Abstracts from Invited Speakers

I-1

CLINICAL PERSPECTIVE ON THE ROLE OF BIOMARKERS IN THE DIAGNOSIS AND MONITORING OF OSTEOARTHRITIS

V. Byers-Kraus
Duke Univ., Durham, NC

Purpose: Evaluating the clinical effectiveness of drugs for OA poses special challenges because of the difficulty in assessing the stage and state of the disease, the slow changes in the current radiographic measure of joint space narrowing, and the duration of the studies necessary to show significant effects. Biomarkers are a potential means of providing quantitative and objective indices to help overcome some of these challenges.
Methods: Before a biomarker is eventually adopted as a clinical tool, it requires biological and clinical validation, ideally in large numbers of patient samples. The evidentiary process linking a biomarker with biology and clinical end points has recently been defined as biomarker qualification.
Results: Although the qualification of OA biomarkers is gaining momentum, the current state of biomarkers for OA is analogous to the state of biomarkers for osteoporosis 30 years ago. Unlike osteoporosis, for which bone density emerged as the gold standard measure, in OA it remains to be determined what surveillance measures will ultimately be adopted. Like osteoporosis, OA has a silent molecular stage, and late stage adverse outcomes. OA has added complexity and heterogeneity with different patterns of joint involvement and different inciting pathologies including genetics, injury, and joint anatomy to name a few.
Conclusions: Increasingly, studies are demonstrating biomarkers with the ability to quantify various dimensions of the OA process with accuracy and precision. Biomarkers used singly and in combination, in conjunction with imaging, demographics, and OA risk factors, provide a rich toolset for evaluating the efficacy of drugs for OA. Increasing use and reliance on a comprehensive toolset, it is hoped, will contribute substantially to development and approval of therapeutics to favorably impact the course of this disease.

I-2

IMMUNOHISTOCHEMISTRY AND CONFOCAL TECHNIQUES

C.M. Brown
McGill Univ. Imaging Facility, Monteal, QC, Canada

Purpose: To provide an introduction to Immunohistochemistry and Confocal Imaging Techniques
Methods: How to perform imaging of immunohistochemistry samples either with a colour camera or blue, green and red filter sets. How to generate high quality confocal images and to separate fluorescence dyes with similar fluorescence spectra. How to remove specimen auto-fluorescence from the signal from specific fluorescence probes.
Results: Proper camera colour balance is required to generate suitable images of immunohistological samples. Three images using blue, green and red filters can be generated using a monochrome camera and overlayed in image processing software in order to image Immunohistological samples without the need for a colour camera. With the proper imaging settings high quality confocal images can be generated from fluorescent samples.

Spectral imaging can be used to separate dyes of similar colour and to remove specimen auto-fluorescence.
Conclusions: Following this presentation attendees should have a clear idea of the main aspects to keep in mind when performing brightfield or confocal imaging.

I-3

STRATEGIES TO USE MODERN IMAGING TECHNOLOGIES TO EVALUATE PATIENTS WITH OA

F.M. Cicuttini
Monash Univ., Melbourne, Australia

Purpose: The radiographic examination of joints is useful in the diagnosis of osteoarthritis (OA) and has enabled significant progress to be made in our understanding of risk factors for OA. However new imaging modalities provide novel opportunities to investigate the pathogenesis of OA through examining the whole joint. The aim of this presentation is to examine current strategies using modern imaging technologies in the evaluation of patients with OA and to consider the potential impact of these new approaches.
OA is a disease of the whole joint. By using improved, non-invasive techniques such as magnetic resonance imaging (MRI) to visualise the whole joint, it is now possible to examine the relative role of different joint structures such as cartilage, bone, menisci, synovium and ligaments simultaneously on the symptomatology and progression of OA. Recent work demonstrates that knee OA is the result of multiple, cumulative changes in joint structure occurring in the apparently healthy, asymptomatic state, which progress until clinical disease presents itself. The use of MRI, a sensitive and non-invasive tool, has enabled the examination of these early changes of OA, prior to radiographic disease, through to the joint with end stage disease. This shows that our current definitions of radiographic tibiofemoral OA fall on the continuum from health to disease, with approximately 10% of articular knee cartilage lost before radiological disease is detected. Since OA is recognised as a multifactorial disease, using a sensitive examination of joint structure, provides opportunities to examine new hypotheses in the pathogenesis of OA. Identifying early changes may provide novel targets for the prevention and treatment of knee OA. At an individual level, they may also help identify those at risk of OA who may benefit from early lifestyle modification. Although much of the current wok has focussed on MRI, other modalities such as ultrasound, scintigraphy, and CT are also being used in the evaluation of patients with OA and provide complementary information.

I-4

NEW APPROACHES TO MEASUREMENT OF OUTCOMES TO ASSESS EFFICACY OF OSTEOARTHRITIS TREATMENT: THE OARSI-OMERACT INITIATIVE

M. Dougados
René Descartes Univ., Paris, France

Purpose: In order to propose a set of criteria for considering total articular replacement (*e.g.* medical treatment failure) to be used as an end-point in clinical trials evaluating potential disease modifying drugs, a joint OARSI-OMERACT initiative is aiming at

proposing a definition of structural progression and a definition of unacceptable symptom state.

Methods: The group in charge of the "unacceptable symptom state" first decided to focus on 2 domains *e.g.* pain and functional impairment, second to propose new tools for evaluating such domains, and third to conduct a cross sectional study in order to propose a threshold of the value in such tools above which the level of symptoms will be considered as not acceptable. For such purpose, the opinion of the orthopedists concerning their visiting patients (*e.g.* good candidate for total articular replacement yes/no) was considered as the gold standard.

Results: An OARSI-OMERACT pain tool and an OARSI-OMERACT function hip/knee tools have been elaborated [1–3]. The study aimed at proposing a threshold in such tools for defining a non acceptable symptom state has recruited more than 2000 patients in more than 10 different countries.

Conclusions: Such joint OARSI-OMERACT initiative should facilitate the conduct of clinical trials to more accurately evaluate not only symptomatic but also disease modifying treatments.

References

[1] Hawker GA, *et al.* Osteoarthritis Cartilage 2008;16:409-14.
[2] Davis AM, *et al.* Osteoarthritis Cartilage 2008;16:551-9.
[3] Perruccio AV, *et al.* Osteoarthritis Cartilage 2008;16:542-50.

I-5

ADVANCES IN THE UNDERSTANDING OF THE ROLE OF AGING IN THE DEVELOPMENT AND PROGRESSION OF OA

J. Haag
Inst. fuer Pathologie, Leipzig, Germany

The prevalence of primary osteoarthritis (OA) increases with age. Aging, therefore, represents a major risk factor for OA. Cartilage degradation is most certainly not simply an effect of wear and tear processes but is significantly influenced by molecular regulatory systems. However, the degree to which regulated aging processes are responsible for the loss of tissue function in OA is not yet fully resolved. Several experimental findings are consistent with a senescent-like state of arthritic chondrocytes and cartilage. These include a shortening of the telomere ends, the expression of senescence markers like SA-βgal, altered cytokine expression profiles and age-dependent modifications of the extracellular matrix. Our own results show an increased transcriptional noise and elevated DNA damage levels in OA chondrocytes.

The process of aging is not an unregulated process but is regulated by evolutionary conserved pathways that safeguard the maintenance of cellular homeostasis. The deregulation of these regulatory networks is linked to the development of aging-associated diseases. In this respect, perturbations of the somatotrophic axis (GH-IGF-Akt) are of special interest. Mice with reduced IGF-1 and insulin signaling (Ames and Snell dwarf mice, Klotho-overexpressing mice) live significantly longer than littermate mice and show an overall reduction of aging-related pathological changes. Interestingly, long-lived Snell dwarfs (which do not produce growth hormone) show a delay of articular cartilage aging and do not develop OA at an age when up to 58% of their wild-type littermates already do.

Our own research provides evidence for a major perturbation of the IGF-Akt-FoxO signaling pathway in human osteoarthritic knee cartilage with reduced FoxO expression levels and an overall reduction of the expression of important stress-response genes concomitant with higher cellular damage levels. The transcript levels of all four known FoxO genes, FoxO1, FoxO3a, FoxO4 and FoxO6 were significantly reduced in the osteoarthritic tissue. This decrease was more pronounced in late osteoarthritic cartilage compared with early degenerated tissues. Several important

FoxO target genes were substantially deregulated. The cell cycle regulators p21, p27 and cyclin G2 were significantly downregulated, while cyclin D1 was markedly upregulated, corresponding to the downregulation of FoxO levels. The FoxO regulated genes involved in oxidative defense also showed differential regulation. The expression of SOD genes was substantially reduced in OA, with SOD2 showing the most substantial decline. mRNA levels of GADD45, a gene involved in DNA repair, were markedly lower in osteoarthritic tissue.

The emerging insights into the mechanisms underlying general aging processes promise to open up new avenues for drug discovery. The identification of pharmacologically tractable drug targets within these conserved aging pathways will make it possible to develop novel therapeutic options for age-related diseases, including primary osteoarthritis. We expect, therefore, that further insights on the role of natural aging processes in the development and progression of primary osteoarthritis, and thus a better understanding of the processes underlying cartilage aging and degradation, will translate into novel ideas and ways of preventing and treating OA.

I-6

BASIC PERSPECTIVE ON THE ROLE OF BIOMARKERS IN THE DIAGNOSIS AND MONITORING OF OSTEOARTHRITIS

D. Heinegård
Lund Univ., Lund, Sweden

An opportunity to detect early stages of process leading to clinical osteoarthritis (OA) is offered by the emerging molecular marker technology. This development will be further advanced when markers with spatial, temporal and process specificity become available. Molecular markers have the potential to provide information the relative activity of the process of degradation and repair attempts, including an inadequate tuning of the synthesis of new constituents of the matrix.

markers with apparent tissue specificity: There are a number of molecules that are released from cartilage as a result of the local process. These include *aggrecan*, where the fragments containing the chondroitin sulfate chains appear early in disease and are formed also in the normal tissue turnover. The fine structure of the glycosaminoglycan side chains change both with age and in disease.

The G1 hyaluronan binding domain is initially retained in the tissue bound to hyaluronan to be released in late stage tissue injury.

Cleavage of Aggrecan at several specific sites by ADAMTS-family members provides unique cleavage sites and neo-epitope antibodies only recognizing fragments with an end corresponding the cleavage are available and applied to studies of joint disease.

Collagen type II epitopes studied include those of the telopeptides at the ends of the molecule and those from cleavages of the triple helical region. Applications of these assays demonstrate increased release of fragmented collagen in osteoarthritis. There are discussions on the specificity of some of these assays.

New synthesis of collagen can be detected by the production and release of the propeptides cleaved of the precursor procollagen II. Such assays have been employed in studies of collagen synthesis in joint disease.

Spatial specificity is possible to obtain by using proteins which are present in only a given tissue compartment.

Interterritorial distribution: Examples of such proteins are *CILP* and *COMP*. Both occur preferentially in the interterritorial matrix of the adult articular cartilage.

COMP is released at an early stage of joint disease. Accordingly elevated COMP levels may serve as a predictor of destruction of the cartilage identified by imaging years later. One shortcoming of many of currently employed assays, including for COMP, is the background provided by turnover in normal cartilages. As a