudinal study, Duclos *et al.*¹⁵ followed the evolution of serum C-telopeptide of type II collagen (CTX-II) concentration in adult (10-month-old) and growing animals (2-month-old) after ACLT. CTX-II was significantly higher in ACLT-adult rabbits than nonoperated adult animals, while in young animals CTX-II was similar in both groups. In adult ACLT-rabbits, the CTX-II evolution showed 2 distinct elevations, one during the 3 first weeks after surgery, followed by a decreased until the fourth week, and a second peak at the twelfth week. In young animals, CTX-II levels increased rapidly and reached a maximum at week 2 and then, decreased over time until the week 20.¹⁵ In experimental OA induced by anterior and posterior cruciate ligaments transection and medial meniscus excision, Zuo *et al.*¹⁶ investigated the evolution of serum bone morphogenetic protein-2 (BMP-2), CTX-II, and cartilage oligomeric protein (COMP). The BMP-2 levels were increased at various times after surgery, and significant differences were observed between the OA and the control groups. CTX-II levels were significantly elevated at different intervals after surgery. Significant differences in the COMP levels from W2 to W20 were observed between the OA and the control groups.¹⁶

Another research observed a significant increase of CTX-II in synovial fluid in rabbit with medial meniscectomy.¹⁷ Moreover, highly significant correlations between synovial fluid CTX-II levels and the macroscopic and microscopic scoring results were found.

Rats

In rats, a variety of experimental OA model exist. The most commonly used rat OA model is the surgically induced medial meniscal tear (MMT), ACLT alone or in combination with (partial) medial meniscectomy (ACLT + pMMx).¹⁸ Intra-articular monoiodoacetate (MIA) injection is also frequently used. Ovariectomy in rats was proposed to mimic postmenopausal OA in humans.¹⁹

In ACLT model, serum level of C2C, a biomarker of type II collagen degradation, was decreased by moderate running on a motorized treadmill (at a speed of 18 m/min for 30 min/d, 3 days per week) while the serum level of CPII, a biomarker of type II synthesis, was decreased by intense running (at a speed of 18 m/min for 60 min/d, 3 d/wk) 2 and 4 weeks after surgery.²⁰ In addition, in all the groups at 2 and 4 weeks after surgery, the Osteoarthritis Research Society International (OARSI) histological score was slightly but significantly correlated positively with the C2C/

CPII ratios.¹⁵ In ACLT rats, serum CTX-II and COMP levels were increased and only CTX-II correlated with the modified Mankin score.²¹

In ACLT and MMX rat model, low-intensity pulsed ultrasound (LIPUS) does not affect the degradation of type II collagen at 7 and 14 days after the operation. In contrast, after 28 days, CPII was significantly increased in LIPUS group compared to control group.²²

The oral administration of high dose of glucosamine hydrochloride (1000 mg/kg/d for 56 days) prevented the increase of CTX-II serum levels²³ and high dose of alendronate (ALN), a potent inhibitor of osteoclastic bone resorption, significantly decreased serum levels of COMP in rats with ACLT-induced OA.²⁴ Another study investigated the effects of physical exercise in healthy young, adult, and aged rats on the expression of lubricin. As expected, the results showed that lubricin levels in rat synovial fluid were decreased with aging.²⁵

In ACLT/pMMX rat model, treatment of OA rat knees with pharmacologic inhibitors of transforming growth factor- α (TGF- α) (AG1478) and CCL2 (RS504393) signaling pathway, significantly increased serum CPII levels but only AG1478 significantly reduced serum C2C levels and the C2C/CPII ratio compared with untreated OA rats. This finding suggested that TGF- α /CCL2 is an important promoter of articular cartilage degradation in posttraumatic OA.²⁶ In another study, oral administration of a high dose of BIS076 (245 mg/kg), a natural porcine cartilage extract associated with hydroxyapatite and vitamin D₃, reduced serum levels of CTX-II and COMP, as well as MMP-3 in OVX and ACLT rats.²⁷

In ovariectomized (OVX) rats, serum COMP level was decreased by a combination of estrogen and progesterone hormones 10 weeks after OVX surgery.²⁸ In another study, CTX-II was also increased in the OVX rats compared with the sham-operated group.¹⁹

Several inflammatory mediators were investigated in MIA-induced OA in rats. Orita *et al.*²⁹ showed a significant increase in the concentrations of tumor necrosis factor- α (TNF- α) and interleukine-6 (IL-6) starting on day 1 after the injection which reached a maximum at day 4, and thereafter, gradually decreased as the post injection period progressed. The concentration of nerve growth factor (NGF) was significantly increased 1 week after the injection.²⁹ In 2010, Swearingen *et al.*³⁰ showed a time-dependent increase in the release of aggrecanase-cleaved aggrecan (NITEGE and ARGS neopeptides) into the synovial fluid of the MIA OA knee joints.

These results were consistent with those of Dufield *et al.*,¹⁵ showing that the levels of aggrecan neopeptides ARGS and AGE3 were elevated in the synovial fluid of rat knee after intraarticular (IA) injection of TNF- α .³¹ Moreover, synovial fluid ARGS levels increased following surgically induced joint instability in the rat meniscal tear (MT) model.³²

In a MIA-induced OA model in rats, serum glycosaminoglycan (GAG) levels significantly decreased after administration of diacetyl rhein.³³

Rats with arthritis induced by Freund's complete adjuvant (FCA) showed elevated levels of synovial fluid IL-1 β , IL-6, and TNF compared with the control group. In MIA rat model, a biphasic elevation of synovial fluid concentration of monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein-3 α (MIP-3 α), and IL-6 were observed.³⁴

Mice

In STR/ort mice, serum level of MMP-3 increased from week 7 to 9.³⁵ In addition, high levels of urinary CTX-II were observed in the STR OA subgroup compared with the STR non-OA.³⁶ In a study that compared wild-type (WT) and biglycan/fibromodulin double-deficient mice, the mean serum concentrations of C2C and Coll2-1 were higher in biglycan/fibromodulin double-deficient mice than in the WT mice at all time points. Mean serum concentrations of Coll2-1NO₂ increased progressively with age in WT mice but not in biglycan/fibromodulin double-deficient mice.³⁷

In mice subjected to noninvasive ACL rupture via tibial compression, serum level of CTX-I was increased in injured mice at all time points compared to uninjured mice. In contrast, serum procollagen type 1 N-terminal propeptide (PINP) levels were not found to be significantly different between any of the experimental groups at any time points.⁸

COMP, MMP-3, and MMP-9 synovial fluid levels also increased in mice after acute joint injury induced by intra-articular fracture.^{38,39}

In a study of Porta *et al.*,⁴⁰ the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) did not modify serum levels of MIA and control groups.

Other studies examined the involvement of inflammatory and oxidative stress biomarkers in the pathogenesis of OA. They demonstrated an increase in serum levels of IL-6, IL-1 β , and TNF- α both in STR/ort mice and in a model of intra-articular fracture.^{41,42} In obese and hyperlipidemic STR mice, serum levels of malondialdehyde (MDA), CTX-II and CPII were elevated compared with levels in the control CBA mice.⁴³ Notably, the level of MDA was correlated with that of CTX-II, but not of CPII.

Guinea Pigs

The Dunkin-Hartley guinea pig strain displays a spontaneous onset of progressive degenerative changes in the knee joints that mimics the pathophysiological processes observed in primary human OA.^{44,45} KS and COMP levels were elevated in the knees of Hartley (OA-prone) compared with the strain 13 (OA-resistant) animals after 2 months of age.^{45,46} Moreover, synovial fluid concentration of KS and

COMP correlated positively with the severity of histological damage in both strains.⁴⁶ In addition, the levels of serum C2C/CPII, representing the ratio of type II collagen degradation and synthesis, were elevated in the OA-prone Hartley compared with strain 13 animal after 4 months of age.^{46,47} Huebner *et al.*⁴⁷ observed abnormalities in collagen network beginning at 2 months of age. Among CTX-II, Coll2-1, and C2C, only serum Coll2-1 increased coincidentally with the appearance of the early disruption of the collagen fibril and correlated with histological severity.⁴⁷ In Dunkin-Hartley guinea pig with ACLT, C2C, stromal cell-derived factor (SDF-1), GAG, MMP-13, and IL-1 β were elevated in the synovial fluid compared with the control group.⁴⁸ Significant positive correlations were found between the SDF-1, GAG, and C2C synovial fluid concentrations and the Mankin score. Huebner *et al.*^{46,49} showed that the Hartley strain exemplified a serum/chemokine profile indicative of a pro-inflammatory state compared with Strain 13. Among the cytokines and chemokines elevated in the Hartley strain, they found IL-6, IL-17, IL-8, and TNF- α . In other research, they also demonstrated that transglutaminase isoenzyme 2 (TG2) concentrations were correlated with synovial fluid COMP.⁵⁰

In another study, dietary omega-3 (n-3) polyunsaturated fatty acid (PUFA) reduced OA in the OA-prone Dunkin-Hartley compared with OA-resistant Bristol Strain1-2 (BS2).⁵¹ McDougall *et al.*⁵² showed that urine concentrations of CTX-II were reduced by cathepsin K inhibitor. Horcjada *et al.*⁴⁴ compared the effects of 3 polyphenols oleuropein, rutin, and curcumin on joint ageing and OA development. Coll2-1 was decreased by rutin and the combination of rutin/curcumin. Fib3-1 and Fib3-2 (2 specific peptides of fibulin-3) were only decreased by the rutin/curcumin mixture, while Coll2-1NO₂ was significantly decreased by all treatments. Moreover, Coll2-1NO₂, Fib3-1, and Fib3-2 were correlated with histological global OA score while Coll2-1NO₂ and Fib3-2 also correlated with synovial histological score.⁴⁴ In a study of metabolic fingerprints, Lamers *et al.*⁵³ showed that purine metabolism is of major importance in OA.

Dogs

In canines, synovial fluid CTX-II levels were elevated early after unilateral cruciate ligament transection.⁵⁴ Coll2-1 and Coll2-1NO₂ also appeared to be OA biomarkers of early structural changes. Indeed, following ACL transection, a significant and rapid increase (2 weeks and 6 weeks, respectively) of these markers was observed in serum.⁵⁵ Moreover, a positive and highly significant correlation was found between Coll2-1 concentrations and the global macroscopic score. Coll2-1NO₂ concentrations were found to be significantly and positively correlated with the size of the osteophytes.⁵⁵ The ratio Coll2-1NO₂/Coll2-1 was negatively correlated with

macroscopic parameters and the histological score, but not with the osteophyte size.

In beagle dogs subjected to cranial cruciate ligament transection (CrCLt), synovial fluid levels of AGEg neoepitope were significantly elevated compared with either contralateral knee or contralateral knee in sham-operated animals.³¹ In a canine model of posttraumatic OA, serum level of KS and HA rise between 1 to 3 weeks after ACLT.⁵⁶

In ACLT dog model, tenascin-C (TN-C), an extracellular matrix glycoprotein, increased in synovial fluid 1 month after ACLT compared with the control group.⁵⁷

In dogs undergoing CrCLt, bone specific alkaline phosphatase (BAP) activity increased in synovial fluid with time after transection and zoledronic acid (25 µg/kg) partially inhibited this change. Synovial fluid C1,2C, CS, and serum CPII levels were significantly increased after transection. Zoledronic had no effects on these biomarkers. Interestingly, significant correlations were detected between synovial fluid BAP activity, serum CPII concentration and synovial fluid CS concentration and radiographic findings when all groups were combined.⁵⁸ In a study published in 2009, Connor *et al.*⁵⁹ showed that a cathepsin K inhibitor (SB-553484) reduced the levels of urinary CTX-I and CTX-II in mature female beagle dogs with partial medial meniscectomy. PF152, a novel highly selective MMP-13 inhibitor, reduced joint lesions in adult dogs with OA induced by partial MMx and decreased biomarkers of type II collagen neoepitope (TIINE) and aggrecan (neoepitopes ARGn or AGEg) degradation.⁶⁰ In another study, high dose of vitamin E (400 UI/animal/day) administrated orally in OA dogs with CrCL transection, reduced inflammation markers including NO_x, IL-1β, and PGE₂ in synovial fluid.⁶¹

During the progression of OA induced by a surgically created medial patellar luxation (MPL) in the left stifle of dog, synovial fluid and serum TIMP-2 levels were significantly decreased, whereas MMP-2 in synovial fluid was significantly increased.⁶² In dogs referred to the clinic, synovial fluid TIMP-1 level was increased in dogs with ACL rupture (ACLR), osteochondritis dissecans (OCD), fragmented coronoid process (FPC), and hip dysplasia (HD) compared to dogs without musculoskeletal diseases.⁶³ Carprofen (2.2 and 4.4 mg/kg), a nonsteroidal inflammatory drug, had no effect on MMP activity in ACLT-induced OA dogs.⁶⁴ Synovial fluid PGE₂ levels were dose-dependently diminished by celecoxib in beagle dog OA induced in one knee according to the groove model showing a neutral effect of celecoxib on the characteristics of experimentally induced OA *in vivo*.⁶⁵

In 2013, Gharbi *et al.*⁶⁶ provided a proteome mapping of dog serum after ACLT surgery. A number of proteins, such as fetuin B and complement C3, were increased in dog OA serum whereas others, such as hyaluronan binding protein 2, complement C1s and C4 were decreased.⁶⁶

Another study investigated the effects of total MMx on biomarkers in synovial lavage fluid. COMP and KS were significantly increased.⁶⁷ Serum levels of COMP and urinary levels of CTX-II were also increased after a joint bleed mimicked by injecting freshly collected (vena puncture) autologous blood intra-articularly in the knee.⁶⁸ Another research observed that a subcutaneous injection of calcitonin (3 units per kg of body weight) significantly decreased the urinary excretion of D-pyridinoline (D-Pyr) and blood levels of KS and HA in dogs after ACLT.⁶⁹

Horses

The published studies investigated the effect of exercises on OA incidence and progression. A study showed that the subchondral bone lysis on standard X-ray was strongly correlated with CPII, and that subchondral bone proliferation at the joint capsule attachment was strongly correlated with PGE₂.⁷⁰ In addition, radial carpal bone edema detected using MRI correlated well with C1,2C increase in the synovial fluid. Synovial fluid levels of Coll2-1 were significantly elevated in tarsocrural joints affected with osteochondrosis compared with unaffected joints.^{71,72} Another study compared sera of box-stall rest, pasture and trained foals.⁷³ Lower serum levels of CPII and higher levels of CTX-I were found in the trained foals compared with the other groups. It was noticed that serum levels of CTX-I and CPII significantly increased with age in all groups.⁷³ In another study, exercise induced a significant increase in the synovial fluid CPII, GAG, C1C, C2C, CTX1 concentrations.⁷⁴ Finally, Fuller *et al.*⁷⁵ in 2010 demonstrated that levels of synovial fluid BAP were significantly higher in the clinically active joint than in the contralateral joint, while KS, GAG, and HA were significantly lower in OA than in the contralateral joint. These biomarkers also showed good correlation with arthroscopy score of cartilage damage.

In an equine metacarpophalangeal groove model, Manninchedda *et al.*⁷⁶ showed that synovial fluid levels of C2C, COMP, CTX-I, osteocalcin (OC), and CS-846 were not significantly different between groups.⁷⁶

Sheep

In sheep undergoing meniscal destabilization (MD), increased levels of dimethyl sulfone (DMSO₂) were observed in serum after 4 weeks by nuclear magnetic resonance spectroscopy, while in ACLT-induced OA sheep, an increase of 3-methylhistidine and a decrease of branched chain amino acids, comprising the essential amino acids valine, leucine, and isoleucine (BCAAs) were identified.⁷⁷ In another study, synovial fluid of MMP-2 was detected 2 and 4 weeks postoperatively in a temporomandibular joint (TMJ) model of OA in sheep.⁷⁸ Moreover, MMP-2 activity correlated with initial articular cartilage destruction rather

than with the progression of OA. In sheep responses to ACL reconstruction injury, a metabolic profiling of synovial fluid identified 6 metabolites such as isobutyrate, glucose, hydroxyproline, asparagine, serine, and uridine, which could be suggested as biomarkers for early postinjury degenerative changes in the knee joints.⁷⁹

Discussion

We have attempted to gather all animal studies in English and French published between 1995 and 2015 and investigating soluble biomarkers in OA. A summary is available in **Table 1**. We also proposed a classification scheme for these biomarkers, largely inspired of the BIPEDS classification (**Table 1**).

In all animal studies that we have selected, soluble biomarkers are viewed as useful indicators of joint tissue metabolism, particularly of bone and cartilage. Some of them are well correlated with histological lesion severity, and can be considered as a burden of disease biomarkers, but none of them can be classified in the prognostic and safety categories.¹⁰

One major concern in animal studies is the method to harvest synovial fluid. Although the collection of synovial fluid in animal models can be challenging, synovial fluid offers the most proximal source for joint turnover information.⁸⁰ The majority of studies in animals have utilized the technique of joint lavage. However, other methods exist for collecting specimens depending on the animal size. In small animal like mice, rats or guinea pigs, Whatman paper can be used to absorb synovial fluid.³⁹ Recently, Seifer *et al.*³⁹ described a method for synovial fluid recovery using Melgisorb (Tendra, Sweden), a calcium sodium alginate compound (CSAC), used as a dressing on wounds to absorb heavy exudate. In dogs, joint lavage with saline has been used to collect synovial fluid and the dilution effect of the lavage has been corrected by normalization to serum urea.⁸⁰

Another concern is the age of animals used and the presence or not of cartilage growth plate. In fact, some of the studies reported in this review were conducted in young immature animals, meaning that biomarker levels can be confounded by the presence of growth plate. This should be kept in mind when interpreting results.

Burden of Disease Biomarkers

Numerous biomarkers measured in serum or synovial fluids were correlated with histologic or macroscopic cartilage lesions severity score in different species like rat, mouse, dog, or guinea pig. Interestingly, some of them increased rapidly after the onset of the disease, before the appearance of the histological structural changes. Most particularly, Coll2-1 is particularly precocious. In serum of guinea pig, Coll2-1 increase was coincident with the early disruption of

the collagen fibril visible by birefringence before the appearance of structural changes observable by conventional microscopy. This finding suggests that the Coll2-1 biomarker may be an early indicator of the onset of chondropathy in the guinea pig.⁴⁷ This also means that biomarkers of type II collagen degradation could be considered as surrogate biomarkers of histology in animal study. This could be advantageous for studying the kinetic of drug candidate efficacy and reduce the number of euthanized animals.

Prognostic Biomarkers

Demonstrating the prognostic value of biomarkers in animal models remains very challenging because in most models, OA is induced using well-standardized methods and lesion progression is predictable and homogenous. To be recognized as a prognostic biomarker, a biomarker has to predict the incidence or the progression of the disease. To our knowledge, there is no biomarker that can be classified in this category based on animal study. This would require a prospective study including pets with OA risk factors and different disease progression rate. This type of information could be helpful for the daily management of care in pets.

Efficacy of Intervention

Soluble biomarkers have been largely used as drug development tools in OA. Not exhaustively, we can mention in rats, diacetylrhein that reduces serum GAG levels,³³ oral glucosamine hydrochloride that prevents the increase of CTX-II levels²³ and alendronate, a potent inhibitor of osteoclastic bone resorption that decreases serum levels of COMP.²⁴ In guinea pigs, prolonged (1 month) administration of cathepsin K inhibitor reduces uCTX-II excretion and oleuropein decreases Coll2-1.^{44,52} In dogs, cathepsin K decreases urinary CTX-I and CTX-II⁵⁹ and PF152 reduces TIINE and aggrecan (neopeptides ARGN or AGE).⁶⁰ Another study in dogs with OA using a supplement with a high dose of vitamin E showed a reduction in inflammation joint markers such as PGE₂ and IL-1 β .⁶¹ These findings indicate that a large panel of biomarkers should be used in the preclinical phase of drugs or other intervention development. This approach would allow a better understanding of their mechanisms of action but also the early detection of potential safety issues, and hence could enhance the drug development efficiency, and possibly reduce the overall cost of OA clinical trial. This also allows the selection of one or more biomarkers sensitive to drug activity that can be used in the next phases of drugs development. For these reasons, the US Food and Drug Administration (FDA), the Osteoarthritis Research Society International (OARSI) and European Medicine Agency (EMA), recently published guidelines recommending a higher level of integration of biomarkers

Table 1. Summary of Studies on Biomarkers in Animal Models of Surgical Osteoarthritis.

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Rabbits							
Zuo et al., 2015	36 mature (24-week-old) New Zealand White male rabbits. Control animals ($n = 3$ for each group, total = 18), 6 experimental OA groups ($n = 3$ for each group; W2, W4, W8, W12, W16, W20)	Hulth method: • medial parapatellar incision, • transecting anterior and posterior cruciate ligaments, • excising medial meniscus	BMP-2	s		A first peak at W2 and a second at W16 with significant differences between the OA and the control group Highest level at W16 Elevated levels from W2 to W20 after surgery, and significant differences compared with the control groups	ELISA, Wuhan EIAab Science Co., Ltd (sensitivity: ≥ 10 pg/mL)
Gao et al., 2013	48 mature (6 months old) white rabbits	PCLT	OPN	sf lavage—injection of 2 ml of sterile Ringer's solution (not corrected for dilution using the urea method)	B	Significant increase after PCLT to reach a maximum after 4 weeks	ELISA, Uscnlife Science & Technology Company, China
Ohnishi et al., 2013							
Ohnishi et al., 2013	12 female Japanese albino rabbits (12 weeks of age)	ACLT	KS	s		No significant differences between pre-ACLT and post-ACLT values	ELISA, Seikagaku Biobusiness, Tokyo, Japan
			HA	s	B	Significant correlation between histological score and post-ACLT values for medial femoral condyle, at week 16	ELISA, Biotech Trading Partners, Inc., Encinitas, CA, USA
			CS	s			ELISA, IBEX Technologies, Inc., Montreal, Canada
Oprenyeszk et al. (2013)	21 HYLAL albino rabbits (21 weeks old) divided into 3 groups: saline ($n = 7$), H hydrogel alone ($n = 7$), AC beads in H hydrogel ($n = 7$)	ACLT	PGE ₂	s		Modified serum levels of PGE ₂ and CRP 6 week after ACLT, compared with the pre-values	ELISA, DetectX, Ann Arbor, Michigan, USA
Duclos et al., 2010	27 male New Zealand rabbits. Skeletally mature (10 months old, $n = 12$), young (2 months old, $n = 15$)	ACLT	CRP	s			Immunoturbidimetry (DiAgam, Ghislenghien, Belgium)
			CTX-II	s	B	Adult rabbits: sCTX-II: significant increase in ACLT rabbits than nonoperated adult animals at weeks 3, 6, 10, 12, 14, 16, and 20 Young rabbits: sCTX-II: similar levels in both groups	ELISA, Nordic Bioscience, Herlev, Denmark

(continued)

Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Lindhorst et al., 2005	51 male New Zealand White rabbits (10 months old)	MMx	CTX-II	sf lavage, injection of 1.5 mL of sterile Ringer's solution (not corrected for dilution using the urea method)	B	Significant increase	ELISA, Matyas 2004 (sf lavage were diluted 1/4 with assay buffer)
Rats							
Appleton et al., 2015	60 male Sprague-Dawley rats (11 to 12 weeks old), 3 time points. 4 groups (N = 5 per treatment group per time point): Sham surgery, OA surgery, AG1478 + OA surgery, RS504393 + OA surgery	ACLT - pMMx	CPII	s	B	Significant decrease in posttraumatic OA surgery compared with sham operated controls	ELISA, Ibex Pharmaceuticals
			C2C	s	B	C2C:CPII ratio: significant increase in posttraumatic OA surgery compared with sham-operated controls	
Ferrandiz et al., 2015	Female Wistar rats (10 weeks old, n = 15 per group). Sham, OVX and OVX + ACLT (3 groups: control, T1: BIS076 163.5 mg/kg and T2: BIS076 245 mg/kg)	ACLT - OVX	CTX-II	s		Enhanced levels with respect to sham-operated rats	ELISA, Nordic Bioscience, Herlev, Denmark (sensitivity of 6.3 pg/mL)
			COMP	s			ELISA, MD Biosciences, Zürich, Switzerland (sensitivity <0.2 U/L)
			MMP-3	s		Significant decrease by BIS076 treatment at the higher dose in control rats	ELISA, R&D Systems (sensitivity of 19.0 pg/mL)
Musumeci et al., 2015	30 male albino Wistar rats. 6 groups (n = 5 per group): young rats (3 months old) sedentary, young rats (3 months old) subject to physical activity, adult rats (12 months old) sedentary, adult rats (12 months old) physical activity, older rats (24 months old) sedentary and older rats (24 months old) physical activity	Common diet, sedentary	lubricin	sf lavage—injection of 100 µL of normal saline in the joint capsule, then aspirating 20 µL of sf (not corrected for dilution using the urea method)		Strikingly decreased with aging	ELISA, Pierce Biotechnology, Rockford, Illinois, USA)

(continued)

Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Finn et al., 2014	Sprague-Dawley (FCA, n = 48) and Lewis male rats (MIA, n = 88)	MIA or FCA	MCP-I MIP-3 α	sf lavage (25 μ L x 4, 0.05 M EDTA, pH 7.5, Sigma) (not corrected for dilution using the urea method)		A biphasic elevation of concentrations after injection of MIA into the joint Elevated levels after FCA injection compared with the control group	ELISA, Meso Scale Discovery, Rockville, MD, USA (sf sample were diluted 1/4 in assay diluents)
Dhanashwar et al., 2013	60 Wistar albino male rats	MIA	IL-1 β IL-6 TNF GAG	s	E	Significant decrease in sGAG after diacetyl rhein administration	ELISA, Erba Diagnostics, India
Yamaguchi et al., 2013	47 Wistar rats (8 weeks old). ACLT rats (3 groups: control group (n = 12) and 2 running groups (moderate n = 11, intense n = 12)) and non-ACLT rats, n = 12 (naïve group)	ACLT	C2C	s	B	Decrease by moderate running at 2 and 4 weeks after surgery	ELISA, Ibex Pharmaceuticals, Montreal, Canada
Yang et al., 2012	37 female Sprague-Dawley rats (7 months old). 5 groups: F-sham control, OVX, OVX- β -estradiol, OVX-progesterone, OVX β -estradiol and progesterone	OVX	CPII COMP	s s	B E	Decrease by intense running at 2 and 4 weeks after surgery Decrease in the group treated with a combination of estrogen and progesterone hormones 10 weeks after surgery	ELISA, MD Biosciences, St. Paul, Minnesota, USA
			OC CTX-I	s s	B, E B, E	Decreased in both hormonal treatments (estrogen and progesterone)	ELISA, Nordic A/S, Herlev, Denmark
Braza-Boils et al., 2011	Male Wistar rats. 2 groups: sham operation and ACLT (n = 10 per group at 24 hours, 6 weeks or 12 weeks)	ACLT	CTX-II COMP HA	s s s	B B B	Progressively increased with the development of OA Levels did not change during the progression of OA	ELISA, Cartilpas, Nordic Bioscience, Herlev, Denmark (sensitivity of 6.3 pg/mL) ELISA, MD Biosciences, Zurich, Switzerland (sensitivity <0.2 U/L) ELISA, Echelon Biosciences, Salt Lake City, Utah (sensitivity 50 ng/mL)

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Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Chockalingam et al., 2011	30 male Lewis rats	MT	ARGS neopeptides	sf lavage (sf urea levels were used to correct sfARGS-aggregran values for dilution)	E	sfARGS-aggregran increased following surgically induced joint instability	A developed ELISA
Orita et al., 2011	60 female Sprague-Dawley rats (6 weeks old)	MIA	TNF- α IL-6	Local tissues		Significant increase starting on day 1 after the injection with a peak on day 4	ELISA, R&D Systems, Minneapolis, Minnesota (sensitivity 14, <5 pg/mL)
			NGF			Significantly increased at 1 week after the injection	ELISA, Millipore, Temecula, California (sensitivity 10 pg/mL)
Duffield et al., 2010	96 male Sprague-Dawley rats. 2 groups (n = 6 per group at various times = 8): vehicle vs. 10 μ g TNF- α	TNF- α	Aggregran (neopeptides ARGS and AGE)	sf lavage with 100 μ L saline (not corrected for dilution using the urea method)	E	Elevated levels of sf aggregran	An immunoaffinity liquid chromatography–tandem mass spectrometry assay
Naito et al., 2010 (a)	20 male Sprague-Dawley rats (10 weeks old). 3 groups: sham operated (n = 5), ACLT + MMx without LIPUS (n = 5), ACLT + LIPUS (n = 5)	ACLT - MMx	CTX-II	s	B	Elevated but no significant difference between +LIPUS and –LIPUS groups	ELISA, Nordic Bioscience Diagnostic A/S, Herlev, Denmark
			CPII	s	B	Significantly increased in LIPUS group compared with control group	ELISA, IBEX technologies Inc., Montreal, Canada
Naito et al., 2010 (b)	18 male Sprague-Dawley rats (10 weeks old). 3 groups: sham operated (n = 6), ACLT without glucosamine (n = 6), ACLT with glucosamine (n = 6)	ACLT	CTX-II	s	B	The oral administration of glucosamine hydrochloride prevented the increase of sCTX-II	ELISA, Nordic Bioscience Diagnostic A/S, Herlev, Denmark
Swearingen et al., 2010	12 male Lewis rats (7-8 weeks old). 2 groups: MIA or saline	MIA	NITEGE ARGS neopeptides	sf lavage with 200 μ L saline (not corrected for dilution using the urea method)	I	A time-dependent increase in sf NITEGE, sf ARGS neopeptides	ELISA
Nemirovskiy et al., 2009	5 male Sprague-Dawley rats	LPS	3-NT	p		Significant increase of p3-NT after LPS stimulation	Immunoaffinity-based liquid chromatography–tandem mass spectrometry method

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Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Hayami et al., 2004	60 male Sprague-Dawley rats (20 weeks old). 5 groups (<i>n</i> = 12 per group): ACLT + vehicle treatment; ACLT + low-dose of ALN; ACLT + high-dose of ALN; sham-operated + vehicle treatment; sham-operated + high-dose ALN treatment	ACLT	COMP	s	B	Significantly diminished by high dose of alendronate (ALN)	ELISA, AnaMar Medical, Uppsala, Sweden)
Hoegh-Andersen et al., 2004	20 virgin female Sprague-Dawley rats (5 or 7 months old)	OVX	CTX-II	s		sCTX-II increased in OVX rats	ELISA, Cartilaps, Nordic Bioscience Diagnostics A/S
Mice							
Khorasani et al., 2015	90 C57BL/6N female mice (10 weeks old). 3 groups: vehicle solution, low dose of alendronate (ALN) (40 µg/kg/dose), high dose of ALN (1,000 µg/kg/dose) at weeks 7, 14, and 56	Tibial compression overload or sham injury	CTX-I	s		Increased levels in injured mice at all time points compared to uninjured mice for all treatment groups	ELISA, CUSABio kit, Wuhan Huamai Biotech Co., Wuhan, China
			PINP	s		No significant differences in levels of serum between any of the experimental groups at any time points	
						No significant differences in levels of MIA and control groups	
Porta et al., 2015	54 Swiss albino CBIKO and CB2KO and WT mice (2-3 months old)	MIA	AEA 2-AG	s s		No significant differences in levels of MIA and control groups	Liquid chromatography-mass spectrometry method
Braza-Boils et al., 2012	10 male STR/ort and 10 male CBA mice (6 weeks old)	STR/ort	MMP-3	s	B	Increased levels from week 7 to 9	ELISA, MD Biosciences, Zurich, Switzerland (sensitivity <0.2 U/L)
Kyostio-Moore et al., 2011	Male STR/ort and CBA mice euthanized at 20 or 40 weeks old (STR/ort, <i>n</i> = 8 or 9; CBA, <i>n</i> = 5 or 6)	STR/ort	IL-1β	s	B	Significant increase at week 40	2 Iplex bead assay (Beadlyte, Upstate, Temecula, California)
Lewis et al., 2011	56 male C57BL/6 (8 weeks old). 4 groups: low-energy fractures (<i>n</i> = 25), high-energy fractures (<i>n</i> = 25), nonfractured controls (<i>n</i> = 3), shams (<i>n</i> =3)	STR/ort	IL-6 IL-1β TNF	s s s		No significant effect of fracture on serum levels of sIL-6, sIL-1β, sTNF at any time point after fracture increase	Bio-Plex Mouse cytokine 23-plex Panel multiplex bead assay, Bio-Rad Laboratories, Inc., Hercules, CA (sample dilution 1/2)

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Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Watari <i>et al.</i> , 2011	10 STR male mice (7 weeks old) and 10 CBA male mice (7 weeks old)	STR/ort	CTX-II CPII MDA	s s s	D D D	Elevated serum levels in STR/ort mice compared with levels in CBA mice	ELISA, Cartilaps, Nordic Bioscience ELISA, IBEX Technologies, Inc., Montreal, Canada ELISA, TBARS, Cayman Chemical Company, USA
Chia <i>et al.</i> , 2010	43 Male BALB/c mice (aged 6-8 weeks). 2 groups: sham-operated ($n = 19$ at 4, 8, and 12 weeks) vs SPS ($n = 24$ at 4, 8, and 12 weeks)	Surgical patellar strengthening (SPS)	MMP-3 MMP-9	sf ?	B	Significant differences between groups (SPS and Sham) after 8 and 16 weeks	ELISA, Uscnlife, Nuhan, China
Sarukawa <i>et al.</i> , 2010	22 male STR/ort mice (8 weeks old) and 12 male CBA mice (8 weeks old, used as controls)	STR OA	CTX-II	u	D	High levels in the STR OA subgroup compared with the STR non OA	ELISA, Cartilaps, Nordic Bioscience, Herlev, Denmark
Seifer <i>et al.</i> , 2008	12 adult male C57BL/6 mice	C57BL/6 high-fat diet	COMP	sf absorption (calcium sodium alginate compound (CSAC) recovery, Melgisorb (Tendra, Göteborg, Sweden)	D	Higher in the fractured knees than nonfractured	ELISA, Clark 1999, with slight modification
Ameys <i>et al.</i> , 2007	15 C57BL/6 WT mice and 15 biglycan/fibromodulin double-deficient (7 weeks old)	Biglycan/fibromodulin double-deficient	C2C Coll2-I	s s	B	The mean concentrations were higher in the biglycan/fibromodulin double-deficient mice than in WT mice	ELISA, Ibex, Montreal, Quebec, Canada
			Coll2-I NO ₂	s		These biomarkers appeared to be useful biomarkers of collagen degradation in OA mice	

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Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Guinea Pigs Horcajada et al., 2015	65 male Dunkin-Hartley (3 weeks old), 4 groups: the control chow diet (n = 15, standard chow diet); standard chow diet with 0.0025% oleuropein (n = 15), or 0.5 % rutin (n = 15), or 0.5 % rutin and 0.25 % curcumin (n = 15) until week 35		Fib3-1 Fib3-2 Coll2-1 Coll2-INO ₂	s	B B B B	Decreased by the rutin/curcumin mixture Decreased by rutin and the combination of rutin/curcumin Significantly decreased by all treatments Coll2-INO ₂ , Fib3-1 and Fib3-2 were correlated with histological global OA score while Coll2-INO ₂ and Fib3-2 also correlated with synovial histological score	ELISA, Artialis SA, Liège, Belgium
Knott et al., 2011	10 OA-prone Dunkin-Hartley (DH) and 10 OA-resistant Bristol Strain-2 (BS2)		n-3 PUFA			Reduced OA in the OA-prone Dunkin-Hartley compared with OA-resistant Strain-2 (BS2)	
Huebner et al., 2010	6 male Dunkin-Hartley (3 weeks of age) and 40 male Dunkin-Hartley (2 months of age)		C2C/CPII Coll2-1	s s	 B	Elevated serum levels in the OA-prone Hartley compared with Strain 13 after 4 months of age Lowest serum levels at 3 weeks of age, increased by 65% from 3 weeks to 4 months of age and remained consistently elevated to 18 months of age	ELISA, IBEX, Montreal, Canada (serum C2C diluted 1/2) ELISA, IBEX, Montreal, Canada (serum Coll2-I diluted 8-fold)
McDougall et al., 2010	Dunkin-Hartley (9-month-old)		Coll2-INO ₂ CTX-II	s u	B E	Serum profile similar to that of Coll-2-I Cathepsin K inhibitor reduced uCTX-II	ELISA, IBEX, Montreal, Canada ELISA, Cartilaps, Nordic Bioscience, Herlev, Denmark

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Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Wei et al., 2010	15 Dunkin-Hartley (3-month-old), 3 groups, baseline control ($n = 5$), primary OA ($n = 4$, 12 months old), ACLT secondary OA ($n = 6$)	ACLT	SDF-1	sf lavage with 100 μ L of isotonic saline solution (not corrected for dilution using the urea method)	B	Elevated synovial fluid levels of SDF-1, GAG, MMP-13, IL-1 β	ELISA, R&D, Minneapolis, Minnesota
			GAG MMP-13 IL-1 β			Significant positive correlations between the SDF-1, GAG, and C2C concentrations in synovial fluid and the Mankin score sFTG-2 is correlated with sfCOMP	Spectrophotometrically Western blot Western blot
Huebner et al., 2009	40 male Dunkin-Hartley (2 months old). Sacrificed at 2, 4, 7, 10, 12 months ($n = 6$ each) and 18 months ($n = 10$)		TG-2	sf absorption with a tared strip of filter paper (+ 10 $\times$ sf weight in normal saline)	B, E		ELISA, Neomarkers, Freemont, California
Huebner et al., 2007	46 male Dunkin-Hartley sacrificed at 3 weeks, 2, 4, 7, 10, 12 months ($n = 6$ per group) and 18 months ($n = 10$)		IL-6 IL-17 IL-8 TNF- α	s	B	Elevated serum levels in the Hartley strain	Bio-Plex Protein Array System (Bio-Rad, Hercules, California)
Huebner et al., 2006	30 male Dunkin-Hartley (2 months of age) sacrificed at 2, 4, 7, 10, 12 months of age ($n = 6$ per group) and 11 Strain 13 at ($n = 3$) and 12 ($n = 8$) months of age	Strain 13	KS COMP sIL-6 sIL-17 purine	sf absorption with a tared strip of filter paper (+ 10 $\times$ sf weight in normal saline) u	B B B	Elevated levels knees of Hartley compared with the strain 13 after 2 months of age Elevated levels from 7 to 12 months of age. Purine metabolism is of major importance in OA	ELISA, Bello 1997 Bio-Plex Protein Array System (Bio-Rad, Hercules, California) NMR
Lamers et al., 2003	46 male Dunkin-Hartley (2 months of age. 3 groups: low dose of vitamin C (2.5-3 mg/d), medium dose (30 mg/d), and high dose (150 mg/d) + 8 male Strain 13	Strain 13	KS COMP	sf absorption with a tared strip of filter paper (+ 10 $\times$ sf weight in normal saline)	B B	Elevated levels in the knees of Hartley (OA-prone) compared to the strain 13 (OA-resistant)	ELISA, Bello 1997
Huebner et al., 2002	Dunkin-Hartley (2 months, $n = 3$; 12 months, $n = 8$) and Strain 13 (2 months, $n = 3$; 12 months, $n = 8$)	Strain 13	KS COMP	sf absorption with a tared strip of filter paper (+ 10 $\times$ sf weight in normal saline)	B B		

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Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Dogs							
Dearmin et al., 2014	21 mixed-breed adult (11-24 months old) male hound-type dogs. 3 groups (<i>n</i> = 7 per group): control, low dose (10 µg/kg) zoledronic acid, high dose (25 µg/kg) zoledronic acid	CrCLt	BAP	sf aspiration		BAP activity in synovial fluid changed significantly from the baseline value throughout the study	ELISA, Metra BAP, Quidel Corp, San Diego, California
			CI,2C		D	Concentration significantly changed from baseline values obtained from the control group at 1 to 9 months after transection	ELISA, IBEX Technologies Inc., Montreal, Quebec, Canada
			CS CPII		E	Not significantly different among treatment groups	
						Zoledronic acid had a chondroprotective effect in dogs with a transected CrCL	
van Vulpen et al., 2014	7 skeletally mature beagle dogs (2 years old)	Joint bleed mimicked	COMP	s	B	Increase levels from baseline to day 2 and from day 2 to day 7 after joint bleed mimicked	ELISA, Novatein Biosciences, Woburn, Massachusetts, USA
Chockalingam et al., 2013	Old skeletally mature male cross-bred hounds (9.6 years old)	ACLT	CTX-II	u			ELISA, Cartilaps, IDS Ltd., Bolder, UK
Gharbi et al., 2013	8 crossbred dogs	ACLT	TN-C	sf aspiration		Increase levels of ACLT dog compared to the fluids of the control group	ELISA, IBL, Japan
			FetuinB C3 C1s C4 hyaluronan binding protein 2	s	I I I I I	Increase levels in dog OA	Proteome mapping
						Decrease levels	
Rhouma et al., 2013	15 adult crossbred dogs (1-4 years old). 2 groups: control (<i>n</i> = 8) and test group (<i>n</i> = 7), which received 400 IU/animal per day of vitamin E for 55 days	CrCLt	NO _x IL-1β	sf using a standard arthrocentesis technique		High dose of vitamin E reduced inflammation joint markers including NO _x , IL-1β, PGE ₂ in synovial fluid	Sievers NO Analyzer, Boulder, Colorado, USA ELISA, Bethyl laboratories, Montgomery, Texas, USA
			PGE ₂				ELISA, Cayman Chemical, Ann Arbor, Michigan, USA

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Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Henrotin et al., 2012	16 adult crossbred dogs (24-48 months old)	ACLT	Coll2-I Coll2-INO ₂	s	B B	Significant increase 2 weeks and 6 weeks, respectively after ACLT Positive and highly significant correlation between Coll2-I concentrations and the global macroscopic score Coll2-INO ₂ significantly correlated with the size of the osteophytes	ELISA, Deberg 2005
Alam et al., 2011	24 skeletally mature, mixed small breed dogs of both genders (age 2-6 years), 2 groups: sham (n = 12) and nonsurgical control group (n = 12)	MPL	MMP-2	sf using a standard arthrocentesis technique	B	Significant increase	SDS polyacrylamide gel electrophoresis, Bio-Rad Laboratories, USA
Dufield et al., 2010	36 skeletally mature beagle dogs. 3 groups: CCLt operated (n = 9), sham-operated (n = 9) and contralateral (n = 18) joints	CrCLt + MMx	TIMP-2 Aggrecan (AGEG)	 sf aspiration	 	Significant decrease Significant increase from transected knee in the same animals or both the control and contralateral knees in sham-operated animals	Biotrak ELISA system (Amersham, UK) Liquid chromatography-tandem mass spectrometry analysis
Settle et al., 2010	60 skeletally mature female beagle dogs (48 months of age). 4 groups (n = 15 per group): control, 0.5 mg/kg PF152, 1.5 mg/kg PF152 or 5 mg/kg PF152	pMMx	Aggrecan CPII	sf aspiration s	 E	sfaggrecan, sCPII levels reduced by a selective MMP-13 inhibitor, PF152	A novel assay was developed
Connor et al., 2009	40 mature female beagle dogs (14-15 months old). 2 groups (n = 20 per group): control or cathepsin K inhibitor at 50 mg/kg	pMMx	CTX-I CTX-II	u	E E	uCTX-I, uCTX-II levels reduced by a cathepsin K inhibitor	ELISA, Nordic Bioscience, Herlev, Denmark
Mastbergen et al., 2006	24 female beagle dogs (1.8 years old). 3 groups (n = 8 per group): celastrol 100 mg/d, 200 mg/d, or placebo	The groove model: Medial incision close to the patellar ligament in the right knee	PGE2	sf aspiration		sfPGE2 levels were dose-dependently diminished by celastrol	EIA, Amersham Bioscience

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Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Matyas <i>et al.</i> , 2004	20 skeletally mixed-breed female dogs	ACLT	CTX-II	sf aspiration		Elevated levels early after unilateral stifle joint	ELISA, Eyre 2000 (sf samples were run at 5-fold dilution)
Hegemann <i>et al.</i> , 2002	26 dogs (control group)	ACLR	TIMP-1	sf aspiration I were diluted 1/100 in saline and sf MMP-3 were diluted 1/8 in saline)	Potential marker for degenerative changes in cartilage	Elevated levels	ELISA, Clark 1991
Lindhorst <i>et al.</i> , 2000	12 male mongrel dogs	MMx	MMP-3 COMP KS	sf aspiration	E B	Significant elevation with a peak levels at 4 weeks in knees undergoing meniscectomy	ELISA, Lark 1994 ELISA, Bello 1997
Pelletier <i>et al.</i> , 2000	26 adult crossbred dogs (2-3 years old). 3 groups: carprofen 2.2 mg/kg/ twice daily/ orally (n = 6), carprofen 4.4 mg/kg/twice daily/ orally (n = 6), control daily/ orally (n = 6), control (n = 11), unoperated dogs and no treatment (n = 3)	ACLT	MMP			No difference in the levels of MMP activity in cartilage between OA and carprofen treated group	Extraction of MMP, Fernandes 1995
Manicourt <i>et al.</i> , 1999	28 mongrel dogs (2-4 years of age). Sham-operated control (n = 6) Operated dogs (n = 22)	ACLT	D-Pyr KS HA	u s s	E	Significant decrease in uD-Pyr and sKS, sHA by injection of calcitonin	
Thonar <i>et al.</i> , 1995		ACLT	antigenic KS HA	s		Serum antigenic KS and sHA rise between 1 and 3 weeks after ACLT	
Horses Maninchedda <i>et al.</i> , 2015	6 French standard breeds	The groove model: Medial incision close to the patellar ligament in the right knee	PGE ₂ C2C COMP	sf aspiration		Not significantly different between groups	ELISA, R&D Systems, Minneapolis, Minnesota, USA ELISA, IBEX Diagnostics, Montreal, Quebec, Canada ELISA, Euro-Diagnostic, Malmo, Sweden

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Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
			CTX-I				ELISA, Immunodiagnostic Systems, Bolder, UK ELISA, Quidel Corporation, San Diego, CA, USA ELISA, Deberg 2005
Verwilghen et al., 2011	63 client-owned horses	OCF	osteocalcin				
			Coll2-I	sf aspiration, under aseptic conditions and placed into tubes containing EDTA (1.6 mg/mL liquid)	B	Significant increase in tarsocrural joints affected with osteochondrosis compared with unaffected joints	
Frisbie et al., 2008	16 horses (2 years old). 2 groups (n = 8 per group): exercise-alone or OA- affected (also exercise)	OCF	CPII C1C C2C GAG	sf aspiration (2-4 mL)	B	Significant increase induced by exercise	ELISA, IBEX Diagnostics, Montreal, Quebec, Canada A modified I, 9 dimethylmethylene blue dye-binding assay ELISA, CrossLaps, Nordic Bioscience Diagnostics, Denmark ELISA, IBEX Diagnostics, Montreal, Quebec, Canada
			Coll				
Kawcak et al., 2008	16 horses (2 years old). 2 groups (n = 8 per group): exercise control or exercise OA	OCF	CPII	sf aspiration (2-4 mL)	B	Subchondral bone lysis on radiographic image is strongly correlated with CPII	ELISA, Assay Design, Ann Arbor, Michigan, USA
			PGE ₂		E	Subchondral bone proliferation at the joint attachment is strongly correlated with PGE ₂	ELISA, CrossLaps, Nordic Bioscience Diagnostics, Denmark ELISA, IBEX Technologies, Montreal, Canada
Billinghurst et al., 2003	43 Dutch Warmblood foals (23 males, 20 females). 3 groups: box (n = 14), training (n = 14), pasture (n = 15)	OCF	CTX-I	s	B	Significant increase with age in all groups	
			CPII		B		

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Table 1. (continued)

Reference	Population/controls (N)	Model	Biomarker(s)	Sample	BIPEDS	Results	Assay
Fuller et al., 2001	22 horses in training (4 years old)		BAP	sf aspiration	B	Significantly higher in the clinically active joint than in the contralateral joint	ELISA, Alkphase-B, MetraBiosystems
Sheep							
Mickiewicz et al., 2015	18 skeletally mature (3-4 years old) female Suffolk-cross sheep. 2 groups: surgical (idealized ACL reconstruction, and sham) and nonsurgical controls	ACL reconstruction	metabolomics analysis		B	Significantly lower in the clinically active joint than in the contralateral joint	ELISA, Thonar 1985 ELISA, Goldberg 1988, with slight modifications
						Identification of 6 metabolites (isobutyrate, glucose, hydroxyproline, asparagine, serine, uridine) which could be suggested as biomarkers for early post-injury degenerative changes in the knee joints	NMR analysis
Maier et al., 2012	36 sheep (4-6 years old) female purebred merino. 3 groups: sham surgery control, MD, or ACLT	ACLT; MD	DMSO	s		Increase in MD after 4 weeks	NMR, Bruker Biospin, Karlsruhe, Germany
			3-methylhistidine			Increase levels in ACLT-induced OA	
			BCAAs			Decrease levels in ACLT-induced OA	
Miyamoto et al., 2002	21 pure merino sheep. 6 groups (n = 3 per group): control group + TMJ group at 2, 4, 6, 8, 12, and 24 weeks after surgery	Temporomandibular joint (TMJ) OA	MMP-2	sf aspiration		sfMMP-2: only detected at 2 and 4 weeks postoperatively Activated MMP-2 activity correlates with initial articular cartilage damage	Zymography, Lever and Balkwill 1997

ACLR = anterior cruciate ligament rupture; ACLT = anterior cruciate ligament transection; CrCLT = cranial cruciate ligament transection; FCA = Freund's complete adjuvant; LPS = lipopolysaccharide; MD = medial meniscus; MIA = medial meniscus; MMT = medial meniscus; MMTx = partial medial meniscus; MMTx = partial medial meniscus; MPL = medial patellar luxation; PCLT = posterior cruciate ligament transection; OCD = osteochondritis; OCF = osteochondral fragmentation; OVX = ovariectomized; BCAAs = branched chain amino acids; DMSO = dimethyl sulfoxide; MCP-1 = monocyte chemoattractant protein 1; MIP-3α = macrophage inflammatory protein 3α; s = serum; sf = synovial fluid; u = urine; AEA = endocannabinoids anandamide; 2-AG = 2-arachidonylglycerol; ARGS neopitopes = aggrecan peptide ending in ARGS; BAP = bone-specific alkaline phosphatase; BCAAs = branched chain amino acids; BMP-2 = bone morphogenetic protein 2; C1,2C = type I and II collagen degradation fragments; C1-2,3C = complement C1-2,3C; C2C = type II collagen cleavage product; Coll2-1 = Sequence of 9 amino acids derived from the α-helix of type II collagen; Coll2-1 NO2 = peptide of 9 amino acids nitrated on its tyrosine residue specific for the type II collagen α-1 chain; COMP = cartilage oligomeric protein; CPII = carboxyl propeptide of type II pro-collagen; CRP = C reactive protein; CS = chondroitin sulfate; CTX-I = C-terminal telopeptide of collagen type I; CTX-II = C-terminal telopeptide of collagen type II; Fib3-1 = Fibulone 3-1; Fib3-2 = Fibulone 3-2; GAG = glycosaminoglycan; HA = hyaluronic acid; IL-1β = interleukin 1-beta; IL-6 = interleukin 6; IL-8 = interleukin 8; IL-17 = interleukin 17; KS = keratan sulfate; MCP-1 = monocyte chemoattractant protein 1; MDA = malondialdehyde (an oxidative stress marker); MIP-3α = macrophage inflammatory protein 3α; MMP-3 = matrix metalloproteinase-3; MMP-13 = matrix metalloproteinase-13; MMP-9 = matrix metalloproteinase-9; NGF = nerve growth factor NITEGE = aggrecanase cleaved aggrecan; NMR, nuclear magnetic resonance; OC = osteocalcin; OPN = osteopontin; PGE2 = prostaglandin E2; PINP = procollagen type I N-terminal propeptide; n-3 PUFA = omega-3 polyunsaturated fatty acid; TN-C = tenascin-C; SDF-1 = stromal cell-derived factor 1; TG-2 = transglutaminase isoenzyme 2; TIMP-1 = tissue inhibitor of matrix metalloproteinase-1; TIMP-2 = tissue inhibitor of matrix metalloproteinase-2; TNF = tumor necrosis factor.

in the development and testing of new drugs to advance decision making on dosing, time and treatment effect, trial design, and risk/benefit analysis.³

Diagnostic Biomarkers

Diagnostic biomarkers ideally differentiate animals with and without the disease. At this time, in guinea pigs, only the ratio C2C/CPII, reflecting type II collagen turnover, have been demonstrated to discriminate OA-prone Hartley and strain 13 animals, a strain that does not develop OA.⁴⁶ This ratio was higher in OA-prone Hartley strain guinea pigs compared to OA-resistant strain 13, indicating an upregulation of collagen degradation in the absence of a compensatory upregulation in collagen synthesis between 2 and 12 months of age.⁴⁶ In mice, several biomarkers discriminate different mouse strain. Serum levels of MDA, CTX-II and CPII were elevated in obese and hyperlipidemic STR/ORT (STR) mice compared with control CBA mice.⁴³ Together, these observations suggest that oxidative stress is associated with the development of OA, possibly via the degradation of type II collagen.⁴³ In parallel, high levels of urinary CTX-II were found in the STR OA subgroup compared with the STR non-OA.³⁶ The models are very relevant to validate biomarker and assess their capacity to discriminate diseased and nondiseased animals.

Safety Biomarkers

In 2011, the BIPED classification was expanded and a "Safety" category to the BIPED system was added.⁸¹ Safety biomarkers could be used in preclinical and clinical applications to monitor the health of the joint tissues, the whole joint organ, or the skeleton in general.⁸¹ For instance, biomarkers reflecting the synthesis of the main proteins of the joint might provide an index of the "joint-protective" effect of a potential treatment.⁸¹ Currently, there are no studies exploring specifically this aspect of joint tissue related biomarkers. A case in point could be evaluation of fibrosis markers in animals treated with MMP inhibitors.

Conclusion

In conclusion, this systematic review indicates that some markers could be valuable to evaluate the burden of OA disease and to assess the therapeutic response in animal models. The most investigated biomarkers in terms of burden of disease assessment are those derived from type II collagen metabolism. Since OA represents a process of matrix damage, turnover, and attempted repair, the use of multiple biomarkers, both anabolic and catabolic, could likely be most accurate in characterizing OA.¹¹ Finally, soluble biomarkers can be a useful "drug development tools" that should be early integrated in the research program of

new drugs. As previously mentioned, biomarkers may be helpful to assess drug safety, to assist with selection of animal models and lead compounds and assess drug mechanisms of action.³ Furthermore, the use of biomarkers at the preclinical stages is helpful to select the doses and the route of administration, to evaluate the residency time in joint, to select a companion biomarker for the next clinical phases and the personalized management of OA, to provide insights into disease pathophysiology and to provide confidence in histological assessment (when synovial fluid markers known to correlate with histology provide consistent results with histology in a subsequent study).

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References

1. Bauer DC, Hunter DJ, Abramson SB, Attur M, Corr M, Felson D, *et al.* Classification of osteoarthritis biomarkers: a proposed approach. *Osteoarthritis Cartilage*. 2006;14(8):723-7. doi:10.1016/j.joca.2006.04.001.
2. Lotz M, Martel-Pelletier J, Christiansen C, Brandi ML, Bruyere O, Chapurlat R, *et al.* Republished: Value of biomarkers in osteoarthritis: current status and perspectives. *Postgrad Med J*. 2014;90(1061):171-8. doi:10.1136/postgrad-medj-2013-203726rep.
3. Kraus VB, Blanco FJ, Englund M, Henrotin Y, Lohmander LS, Losina E, *et al.* OARSI Clinical Trials Recommendations: soluble biomarker assessments in clinical trials in osteoarthritis. *Osteoarthritis Cartilage*. 2015;23(5):686-97. doi:10.1016/j.joca.2015.03.002.
4. Henrotin Y, Addison S, Kraus V, Deberg M. Type II collagen markers in osteoarthritis: what do they indicate? *Curr Opin Rheumatol*. 2007;19(5):444-50. doi:10.1097/BOR.0b013e32829fb3b5.
5. Kraus VB. Osteoarthritis year 2010 in review: biochemical markers. *Osteoarthritis Cartilage*. 2011;19(4):346-53. doi:10.1016/j.joca.2011.02.002.
6. van Spil WE, DeGroot J, Lems WF, Oostveen JC, Lafeber FP. Serum and urinary biochemical markers for knee and hip osteoarthritis: a systematic review applying the consensus BIPED criteria. *Osteoarthritis Cartilage*. 2010;18(5):605-12. doi:10.1016/j.joca.2010.01.012.
7. Ward BD, Furman BD, Huebner JL, Kraus VB, Guilak F, Olson SA. Absence of posttraumatic arthritis following

- intraarticular fracture in the MRL/MpJ mouse. *Arthritis Rheum.* 2008;58(3):744-53. doi:10.1002/art.23288.
8. Khorasani MS, Diko S, Hsia AW, Anderson MJ, Genetos DC, Haudenschild DR, *et al.* Effect of alendronate on post-traumatic osteoarthritis induced by anterior cruciate ligament rupture in mice. *Arthritis Res Ther.* 2015;17(1):30. doi:10.1186/s13075-015-0546-0.
 9. Malesud CJ. Markers of osteoarthritis and cartilage research in animal models. *Curr Opin Rheumatol.* 1993;5(4):494-502.
 10. Poole R, Blake S, Buschmann M, Goldring S, Lavery S, Lockwood S, *et al.* Recommendations for the use of preclinical models in the study and treatment of osteoarthritis. *Osteoarthritis Cartilage.* 2010;18(Suppl 3):S10-6. doi:10.1016/j.joca.2010.05.027.
 11. Teeple E, Jay GD, Elsaid KA, Fleming BC. Animal models of osteoarthritis: challenges of model selection and analysis. *AAPS J.* 2013;15(2):438-46. doi:10.1208/s12248-013-9454-x.
 12. Opreyeshcz F, Chausson M, Maquet V, Dubuc JE, Henrotin Y. Protective effect of a new biomaterial against the development of experimental osteoarthritis lesions in rabbit: a pilot study evaluating the intra-articular injection of alginate-chitosan beads dispersed in an hydrogel. *Osteoarthritis Cartilage.* 2013;21(8):1099-107. doi:10.1016/j.joca.2013.04.017.
 13. Ohnishi A, Osaki T, Matahira Y, Tsuka T, Imagawa T, Okamoto Y, *et al.* Evaluation of the chondroprotective effects of glucosamine and fish collagen peptide on a rabbit ACLT model using serum biomarkers. *J Vet Med Sci.* 2013;75(4):421-9. doi:10.1292/jvms.12-0240.
 14. Gao SG, Cheng L, Zeng C, Wei LC, Zhang FJ, Tian J, *et al.* Usefulness of specific OA biomarkers, thrombin-cleaved osteopontin, in the posterior cruciate ligament OA rabbit model. *Osteoarthritis Cartilage.* 2013;21(1):144-50. doi:10.1016/j.joca.2012.09.006.
 15. Duclos ME, Roualdes O, Cararo R, Rousseau JC, Roger T, Hartmann DJ. Significance of the serum CTX-II level in an osteoarthritis animal model: a 5-month longitudinal study. *Osteoarthritis Cartilage.* 2010;18(11):1467-76. doi:10.1016/j.joca.2010.07.007.
 16. Zuo H, Jiang L, Qu N, Wang J, Cui X, Yao W. The biomarkers changes in serum and the correlation with quantitative MRI markers by histopathologic evaluation of the cartilage in surgically-induced osteoarthritis rabbit model. *PLoS One.* 2015;10(4):e0124717. doi:10.1371/journal.pone.0124717.
 17. Lindhorst E, Wachsmuth L, Kimmig N, Raiss R, Aigner T, Atley L, *et al.* Increase in degraded collagen type II in synovial fluid early in the rabbit meniscectomy model of osteoarthritis. *Osteoarthritis Cartilage.* 2005;13(2):139-45. doi:10.1016/j.joca.2004.10.017.
 18. Gerwin N, Bendele AM, Glasson S, Carlson CS. The OARSI histopathology initiative—recommendations for histological assessments of osteoarthritis in the rat. *Osteoarthritis Cartilage.* 2010;18(Suppl 3):S24-34. doi:10.1016/j.joca.2010.05.030.
 19. Hoegh-Andersen P, Tanko LB, Andersen TL, Lundberg CV, Mo JA, Heegaard AM, *et al.* Ovariectomized rats as a model of postmenopausal osteoarthritis: validation and application. *Arthritis Res Ther.* 2004;6(2):R169-80. doi:10.1186/ar1152.
 20. Yamaguchi S, Aoyama T, Ito A, Nagai M, Iijima H, Zhang X, *et al.* Effects of exercise level on biomarkers in a rat knee model of osteoarthritis. *J Orthop Res.* 2013;31(7):1026-31. doi:10.1002/jor.22332.
 21. Braza-Boils A, Alcaraz MJ, Ferrandiz ML. Regulation of the inflammatory response by tin protoporphyrin IX in the rat anterior cruciate ligament transection model of osteoarthritis. *J Orthop Res.* 2011;29(9):1375-82. doi:10.1002/jor.21411.
 22. Naito K, Watari T, Muta T, Furuhashi A, Iwase H, Igarashi M, *et al.* Low-intensity pulsed ultrasound (LIPUS) increases the articular cartilage type II collagen in a rat osteoarthritis model. *J Orthop Res.* 2010;28:361-9. doi:10.1002/jor.20995.
 23. Naito K, Watari T, Furuhashi A, Yomogida S, Sakamoto K, Kurosawa H, *et al.* Evaluation of the effect of glucosamine on an experimental rat osteoarthritis model. *Life Sci.* 2010;86(13-14):538-43. doi:10.1016/j.lfs.2010.02.015.
 24. Hayami T, Pickarski M, Wesolowski GA, McLane J, Bone A, Destefano J, *et al.* The role of subchondral bone remodeling in osteoarthritis: reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. *Arthritis Rheum.* 2004;50(4):1193-206. doi:10.1002/art.20124.
 25. Musumeci G, Castrogiovanni P, Trovato FM, Imbesi R, Giunta S, Szychlinska MA, *et al.* Physical activity ameliorates cartilage degeneration in a rat model of aging: a study on lubricin expression. *Scand J Med Sci Sports.* 2015;25(2):e222-30. doi:10.1111/sms.12290.
 26. Appleton CT, Usmani SE, Pest MA, Pitelka V, Mort JS, Beier F. Reduction in disease progression by inhibition of transforming growth factor α -CCL2 signaling in experimental posttraumatic osteoarthritis. *Arthritis Rheumatol.* 2015;67(10):2691-701. doi:10.1002/art.39255.
 27. Ferrándiz ML, Terencio MC, Carceller MC, Ruhi R, Dalmau P, Vergés J, *et al.* Effects of BIS076 in a model of osteoarthritis induced by anterior cruciate ligament transection in ovariectomized rats. *BMC Musculoskelet Disord.* 2015;16:92. doi:10.1186/s12891-015-0547-9.
 28. Yang JH, Kim JH, Lim DS, Oh KJ. Effect of combined sex hormone replacement on bone/cartilage turnover in a murine model of osteoarthritis. *Clin Orthop Surg.* 2012;4(3):234-41. doi:10.4055/cios.2012.4.3.234.
 29. Orita S, Ishikawa T, Miyagi M, Ochiai N, Inoue G, Eguchi Y, *et al.* Pain-related sensory innervation in monoiodoacetate-induced osteoarthritis in rat knees that gradually develops neuronal injury in addition to inflammatory pain. *BMC Musculoskelet Disord.* 2011;12:134. doi:10.1186/1471-2474-12-134.
 30. Swearingen CA, Chambers MG, Lin C, Marimuthu J, Rito CJ, Carter QL, *et al.* A short-term pharmacodynamic model for monitoring aggrecanase activity: injection of monosodium iodoacetate (MIA) in rats and assessment of aggrecan neopeptide release in synovial fluid using novel ELISAs. *Osteoarthritis Cartilage.* 2010;18(9):1159-66. doi:10.1016/j.joca.2010.02.019.
 31. Dufield DR, Nemirovskiy OV, Jennings MG, Tortorella MD, Malfait AM, Mathews WR. An immunoaffinity liquid chromatography-tandem mass spectrometry assay for detection of endogenous aggrecan fragments in biological fluids:

- use as a biomarker for aggrecanase activity and cartilage degradation. *Anal Biochem.* 2010;406:113-23. doi:10.1016/j.ab.2010.06.044.
32. Chockalingam PS, Sun W, Rivera-Bermudez MA, Zeng W, Duffield DR, Larsson S, *et al.* Elevated aggrecanase activity in a rat model of joint injury is attenuated by an aggrecanase specific inhibitor. *Osteoarthritis Cartilage.* 2011;19(3):315-23. doi:10.1016/j.joca.2010.12.004.
 33. Dhaneshwar S, Patil D. Chondromodulating chimeric prodrugs of diacetylrhein: synthesis and evaluation in monoiodoacetate-induced hyperalgesia. *Med Chem.* 2013;9(3):449-58.
 34. Finn A, Angeby Moller K, Gustafsson C, Abdelmoaty S, Nordahl G, Ferm M, *et al.* Influence of model and matrix on cytokine profile in rat and human. *Rheumatology.* 2014;53(12):2297-305. doi:10.1093/rheumatology/keu281.
 35. Braza-Boils A, Ferrandiz ML, Terencio MC, Alcaraz MJ. Analysis of early biochemical markers and regulation by tin protoporphyrin IX in a model of spontaneous osteoarthritis. *Exp Gerontol.* 2012;47(5):406-9. doi:10.1016/j.exger.2012.03.005.
 36. Sarukawa J, Takahashi M, Doi M, Suzuki D, Nagano A. A longitudinal analysis of urinary biochemical markers and bone mineral density in STR/Ort mice as a model of spontaneous osteoarthritis. *Arthritis Rheum.* 2010;62(2):463-71. doi:10.1002/art.27202.
 37. Ameys LG, Deberg M, Oliveira M, Labasse A, Aeschlimann JM, Henrotin Y. The chemical biomarkers C2C, Coll2-1, and Coll2-INO2 provide complementary information on type II collagen catabolism in healthy and osteoarthritic mice. *Arthritis Rheum.* 2007;56:3336-46. doi:10.1002/art.22875.
 38. Chia WT, Pan RY, Tseng FJ, Chen YW, Feng CK, Lee HS, *et al.* Experimental osteoarthritis induced by surgical realignment of the patella in BALB/c mice. *J Bone Joint Surg Br.* 2010;92(12):1710-6. doi:10.1302/0301-620x.92b12.23366.
 39. Seifer DR, Furman BD, Guilak F, Olson SA, Brooks SC 3rd, Kraus VB. Novel synovial fluid recovery method allows for quantification of a marker of arthritis in mice. *Osteoarthritis Cartilage.* 2008;16(12):1532-8. doi:10.1016/j.joca.2008.04.013.
 40. Porta CL, Andreea Bura S, Llorente-Onaindia J, Pastor A, Navarrete F, García-Gutiérrez MS, *et al.* Role of the endocannabinoid system in the emotional manifestations of osteoarthritis pain. *Pain.* 2015;156(10):2001-12. doi:10.1097/j.pain.0000000000000260.
 41. Kyostio-Moore S, Nambiar B, Hutto E, Ewing PJ, Piraino S, Berthelette P, *et al.* STR/ort mice, a model for spontaneous osteoarthritis, exhibit elevated levels of both local and systemic inflammatory markers. *Comp Med.* 2011;61(4):346-55.
 42. Lewis JS, Hembree WC, Furman BD, Tippets L, Cattel D, Huebner JL, *et al.* Acute joint pathology and synovial inflammation is associated with increased intra-articular fracture severity in the mouse knee. *Osteoarthritis Cartilage.* 2011;19(7):864-73. doi:10.1016/j.joca.2011.04.011.
 43. Watari T, Naito K, Sakamoto K, Kurosawa H, Nagaoka I, Kaneko K. Evaluation of the effect of oxidative stress on articular cartilage in spontaneously osteoarthritic STR/OrtCrj mice by measuring the biomarkers for oxidative stress and type II collagen degradation/synthesis. *Exp Ther Med.* 2011;2(2):245-50. doi:10.3892/etm.2011.196.
 44. Horcajada MN, Sanchez C, Membrez Scalfo F, Drion P, Comblain F, Taralla S, *et al.* Oleuropein or rutin consumption decreases the spontaneous development of osteoarthritis in the Hartley guinea pig. *Osteoarthritis Cartilage.* 2015;23(1):94-102. doi:10.1016/j.joca.2014.08.016.
 45. Huebner JL, Hanes MA, Beekman B, TeKoppele JM, Kraus VB. A comparative analysis of bone and cartilage metabolism in two strains of guinea-pig with varying degrees of naturally occurring osteoarthritis. *Osteoarthritis Cartilage.* 2002;10(10):758-67. doi:10.1053/joca.2002.0821.
 46. Huebner JL, Kraus VB. Assessment of the utility of biomarkers of osteoarthritis in the guinea pig. *Osteoarthritis Cartilage.* 2006;14(9):923-30. doi:10.1016/j.joca.2006.03.007.
 47. Huebner JL, Williams JM, Deberg M, Henrotin Y, Kraus VB. Collagen fibril disruption occurs early in primary guinea pig knee osteoarthritis. *Osteoarthritis Cartilage.* 2010;18(3):397-405. doi:10.1016/j.joca.2009.09.011.
 48. Wei L, Fleming BC, Sun X, Teeple E, Wu W, Jay GD, *et al.* Comparison of differential biomarkers of osteoarthritis with and without posttraumatic injury in the Hartley guinea pig model. *J Orthop Res.* 2010;28(7):900-6. doi:10.1002/jor.21093.
 49. Huebner JL, Seifer DR, Kraus VB. A longitudinal analysis of serum cytokines in the Hartley guinea pig model of osteoarthritis. *Osteoarthritis Cartilage.* 2007;15(3):354-6. doi:10.1016/j.joca.2006.10.014.
 50. Huebner JL, Johnson KA, Kraus VB, Terkeltaub RA. Transglutaminase 2 is a marker of chondrocyte hypertrophy and osteoarthritis severity in the Hartley guinea pig model of knee OA. *Osteoarthritis Cartilage.* 2009;17(8):1056-64. doi:10.1016/j.joca.2009.02.015.
 51. Knott L, Avery NC, Hollander AP, Tarlton JF. Regulation of osteoarthritis by omega-3 (n-3) polyunsaturated fatty acids in a naturally occurring model of disease. *Osteoarthritis Cartilage.* 2011;19(9):1150-7. doi:10.1016/j.joca.2011.06.005.
 52. McDougall JJ, Schuelert N, Bowyer J. Cathepsin K inhibition reduces CTXII levels and joint pain in the guinea pig model of spontaneous osteoarthritis. *Osteoarthritis Cartilage.* 2010;18(10):1355-7. doi:10.1016/j.joca.2010.07.014.
 53. Lamers RJ, DeGroot J, Spies-Faber EJ, Jellema RH, Kraus VB, Verzijl N, *et al.* Identification of disease- and nutrient-related metabolic fingerprints in osteoarthritic guinea pigs. *J Nutr.* 2003;133(6):1776-80.
 54. Matyas JR, Atley L, Ionescu M, Eyre DR, Poole AR. Analysis of cartilage biomarkers in the early phases of canine experimental osteoarthritis. *Arthritis Rheum.* 2004;50(2):543-52. doi:10.1002/art.20027.
 55. Henrotin Y, Martel-Pelletier J, Msika P, Guillou GB, Deberg M. Usefulness of specific OA biomarkers, Coll2-1 and Coll2-INO2, in the anterior cruciate ligament OA canine model. *Osteoarthritis Cartilage.* 2012;20(7):787-90. doi:10.1016/j.joca.2012.03.016.
 56. Thonar EJ, Masuda K, Lenz ME, Hauselmann HJ, Kuettner KE, Manicourt DH. Serum markers of systemic disease processes in osteoarthritis. *J Rheumatol Suppl.* 1995;43:68-70.
 57. Chockalingam PS, Glasson SS, Lohmander LS. Tenascin-C levels in synovial fluid are elevated after injury to the human and canine joint and correlate with markers of inflammation and matrix degradation. *Osteoarthritis Cartilage.* 2013;21(2):339-45. doi:10.1016/j.joca.2012.10.016.

58. Dearmin MG, Trumble TN, Garcia A, Chambers JN, Budsberg SC. Chondroprotective effects of zoledronic acid on articular cartilage in dogs with experimentally induced osteoarthritis. *Am J Vet Res*. 2014;75(4):329-37. doi:10.2460/ajvr.75.4.329.
59. Connor JR, LePage C, Swift BA, Yamashita D, Bendele AM, Maul D, et al. Protective effects of a cathepsin K inhibitor, SB-553484, in the canine partial medial meniscectomy model of osteoarthritis. *Osteoarthritis Cartilage*. 2009;17(9):1236-43. doi:10.1016/j.joca.2009.03.015.
60. Settle S, Vickery L, Nemirovskiy O, Vidmar T, Bendele A, Messing D, et al. Cartilage degradation biomarkers predict efficacy of a novel, highly selective matrix metalloproteinase 13 inhibitor in a dog model of osteoarthritis: confirmation by multivariate analysis that modulation of type II collagen and aggrecan degradation peptides parallels pathologic changes. *Arthritis Rheum*. 2010;62(10):3006-15. doi:10.1002/art.27596.
61. Rhouma M, de Oliveira EI, Warrak A, Troncy E, Beaudry F, Chorfi Y. Anti-inflammatory response of dietary vitamin E and its effects on pain and joint structures during early stages of surgically induced osteoarthritis in dogs. *Can J Vet Res*. 2013;77(3):191-8.
62. Alam MR, Ji JR, Kim MS, Kim NS. Biomarkers for identifying the early phases of osteoarthritis secondary to medial patellar luxation in dogs. *J Vet Sci*. 2011;12(3):273. doi:10.4142/jvs.2011.12.3.273.
63. Hegemann N, Kohn B, Brunnberg L, Schmidt MF. Biomarkers of joint tissue metabolism in canine osteoarthritic and arthritic joint disorders. *Osteoarthritis Cartilage*. 2002;10(9):714-21. doi:10.1053/joca.2002.0820.
64. Pelletier JP, Lajeunesse D, Jovanovic DV, Lascau-Coman V, Jolicœur FC, Hilal G, et al. Carprofen simultaneously reduces progression of morphological changes in cartilage and subchondral bone in experimental dog osteoarthritis. *J Rheumatol*. 2000;27(12):2893-902.
65. Mastbergen SC, Marijnissen AC, Vianen ME, Zoer B, van Roermund PM, Bijlsma JW, et al. Inhibition of COX-2 by celecoxib in the canine groove model of osteoarthritis. *Rheumatology*. 2006;45(4):405-13. doi:10.1093/rheumatology/kei187.
66. Gharbi M, Sanchez C, Mazzucchelli G, De Pauw E, Henrotin Y. Identification of differential pattern of protein expression in canine osteoarthritis serum after anterior cruciate ligament transection: a proteomic analysis. *Vet J*. 2013;197(3):848-53. doi:10.1016/j.tvjl.2013.05.037.
67. Lindhorst E, Vail TP, Guilak F, Wang H, Setton LA, Vilim V, et al. Longitudinal characterization of synovial fluid biomarkers in the canine meniscectomy model of osteoarthritis. *J Orthop Res*. 2000;18(2):269-80. doi:10.1002/jor.1100180216.
68. van Vulpen LF, van Meegeren ME, Roosendaal G, Jansen NW, van Laar JM, Schutgens RE, et al. Biochemical markers of joint tissue damage increase shortly after a joint bleed; an explorative human and canine in vivo study. *Osteoarthritis Cartilage*. 2015;23(1):63-9. doi:10.1016/j.joca.2014.09.008.
69. Manicourt DH, Altman RD, Williams JM, Devogelaer JP, Druetz-Van Egeren A, Lenz ME, et al. Treatment with calcitonin suppresses the responses of bone, cartilage, and synovium in the early stages of canine experimental osteoarthritis and significantly reduces the severity of the cartilage lesions. *Arthritis Rheum*. 1999;42(6):1159-67. doi:10.1002/1529-0131(199906)42:6<1159::aid-anr12>3.0.co;2-q.
70. Kawcak CE, Frisbie DD, Werpy NM, Park RD, McIlwraith CW. Effects of exercise vs experimental osteoarthritis on imaging outcomes. *Osteoarthritis Cartilage*. 2008;16(12):1519-25. doi:10.1016/j.joca.2008.04.015.
71. Verwilghen DR, Enzerink E, Martens A, Franck T, Balligand M, Henrotin Y, et al. Relationship between arthroscopic joint evaluation and the levels of Coll2-1, Coll2-1NO(2), and myeloperoxidase in the blood and synovial fluid of horses affected with osteochondrosis of the tarsocrural joint. *Osteoarthritis Cartilage*. 2011;19(11):1323-9. doi:10.1016/j.joca.2011.08.002.
72. Verwilghen DR, Martens A, Busschers E, Franck T, Deberg M, Henrotin Y, et al. Coll2-1, Coll2-1NO2 and myeloperoxidase concentrations in the synovial fluid of equine tarsocrural joints affected with osteochondrosis. *Vet Res Commun*. 2011;35(7):401-8. doi:10.1007/s11259-011-9487-5.
73. Billinghamhurst R. Significant exercise-related changes in the serum levels of two biomarkers of collagen metabolism in young horses. *Osteoarthritis Cartilage*. 2003;11(10):760-9. doi:10.1016/s1063-4584(03)00152-3.
74. Frisbie DD, Al-Sobayil F, Billinghamhurst RC, Kawcak CE, McIlwraith CW. Changes in synovial fluid and serum biomarkers with exercise and early osteoarthritis in horses. *Osteoarthritis Cartilage*. 2008;16(10):1196-204. doi:10.1016/j.joca.2008.03.008.
75. Fuller CJ, Barr AR, Sharif M, Dieppe PA. Cross-sectional comparison of synovial fluid biochemical markers in equine osteoarthritis and the correlation of these markers with articular cartilage damage. *Osteoarthritis Cartilage*. 2001;9(1):49-55. doi:10.1053/joca.2000.0349.
76. Maninchedda U, Lepage OM, Gangl M, Hilalret S, Remandet B, Meot F, et al. Development of an equine groove model to induce metacarpophalangeal osteoarthritis: a pilot study on 6 horses. *PLoS One*. 2015;10(2):e0115089. doi:10.1371/journal.pone.0115089.
77. Maher AD, Coles C, White J, Bateman JF, Fuller ES, Burkhardt D, et al. ¹H NMR spectroscopy of serum reveals unique metabolic fingerprints associated with subtypes of surgically induced osteoarthritis in sheep. *J Proteome Res*. 2012;11(8):4261-8. doi:10.1021/pr300368h.
78. Miyamoto K, Ishimaru J, Kurita K, Goss AN. Synovial matrix metalloproteinase-2 in different stages of sheep temporomandibular joint osteoarthritis. *J Oral Maxillofac Surg*. 2002;60(1):66-72.
79. Mickiewicz B, Heard BJ, Chau JK, Chung M, Hart DA, Shrive NG, et al. Metabolic profiling of synovial fluid in a unilateral ovine model of anterior cruciate ligament reconstruction of the knee suggests biomarkers for early osteoarthritis. *J Orthop Res*. 2015;33(1):71-7. doi:10.1002/jor.22743.
80. Kraus VB, Huebner JL, Fink C, King JB, Brown S, Vail TP, et al. Urea as a passive transport marker for arthritis biomarker studies. *Arthritis Rheum*. 2002;46(2):420-7.
81. Kraus VB, Burnett B, Coindreau J, Cottrell S, Eyre D, Gendreau M, et al. Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. *Osteoarthritis Cartilage*. 2011;19(5):515-42. doi:10.1016/j.joca.2010.08.019.