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Review Articles

THE PAST AND CURRENT UPDATES ON DIAGNOSTIC ASPECTS OF OSTEOARTHRITIS

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SUMMARY

Osteoarthritis (OA) is a progressive joint disease leading to the destruction of joint structures, which in turn causes severe and chronic pain to the patient. Since OA is a troubling and disruptive disease, numerous researches have been done into diagnosing this disease, both in the early and the late stages of the disease. Diagnostic modalities such as radiography, computed-tomography (CT), micro-computed tomography (μ-CT), and magnetic resonance imaging (MRI) have been used in OA research. Not only that, more advance measurements and criteria have been established to standardize OA research. Currently, the OA research has been delving into proteomic studies to search for potential disease biomarkers. Biomarkers such as urinary C-terminal telopeptide of collagen type 2 (uCTX-II) and cartilage oligometric protein (COMP) have shown potential to be both diagnostic and prognostic biomarkers. For this review paper, the developments in diagnostic modalities are discussed focusing more on proteomic and biomarker studies.

Keywords: biomarkers, computed tomography, osteoarthritis, radiography

INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease characterised by metabolic, biochemical, and structural changes of articular cartilage, subchondral bone, synovial membrane, and periarticular structures (Yamagiwa *et al.*, 2003; Castañeda *et al.*, 2012, Lee *et al*., 2013). Initially, OA was thought to be a disease of the cartilage, however as more and more extensive studies and researches were done over the years and it was unraveled that OA is a disease of organ level failure which includes ligaments, muscles, nerve, bone, and meniscus (Brandt *et al*., 2006). OA is a heterogeneous disease with many contributing risk factors that are able to induce or accelerate disease progression (Sowers & Karvonen-Gutierrez, 2010; Castañeda *et al.*, 2012). There are various contributing risk factors which can be broadly categorized into non-genetic and genetic factors (Sharma *et al.*, 2013). There are several on-going theories on aetiopathogenesis of OA that are currently being debated whether it is a cartilage or bone driven disease (Buckwalter *et al*., 2013; Houard *et al.,* 2013). Despite that argument, evidence suggests that the pathological changes occurring in the osteoarthritic joint eventually leads to its destruction and failure (Sokolove & Lepus, 2013). Simply put, OA is a troubling and painful disease that comes and never leaves and as it progresses, the pain worsens and this will affect the quality of life in one way or another.

There are different definitions for early OA but for this article, a general description of early OA is classified based on three criteria (Luyten *et al.,* 2012): (i) pain in the knee, (ii) standard radiographs with Kellgren-Lawrence grade 0 or I or II (osteophytes only) and (iii) at least one of the two following structural criteria, arthroscopic findings of cartilage lesions or magnetic resonance imaging (MRI) findings demonstrating articular cartilage degeneration and/or meniscal degeneration, and/or subchondral bone marrow lesions (BMLs).

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Diagnostic modalities of osteoarthritis

Currently, many methods of diagnosis of OA had been developed over the decades. However, for a patient suspected of OA to be first presented, they would need to have the main symptoms of joint pain, especially unbearable after weight-bearing activities and loss of range of mobility to carry out day-to-day activities (Bijlsma *et al*., 2011).

Radiography

Radiography is usually the first line diagnostic tool for diagnosing OA (Kim *et al*., 2015). Plain radiography of the joint will be taken to rule in or to rule out OA. It is fast, low cost, and easily accessible in treatment centers. Plain radiograph is the gold standard in imaging osteoarthritic joints (Bijlsma *et al.*, 2011). In 1956, Kellgren and Lawrence introduced the guidelines of radiological grading of OA (Table 1) and these guidelines are still being used up till now (Nojiri *et al.*, 2006; Fernandes *et al.*, 2015; Li *et al.*, 2015; Van Spil *et al.*, 2015). Albeit being a very useful and accessible tool to diagnose OA, radiograph has its limitations of producing two dimensional image and limited ability to visualize the articular cartilage, synovium, menisci, and other non-osseous joint structures. In addition, small osteophytes might not visualised in radiographs if hidden by overlying boney structure in radiographs. If more images were taken in various positions, it will subject the patient to more exposure to radiation. Radiography is more useful in detecting OA in the late stages, where osseous changes have already taken place compared to early OA where only subtle changes occur (Hayashi *et al*., 2014; Kim *et al*., 2015).

Table 1. Kellgren and Lawrence grading system for osteoarthritis

Classification	Radiographic findings
Grade 0	No radiographic features of OA are present
Grade 1	Doubtful joint space narrowing (JSN) and possible osteophytic lipping
Grade 2	Definite osteophytes and possible JSN on anteroposterior weight-bearing radiograph
Grade 3	Multiple osteophytes, definite JSN, sclerosis, possible bony deformity
Grade 4	Large osteophytes, marked JSN, severe sclerosis and definite bony deformity

Computed tomography

As technology advances, better machines and equipment have been engineered. Computed tomography (CT) is a technique that is well suited for bone imaging. It is a better diagnostic tool in comparison to radiography as the images are not superimposed and can be evaluated in different reconstructed views. Currently, CT has been widely used in the study of changes of different structures of the joints in events of OA. It produces three dimensional (3D) images with the option of adding contrast agent to enhance certain regions especially the cartilage (Bijlsma *et al.*, 2011; Siebelt *et al.*, 2011). On the downside, it is more costly and only available in certain hospitals and treatment centers. Not to mention, the patient is exposed to higher dosage of radiation. Furthermore, the spatial resolution of the commercial CT makes its impractical to be used for imaging laboratory animals.

Micro-computed tomography

In addition to the traditional CT, micro computed tomography (micro-CT) has been further developed for the investigation of OA in laboratory animals. Micro-CT is a small scale radiographic imaging in 3D with high resolution imaging. It has been used in an extensive range of OA studies using animal models both ex vivo and in vivo, focusing on bone morphology and microarchitecture (Bouxsein *et al.*, 2010, Lau *et al*., 2013). To enhance the targeted area such as changes in the articular cartilage or subchondral bone, radiopaque contrast agents such as barium sulfate (Leng *et al*., 2008), sodium and meglumine ioxaglate (Kotwal *et al.*, 2012; Lau *et al*., 2013) had been added in to the bone or joint space in order to highlight these specific regions. So far, micro-CT has been used more for research purposes rather than for clinical diagnosis. Despite that, the results has been translated into the clinical applied CT arthrography (Siebelt *et al*., 2011)

As technology progresses, four dimensional (4D) micro-CT combines in vivo micro-CT imaging and a computational approach for direct visualisation of images. By creating 3D spatial and one dimension (1D) temporal CT images, this technique has allowed for achieving the 3D measures comparable to traditional 2D histology with the additional capability of temporal characterization of the formation, resorption, remodeling, and bone turnover events (Birkhold *et al.*, 2015). However, even though 4D micro-CT has not yet been applied to the study of OA thus far, it is only a matter of time before it is used to monitor changes of the articular cartilage and subchondral bone.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) has been used widely used in the diagnosis of OA. Based on past literature, MRI is also an important tool to diagnose and stage early OA (Madry *et al.*, 2016). As it is known for its ability to image soft tissues, MRI is used in imaging joint structures including articular cartilage, menisci, ligaments, and synovium. As degeneration of articular cartilage is one of the hallmarks of OA progress, MRI has been extensively used in many recent OA studies (Sharma *et al*., 2014; Wise *et al.,* 2016). The capability of MRI to provide exquisite contrast of the morphological and physiological conditions of the articular cartilage had shed light in the events of pathogenesis of OA.

Delayed Gadolinium-enhanced MRI (dGEMRIC) has been introduced in OA studies to quantitatively estimate glycosaminoglycan content of cartilage (Nojiri *et al*., 2006; Madry *et al*., 2016). T2 mapping or diffusion-weighted imaging (DWI) together with MRI was also used to provide qualitative and quantitative information to evaluate tissue changes and structural integrity (Braun & Gold, 2012; Madry *et al*., 2016). Since MRI has a higher sensitivity for the detection of OA as compared to radiographs, it has been greatly used for both diagnosing and monitoring OA progression, not to mention pre and post-operative surgical treatments involving the joint. Nonetheless, MRI has its disadvantages in its high cost, acquisition time, and the need for a skilled operator due to its complex techniques.

Concurrently, researchers then turned to proteomics to search for disease biomarkers related to OA. A disease biomarker can be defined as a measurable indicator of a specific biological state, particularly the one that provides essential details about the risk, presence or stage of a disease (Rifai *et al.*, 2006). It can be used for very broad clinical applications, both for diagnostic purposes and as a prognostic indicator. Currently, disease biomarkers are the most promising method for diagnosing early OA (Madry *et al*., 2016). More on the current proteomic studies and possible biomarkers will be discussed.

Proteomic studies in diagnosing early OA

The Biomarkers Definition Working Group defined biomarkers as a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, pathogenic process, or pharmacologic response to a therapeutic intervention. As comprehensive proteomic studies uses clinical samples such as tissues or body fluids, it is better defined as clinical proteomics (Paik *et al*., 2008). In clinical proteomics, the main aim is the discovery of biomarkers, specifically disease biomarkers.

The process in proteomics depends on the separation of a large number of proteins which are identified by mass spectrometry (MS) (Ruiz-Romero and Blanco, 2010). Separation of proteins can be carried out with methods using two dimensional gel electrophoresis (2DGE) and liquid chromatography (LC). These individual proteins could be identified via MS. MS protein identification is performed through a process of protein or peptide ionisation, ion separation and lastly detected according to the instrument used. Peptide ionisation can be done using matrix-assisted laser desorption/ionisation (MALDI) source or in solution for electrospray ionisation (ESI) sources. Then, these ions are separated according to their mass/charge relationships *(m/z)* by using a time-of-flight (TOF), quadrupole or ion trap analyser. The ion masses are measured in the detectors to form a mass spectrum according to its *m/z* values. These values will then be compared to the database to determine the protein identified (Mateos *et al*., 2012; Hrabák *et al*., 2013).

Proteomics in OA research is mainly conducted to (i) understand the pathophysiology and disease mechanism of OA development, (ii) find potential disease biomarkers, and (iii) identify new therapeutic targets (Gharbi *et al.*, 2011). Proteomic research permits the discovery of novel protein biomarkers not only for diagnosis but also for the planning of therapeutic treatment. These biomarkers will help in developing a therapeutic treatment plan that caters to different individuals at different stages and progress of the disease (Cho, 2007). Proteomics also provides the opportunity for the study of novel molecular targets for drug discovery.

Selected OA proteomic studies published between 2010 and 2015

Proteomics research for biomarkers discovery of OA can be divided into two approaches which are (i) target-specific and (ii) global/non-directed (Ruiz-Romero and Blanco, 2010). The target-specific approach focuses on particular biomarker/s and often uses antibodies via Western blot analysis, enzyme-linked immunosorbent assay (ELISA), or antibody arrays (Ruiz-Romero and Blanco, 2010). On the other hand, the global or non-directed approach screens and profiles all proteins that were identified.

Over the years, many methods and strategies were developed for uncovering biomarkers in OA. Some of the methods and strategies that were used over the last five years are listed in Table 2. Several researches on finding the potential biomarkers of OA were done using 2DGE followed by MS analysis. In addition, various methods of sample preparations such as high abundance protein depletion were studied before by analysing by MS and bioinformatics tools. For example, Bennike (2014) prepared his samples with different trypsin digestion protocols and later analysed them by high resolution/high-accuracy MS systems for shotgun proteomic analysis. Another highly popular method is one dimensional gel electrophoresis (1DGE) followed by MS analysis.

Other methods that have been popular in this field are gel free methods or also known as shotgun proteomics. Proteins are separated by high performance liquid chromatography (HPLC) followed by MS analysis (Gharbi *et al.*, 2011). This method has a higher resolution and also easier to perform because the procedures are automated.

Table 2. Some of the strategies for proteomic research for osteoarthritis

Proteomic approaches	Mass spectrometer	References
1DGE	LC-MS/MS	Garner *et al*., 2013 Balakrishnan *et al.,* 2014
1DGE, In-gel digestion and iTRAQ labeling	LC-MS/MS	Lourido *et al*., 2014
1DGE, Filter-Aided Sample Preparation (FASP) Digestion, Urea In-Solution Digestion, In-gel digestion	LC-MS/MS	Bennike *et al*., 2014 Bennike *et al*., 2015
Western blot	Surface enhanced laser desorption/ionization (SELDI)-TOF-MS	Han *et al*., 2012
2DGE	- MALDI-TOF-MS	Kong *et al*., 2012 Chiaradia *et al*., 2012 Chen *et al*., 2011
2D Difference Gel Electrophoresis (2DIGE) and Western Blot	LC-MALDI-TOF/TOF	Fernandez-Costa *et al*., 2012
Nano Liquid Chromatography (nanoLC) and Western Blot	MALDI-TOF/TOF	Mateos *et al*., 2012
FFPE (formalin-fixed paraffin-embedded)	LC-MS/MS	Hayashi *et al.,* 2015
Protein chip array	SELDI-TOF-MS	de Seny *et al*., 2011
Selected reaction monitoring (SRM)	MS	Ritter *et al*., 2014

Table 3. Potential biomarkers for osteoarthritis

Area	Group	Potential Biomarkers	Function
Joint inflammation	Innate immune system	Chemokines: • Interferon gamma inducible protein 10 (CXCL-10) • Serum fractalkine (CX3CL1) • SF CXCL12 chemokine ligand 2 (SF MCP-1/CCL2)	Act as chemoattractants to guide cells to migrate to a specific location
		Cytokines: • IL-1α • IL-18 • TNF-α (effectively used to monitor efficacy of various OA treatment in rabbits)	Small proteins released from various cells
		Macrophage: • CD14 • CD163	Associated with response to inflammation
		Complement: • C3a • C5b-9	Assists antibodies and phagocytic cells with the clearance of foreign objects
		C-reactive protein (CRP)	Central component of the innate immune inflammatory response
		Adipokines/Hormones: • Adiponectin • Leptin • Adipsin • Visfatin • Estradiol • Urinary estrogen metabolite 2-hydroxyestrone	Mediates inflammatory effects and cartilage catabolism in OA
Articular cartilage	Structural protein	Type II collagen	Bind to other matrix macromolecules i.e. types IX and XI collagen and is critical for cartilage stability
	Type II collagen degradation	Helical fragments: • Helix-II • Coll 2-1 • Coll 2-1 NO_2	Play a role in inflammatory processes and cartilage destruction of the joint
	Proteoglycan	• ADAMTS4 • ADAMTS5	Protein core to which many chondroitin and keratin-sulphate chains are attached to
	Cartilage degradation	Cartilage oligomeric matrix protein (COMP)	Aids in inflammatory proliferation of synovial membrane, regulation of fibril network and maintain the mature collagen network (Sharma *et al*., 2013)
		Amino-terminal type II procollagen propeptide (PIINP)	Reflects the rate of synthesis of collagen type II
		Carboxy-terminal type II procollagen propeptide	
		YLK-40 Glycoprotein: noncollagenous protein	Aids in tissue inflammation, immunity, and/or remodeling

		Hyaluronic acid (HA)	Provides viscoelasticity of synovium fluid and cartilage
	Remodeling of calcified cartilage	*C*-terminal telopeptide of type II collagen (CTX-II)	Provides strength, integrity and maintain shape of tissue
	Cartilage turnover	Chondroitin sulfate 846 epitope (CS 846)	Provides hydrated gel structure of the cartilage
Synovial fluid	Angiogenetic factor	Vascular endothelial growth factor (VEGF)	Increases vascularity within articular cartilage
		Sphingosine 1-phosphate (S1P)	Regulation of cyclooxygenase-2 (COX-2), VEGF, and inducible nitric oxide synthase (iNOS), MMP-13, and ADAMTS-4 (cartilage chondrocytes)
Chondrocytes	Osteoblasts	RANKL	Causes elevated osteoclastogenesis during OA
Bone	Bone degradation	Amino-terminal type II procollagen propeptide (PIINP)	Reflects the rate of synthesis of collagen type II
		Carboxy-terminal type II procollagen propeptide	
	Anabolic bone turnover	Osteocalcin (OC)	Needed for bone mineralization and recruitment of osteoblast and osteoclast
	Type I collagen degradation	*N*-terminal type I collagen telopeptides (NTX I)	Maintains bone remodeling process
Subchondral bone	Growth factors	• Insulin-like growth-factor 1 (IGF-1) • Transforming growth-factor β (TGF β)	Contributes to bone formation

Potential biomarker discovery

According to Lotz (2013), suitable biomarkers for OA should be structural molecules or a fragment linked to cartilage, bone or synovium of the joint which only relates to joints specifically that affected the joint. Based on the past proteomic studies conducted, many potential biomarkers were uncovered. These biomarkers are listed in Table 3.

Disease biomarkers are the future of diagnosing OA. From there, a tailored multimodal treatment using Disease Modifying OA Drugs (DMOAD) concurrently with physiotherapy and muscle strengthening exercise can be used to combat this troubling disease. The possibility of usage for pinpointing the area of damage, the progression of disease, and monitoring the treatment outcome is endless and will open doors to many more studies.

Nonetheless, out of these biomarkers which were discovered, only a handful showed potential to be biomarkers. A few of those included urinary C-terminal telopeptide of collagen type 2 (uCTX-II) and serum cartilage oligomeric protein (COMP) showed high potential to be both diagnostic and prognostic biomarkers (Dam *et al*., 2009; Lotz *et al*., 2013). However, till now, there is still no "the one" biomarker that has been sufficiently validated to be the gold standard regardless of the extensive amount of research that had been done (Madry *et al.,* 2016).

Conversely, there are also limitations to their uses. If other physiological and pathological changes do occur in other parts of the body other than the joints, systemic biomarkers will also be circulated might interfere with or provide a false diagnosis. Besides that, the process of detecting disease biomarkers requires a high cost and may not be feasible to be used in the clinical setting as compared to other diagnostic modalities.

CONCLUSION

More cutting-edge technology and equipment have greatly advanced the OA study in the hope of developing a sustainable method to diagnose early onset of OA. Ultimately, these studies hope to arrest this disease before it develops further and to finally to uncover a cure for this debilitating disease. Engineers will continue to develop higher resolution imaging modalities and scientist will continue finding new and noble methods to uncover "the one" biomarker and eventually piece together the puzzle on the pathogenesis of OA.

DECLARATION OF INTEREST

The authors report no conflicts of interest.

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