Osteoarthritis and Cartilage

OARSI Osteoarthritis RESEARCH SOCIETY INTERNATIONAL

Review

Osteoarthritis year 2011 in review: biochemical markers of osteoarthritis: an overview of research and initiatives

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SUMMARY

The "year in review" session is a key moment of the Osteoarthritis Research Society International (OARSI) Congress. This is a unique opportunity for opinion leaders to summarize and comment the recent advances in one particular field of osteoarthritis research. This review is a summary of selected studies related to soluble biomarkers published between September 1st, 2010 and August 30th, 2011 and identified by a pubmed search using the terms "biomarkers" and "osteoarthritis". In addition, I have selected some works presented during the 2011 OARSI Congress. This year was dominated by the publication of a consensus paper on the qualification of osteoarthritis (OA) biomarkers by the OARSI/ Food Drug Administration (FDA) Osteoarthritis Biomarkers Working Group, and of proteomes of chondrocyte vesicles, urine and serum.

Introduction

The development of new treatments for osteoarthritis (OA) is disabled by the lack of a good primary clinical outcome for measuring cartilage structural changes. Until now, the gold standard was the measurement of joint space width on standard X-ray. This parameter lacks of sensitivity and reproducibility and fails to detect the early metabolic changes preceding the appearance of structural changes. As a consequence, to assess structural changes in clinical trials requires a large population and a long-term follow-up. The academic and industrial researchers need of new tools for detecting the early metabolic changes occurring in joint tissues before the appearance of imaging lesions. The development of these tools will allow healthcare providers to administer treatment earlier and potentially improve efficacy. Healthcare providers also need tools to identify fast progressors and more sensitive means to evaluate response to treatment. This personalized medicine approach will contribute to the improvement of quality and efficacy of the OA patient management.

Soluble biomarkers are good candidate to fit these purposes. The scientific community's interest in OA biochemical markers has increased during the last decade. The number of paper published in this field between 2000 and 2010 has increased five-fold (Fig. 1). This review sought to highlight some biomarker studies published

over the prior year, since the 2010 meeting of the Osteoarthritis Research Society International (OARSI) in Brussels and referenced in the pubmed database. This is not a systematic review and the paper selection was based on the expert opinion of the author.

OARSI/FDA Osteoarthritis Biomarkers Working group white paper

This year 2011 was marked by the publication of a consensus document for the application of *in vitro* (biochemical and other soluble) biomarkers in the development of drugs for OA by the OARSI/Food Drug Administration (FDA) biomarkers working groups[1]. This group attempts to respond to specific questions posed by the FDA. The OARSI/FDA working group document summarizes definition and classification systems for biomarkers, applications and potential utility of biomarkers for development of OA therapeutics, provides guidelines for biomarkers qualification and a research agenda to advance the science of OA-related research. This guide gives also precise advices for sample acquisition and handling and a glossary of the term related to biomarker research. This paper should be a reference for researchers working in the biomarker research field.

Biological fluid proteomes as sources of new biomarkers

The proteomic corresponds to a wide variety of techniques to study the protein content of cells, tissues or biological fluids and to isolate proteins of interest. This approach allows the identification of new biomarkers by comparing the proteome of normal and

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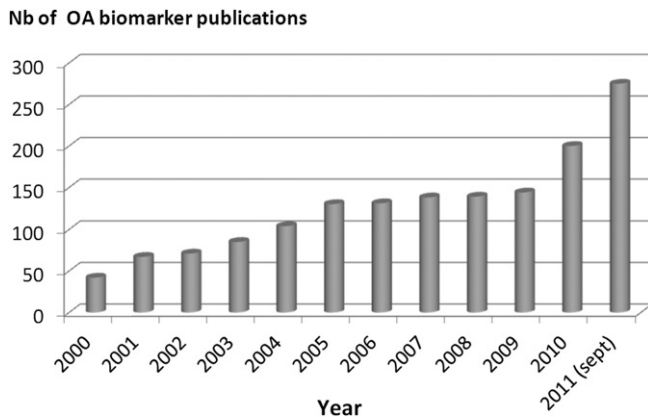
Nb of OA biomarker publications

Fig. 1. Yearly number of papers in pubmed database identified by introducing the research words "biomarkers" and "osteoarthritis".

disease affected biological material. This year, papers describing the proteomes of OA chondrocytes vesicles, urine and serum have been published. In serum, De Seny et al.[2] have identified four novel biomarkers for OA: V65 vitronectin fragment, cf3 peptide, Connective Tissue-Activating Peptide III (CTAPIII) and an unknown protein. V65 vitronectin fragment and cf3, a fragment of C3b, were found to be expressed at higher levels in sera of OA patients compared to controls subjects and patients with rheumatoid arthritis, and that watever the radiological severity of OA. CTAPIII was significantly lower in the OA patients population compared to control subjects, and among OA subsets, CTAPIII remained significantly reduced in OA patients with the worst radiographic disease.

Mass spectrometry analysis identified 13 proteins with an abundance decreased or increased by more than 1.5 fold in urine of OA patients compared to normal subjects[3]. The proteins found to be increased in urine were mainly β-actin, α1-microglobulin, fibulin-3, apoptosis-inducing factor-2 and the Zn-α2-glycoprotein precursor and the decreased proteins were serpin β1 and β3, mannan binding lectin serine protease-2 precursor, kinninogen-1 precursor and α1-antitrypsin. Interestingly, two mostly increased spots in the proteome of OA patients contained specific sequences of fibulin-3 (so called Fib3-1 and Fib3-2), the only extracellular matrix protein found to be significantly modified in the proteome of urine of OA patients. Fibulin-3 is widely distributed in various tissues. The protein is also present in blood vessels of different sizes, and is capable of inhibiting vessel development and angiogenesis both *in vitro* and *in vivo*[4]. During development, the protein is expressed in the mesenchyme giving rise to cartilage and bone, and plays a role in organizing the development of skeletal system[5]. Moreover, fibulin-3 is intimately associated with Tissue Inhibitor of MetalloProteases (TIMPs)-3, an inhibitor of metalloproteinases involved in the pathogenesis of OA[6–8]. The overexpression of fibulin-3 suppressed chondrocyte differentiation by inhibition of cartilage nodule formation, proteoglycan production, matrix gene expression[9].

Articular cartilage vesicles (ACVs) are extracellular organelles found in normal articular cartilage. Rosenthal et al.[10] have analyzed the proteome of these vesicles from normal and OA human cartilage. Six proteins were found only in normal ACVs, while nine proteins were seen exclusively in OA ACVs. Many of the proteins that were exclusive to OA, such as fibrinogen, complement, immunoglobulins, and apolipoproteins, are typical markers of inflammation. Levels of nine proteins were significantly increased in OA ACVs including transforming growth factor β-induced protein big-H3, integring-binding protein DEL-1, serum amyloid P-component precursor and vitronectin precursor. The primary role

for ACVs was in the pathologic mineralization of cartilage. The presence of inflammation markers in OA ACVs supports a role of ACVs in OA-related inflammation.

New potential biomarkers for OA research

We have developed new immunoassays for measuring in serum two fragments of fibulin-3, so called Fib3-1 and Fib3-2[3]. By comparison with age-matched healthy subjects, these epitopes were found to be elevated in serum of OA patients. They discriminate between OA and normal populations with high specificity and sensitivity, indicating that these fragments are good diagnostic biomarkers. Of course, this needs to be confirmed in independent trials.

CIIM was an MMP-released neo-epitope located in the C-terminal area of the triple helicoidal part of the type II collagen molecule[11]. Mean serum CIIM was significantly higher in individuals with mild and severe OA than in younger individuals without OA. However, the advantages of this new biomarker over existing type II collagen biomarkers, like Coll2-1 or C2C, should be demonstrated.

D-COMP is a desaminated cartilage oligomeric matrix protein (COMP) biomarker[12]. Interestingly, in 450 participants from the Johnstone County osteoarthritis project, D-COMP was associated with hip but not knee OA severity as defined by increasing Kellgren–Lawrence (KL) grade. In contrast, total COMP was associated with knee but not hip K&L grade. D-COMP is the first biomarker to show specificity for a particular joint site.

Advances in OA biomarkers qualification

Obesity is the main modifiable risk factor for the onset of the knee. The observation that obesity is also a risk factor for OA of non-weight bearing joints such as the hand has suggested that the link between obesity and OA might also occur through systemic inflammation. Richette et al.[13] have demonstrated that a massive weigh loss (>10%) after bariatric surgery improved pain and function in obese patients with knee OA. As expected, weight loss resulted in a significant increase in serum levels of adiponectin and a significant decrease in that of leptin and inflammatory markers like interleukin(IL)-6, hs C-Reactive Protein (CRP), orosomucoid and fibrinogen. In parallel, massive weight loss resulted in a significant increase in the level of PIIANP, a marker of type II collagen synthesis, and a decrease in that of COMP, a marker of cartilage degradation. These results are the first which associate weight loss and cartilage biomarkers, helping us to understand disease and the link between obesity and arthritis.

Several studies have found an association of uric acid and OA. Based on these previous observations, Denoble et al.[14] have postulated that uric acid could increase risk for OA through inflammasome activation. In this cohort of subjects with knee OA, synovial fluid uric acid was strongly and positively correlated with OA severity evaluated by both radiograph and bone scintigraphy and with two inflammatory cytokines IL-18 and IL-1β. They conclude that uric acid is a marker of knee OA severity.

The relationship between biomarkers levels and incident radiographic OA has been poorly investigated. Recently, Golightly et al.[15] have reported that higher baseline COMP and hyaluronic acid serum levels predicted incident joint space narrowing and higher COMP levels predicted incident knee osteophytes. These results represent detection of a molecular stage of OA prior of radiographic manifestation.

In an exploratory study, Eckstein et al.[16] compared a large set of molecular markers in biological fluids between knees with or without structural progression, using MRI-based cartilage loss as an

outcome. They found that none of the 16 molecular markers (including uCTX-II, uTUNE, s COMP, sPIINP, etc) investigated were associated with longitudinal cartilage thinning in radiographic knee OA In contrast, Berry et al[17] found that low level (less than the mean) but not high level (greater than or equal to the mean) of COMP and PIIANP, but not C2C, were associated with reduced medial cartilage volume loss over 2 years. PIIANP was also associated with a significantly reduced risk of knee joint replacement. In the Pelletier's phase III clinical trial in patients with knee OA[18], higher baseline values of IL-6, CRP and COMP were predictive of cartilage volume loss over 2 years. However, over time a reduction in MMP-1 and MMP-3 levels correlated best with reduction in volume loss and the effect of licofelone, a COX/LOX inhibitor,

OARSI OA Biomarkers Global initiative

The OARSI OA Biomarker Global Initiative aims to develop biomarkers by creating a consortium of researchers, managing research projects and organizing a series of mini-symposium and workshops. A mini-symposium was organized during the 2011 OARSI Congress. The goal of this mini-symposium was to exchange recent information and ideas among members of the biomarkers community. This mini-symposium was marked by the presentation of the Foundation for the NHI (fNHI) biomarkers project aiming to assess the responsiveness of the OA biochemical and imaging markers and to establish the predictive validity of these biomarkers using the data set of NHI funded multi-center longitudinal, prospective, observational study of knee OA. This 2.5-years project will assess 13 biochemical markers including hyaluronan, COMP, collagen I and II epitopes, aggrecan and MMP-3 in serum and urine of knee OA patients. A third workshop will be held July 12–14 2012 in South Carolina and will cover biochemical biomarkers and radiological imaging.

Concluding remarks

Research in OA biomarkers is in a growing phase stimulated by the OARSI OA Biomarker Global Initiative and industry who are expecting new tools to accelerate drug discovery and development. We speculate that biomarkers predictive of OA progression at the individual level will be available in the near future. These biomarkers will be helpful for the development of personalized management for the treatment of OA patients. The early detection of the progressor patients will allow preventative approach for managing the disease and a better use of the existing therapeutic modalities. Many biomarkers are tested as companion biomarkers by industry to rapidly detect patients who respond to a particular treatment. This attractive approach will be helpful to increase the efficacy of the OA pharmacological management and probably to better control drug consumption.

Conflict of interests

Yves Henrotin is the founders of the University of Liège spin-off company Artialis SA.

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