Biomarkers of lameness in dairy animals: The challenge of translating information from *in vitro* systems

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Other Affiliations

Arthritis Research UK
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Co-ordination of EU FP7 Project D-BOARD
5 year project, started October 2012, ends September 2017

Other Responsibilities

**OARSI**
- Member of the **Board of Directors**
- Former Co-Chair of the **Communications Committee**
- Current Chair of the **Strategic Alliances Committee**

**The European Commission**
- Served on expert evaluation panels responsible for the area of **Aging and Chronic Diseases**

**BBSRC**
- Served on **Committee A (Animal disease, health and welfare)**
- Member of the **Industrial CASE Studentship Committee**
- Served on the **Portfolio Monitoring Group**
This DairyCare Conference focuses on the Health, Welfare and the Lameness/Reproduction interface in dairy animals

A topic that is of interest to members of this COST Action is the use of biomarkers to detect and assess health/welfare problems, including lameness.

This presentation will provide an overview of the pathophysiologic changes that occur in inflammatory and degenerative diseases of joints and discuss the role of biomarkers in disease diagnosis, monitoring and treatment.
Lameness in Dairy Cattle

Definition

Lameness is “a disturbance of locomotion”

“Deviation in gait and posture”

DairyCo:
“The best all-encompassing definition of lameness includes any abnormality which causes a cow to change the way that she walks, and can be caused by a range of foot and leg conditions, themselves caused by disease, management or environmental factors.”

http://www.dairyco.org.uk/

Causes of Cattle Lameness:

1. Digital Dermatitis – Highly contagious and erosive infection
2. White Line Lesions - Disruption in the wall of the horn
Osteoarthritis (OA)

- The most common form of arthritis in humans and companion animals
- A major cause of joint pain, inflammation and loss of mobility
Osteoarthritis (OA)

- Characterized by progressive deterioration and loss of articular cartilage
- Primarily affects load-bearing synovial joints (knees, hips) but other joints may be affected (hands)
Loss of Homeostasis Leads to Osteoarthritis

Articular cartilage

Calcified cartilage

Subchondral trabecular bone

Trauma
Inflammation
Genetic defects
Aging

Synthesis
Degradation

Phenotypic Modulation

Articular cartilage

Tidemark duplication
Subchondral cortical bone
Vascular invasion

Slide courtesy of Dr. Mary Goldring, Hospital for Special Surgery, New York
Global Prevalence of Obesity

**OBESITY:** The percentage of the population older than 15 with a body-mass index greater than 30.

- **USA:** 31%
- **Mexico:** 24%
- **UK:** 23%
- **Slovak Republic:** 22%
- **Greece:** 22%
- **Australia:** 22%
- **New Zealand:** 21%
- **Hungary:** 19%
- **Czech Republic:** 15%

- **Canada:** 14%
- **Spain:** 13%
- **Ireland:** 13%
- **Germany:** 13%
- **Portugal:** 13%
- **Finland:** 13%
- **Turkey:** 12%
- **Belgium:** 12%
- **Poland:** 11%

- **Netherlands:** 10%
- **Sweden:** 10%
- **Denmark:** 10%
- **France:** 9%
- **Austria:** 9%
- **Italy:** 9%
- **Norway:** 8%
- **Japan:** 3%
- **Korea:** 3%
Obesity is a Major Risk Factor for OA

Strong evidence for a link between obesity and OA

Obesity is a complex metabolic and inflammatory syndrome

– Adipokines, (adipocytokines) play important roles in the onset of disease

Lifestyle changes for OA patients:

– Weight loss and calorie restriction

Evidence from Epidemiological Studies

First National Health and Nutrition Examination Survey (HANES I)

- Obese women 4x greater risk of developing knee OA
- Obese men 5x greater risk of developing knee OA


Framingham study

- Overweight individuals in their thirties without OA were at greater risk of developing the disease later in life


Different studies with repeated X-rays over time

- Being overweight significantly increases the risk of developing knee OA

Obesity - Shifting the Paradigm in Osteoarthritis

Obesity versus Osteoarthritis

- mechanical overload
- systemic inflammation/metabolic factors

Obesity

Osteoarthritis
The Role of Inflammation in the Pathogenesis of OA

Inflammatory mediators released may contribute to other degenerative diseases

The Co-Morbidity Hypothesis
Systemic Effects and Potential Consequences of OA Derived Inflammatory Mediators

Low-grade inflammation
(adipokines, cytokines, lipid mediators, ROS, etc.)

Adipose tissue

Hyperglycemia, Ox-LDL

Cell senescence (inflammaging)

OA

Systemic effects of OA-derived inflammatory mediators

• Alzheimer disease
• Stroke

• Myocardial infarction
Emerging Concepts in OA

Different Phenotypes of OA

- Inflammatory OA
- Cartilage-driven OA
- Bone-driven OA
- Traumatic/acute OA
Inflammation is now well accepted as a feature of osteoarthritis but we have known about this for almost 40 years.

George E. Ehrlich (1929-2014)

In a paper published in 1975 Ehrlich described a cohort of predominantly menopausal females who presented with a deforming and inflammatory osteoarthritis, some of whom went on to develop changes characteristic of rheumatoid arthritis.

Joint Trauma:

Strong Risk Factor for the Development of OA in Athletes

Joint injury is associated with an increased risk of developing post-traumatic OA (PTOA)
Elite and professional athletes (engaged in high impact sports) are at substantially increased risk for injury and for subsequent development of OA in the affected joints, even without major injury*

- Mechanical Factors
  - Injury
  - Surgery
  - Muscle weakness
  - Joint deformity
  - Repetitive joint loading

*Arden and Nevitt (2006)
Best Practice & Research Clinical Rheumatology 20, 3–25
ABNORMAL LOADING
OBESITY
(Mechanical Stress + Adipokines)

CYTOKINES
CHEMOKINES
TLR/RAGE Ligands
ADIPOKINES

ECM DEGRADATION PRODUCTS

Cartilage

MMPs
ADAMTS

NOS2
COX2

Otero, Loeser, Goldring – OARSI Primer
Pathogenesis of osteoarthritis

- Macrophage
- Synoviocyte
- Mast cell
- T cell
- NF-κB
- IL-1β
- TNF-α
- MMPs
- IL-6
- RANKL
- Osteoclast
- SUBCHODRAL BONE
- SYNOVIAL MEMBRANE
- SYNOVIAL FLUID
- ARTICULAR CARTilage

Provided by Patrick du Souich
Catabolic and Anabolic Factors Regulating Chondrocyte Function

What is a biomarker?
What is the rationale for identifying new biomarkers of OA?
Can biomarkers help us define “early”, “pre-radiographic” OA?
A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework

Biomarkers Definitions Working Group *Bethesda, Md*
The Gold Standard (Radiography) is Inadequate
Rationale for Identifying Early Osteoarthritis Biomarkers

Early detection will facilitate earlier diagnosis and treatment because OA which is characterised by a prolonged pre-clinical ‘molecular’ phase, a ‘pre-radiographic’ phase, and a ‘recalcitrant radiographic’ phase by which time there are extensive structural changes to joints along with pain and loss of function.

Biomarkers could provide an early warning of joint degeneration which could prompt earlier, more targeted treatment.

Osteoarthritis and Cartilage 19 (2011) 515–542
Biomarkers may aid in the phenotyping of OA patients, as they are direct measures of joint destruction.

Convergence of pathways leads to tissue destruction.


Slide courtesy of Dr. Anne-Christine Bay-Jensen, Nordic Bioscience Company
Bone and Joint Biomarkers

**Inflammation**
- VICM; Citrullinated and degraded vimentin
- CRPM; MMP degraded CRP
- Liver derived CRP*

**Joint destruction**
- CTX-I; Bone resorption
- C2M; Cartilage degradation
- ICTP*; Connective tissue degradation by MMPs
- AGNx1; ADAMTS degraded aggrecan
- C6M; Interstitial matrix destruction by MMPs

**Proteases**
- MMP3*
- Active MMP3
- Active MMP9
- Active MMP13
- Active ADAMTS 4/5

**Joint formation**
- Osteocalcin; bone formation
- PINP*; Connective tissue formation
- PIIANP*; Cartilage dedifferentiation
- P2NP; Cartilage formation

**Synovial turnover**
- C3M; MMP degraded type III collagen
- C1M; MMP degraded type I collagen
- VCANM; Versican degraded by MMPs
To bring together a consortium of **specialist academic institutions** and **leading SMEs** from strategically important geographical regions of Europe to use advanced analytical (omics-based) technologies to **identify new biomarkers** and **develop sensitive diagnostic tests** capable of **subclinical disease diagnosis** for OA.
D-BOARD: “Omics-Based” Focus on novel OA Biomarkers

D-BOARD uses an “omics-based” approach to identify **new** OA biomarkers and develop new biomarker assays.
Translating Information from *In Vitro and Animal* Models to Human Subjects and Patients
Candidate Biomarker Discovery (WP1, WP2, WP3, WP5)

Biomarker Verification § (WP1, WP4, WP6)

Biomarker Assay Development and Optimization (WP1, WP6)

Biomarker Validation ¶ (WP1, WP4, WP6)

Biomarker Qualification * (WP1, WP6)

Product Development and Commercialization (WP6)

§ Independent scientific and analytical verification of the biomarker – this process involves verification of the analytical performance characteristics and clinical correlation of a biomarker with a biological process or clinical outcome

* Linking a biomarker to a clinical endpoint - Qualification is a process applied to a particular biomarker to support its use as a surrogate endpoint in drug discovery, development or post approval and, where appropriate, in regulatory decision making

¶ Assessing all technical aspects of the biomarker assay
SMEs and Collaborating Companies in D-BOARD

NORDIC BIOSCIENCE
BIOMARKER & RESEARCH

BiOMEDIQ

TNO innovation for life

BiOTalentum

artialis
Passion for joint health

QuickZyme Biosciences

Novel Diagnostics and Biomarkers for Early Identification of Chronic Inflammatory Joint Diseases

European Commission

SEVENTH FRAMEWORK PROGRAMME
External Advisory Committee

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Duke University Medical Center
Durham, NC

**Linda Sandell, PhD**
Washington University
St. Louis, MO

**David Hunter, MD, PhD**
St. Leonards, Australia
D-BOARD WP1 (Proteomics)

PROTEOMIC APPROACHES in OA

Biological fluids
- Serum/Plasma
- Urine
- Synovial fluid

Tissues
- Cartilage
- Bone
- Synovial membrane

Cells
- Cartilage extract
- Bone explant
- Synovial membrane explant
- Secretome

Cell types
- Chondrocytes
- Osteoblasts
- Synoviocytes
- Osteoclasts
The APPROACH Consortium

APPRO\textit{OA}Ch – \textit{Applied} Public-Private Research enabling \textit{OsteoArthritis} Clinical Headway

Paul-Peter Tak (Glaxosmithkline Research and Development Ltd)

Harrie Weinans, PhD (UMCU, Managing Entity)

Jonathan Larkin, PhD (GSK, Project Coordinator)
The goal of APPROACH

Aim: Find the right patient for the right treatment

Biomarkers
  • Reporters
  • Targets

Models $\rightarrow$ Guidelines
Proteomic and metabolomic tools are being used to study metabolic profiles of articular cartilage and synovial tissue in inflammatory culture models and in sera from patients with osteoarthritis (OA)

- **Proteomic and metabolic profiling** of biological fluids and joint tissues can provide a global view of the physiologic state of the intra-articular environment of an osteoarthritic joint

- **Advances in analytical techniques** will enable improved metabolic profiling of different stages of disease
Healthy cartilage

Inflammatory cartilage

Healthy chondrocyte

Chondrocyte in an Inflammatory Environment

• CHONDRION: Chondrocyte Ion Channel Function and Regulation in Health and Disease

Dr Csaba Matta
Marie Curie Research Fellow

CHONDRION

UNIVERSITY OF SURREY
The **ADVANCE Study** is a cohort study investigating the long term cardiovascular, musculoskeletal and other health and psychosocial related outcomes of UK armed services physical battlefield trauma patients over a 20yr period.

- There have been no similar prospective cohort studies in military trauma populations.
- Trauma and amputation are risk factors for development for OA.
- N= 600 battlefield trauma casualties  N=600 non exposed controls. BL and FU: 5yrs, 10yrs, 15yrs and 20yrs.
- The “non exposed” group will be matched for age, sex, rank, role and deployment.

The **BIO-MIL-OA study** is a sub study of the ADVANCE study investigating radiographic and pain outcomes and predictive biomarkers of OA in military trauma patients and matched “controls”.

- **Aims of the study**
  - To identify predictive biomarkers of OA in a young military combat casualty cohort.
  - To identify predictive biomarkers of OA in a young non injured active military cohort.
  - To identify pain outcomes in military combat casualties and active age matched controls.

**Funding**

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<tr>
<td>0-5yrs</td>
<td>£1.62M</td>
</tr>
<tr>
<td>Total: 0-20yrs</td>
<td>£6.48M</td>
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**PI:** Wing Commander Alex Bennett, PhD

**Co-I:** Ali Mobasher, D.Phil
In Vitro Models

Primary Cultures

Co-cultures

3-Dimensional Alginate Culture

3-Dimensional Explant Culture

Figure 1: Different models of co-culture using ThinCert™ cell culture inserts. Two cell populations that are co-cultivated in different compartments (inset and wall) stay physically separated, but may communicate via paracrine signaling through the pores of the membrane (a, b). Alternatively, both cell populations may be co-cultivated in the upper compartment (a), thus allowing extensive and direct cell-cell interactions (c).
3-Dimensional Explant Culture

Cartilage is harvested from synovial under sterile conditions and pieces (explants) are placed in a culture dish containing growth media

- Advantages of the Cartilage Explant Culture Model:
  - Cells remain in their original 3-dimensional environment
  - The extracellular matrix around them precisely mimics their in vivo environment
  - Ideal for studies of extracellular matrix synthesis and degradation
  - Suitable for proteomic work
Proteomic Studies on the Secretome using Explant Models of Articular Cartilage

High throughput proteomic analysis of the secretome in an explant model of articular cartilage inflammation

Abigail L. Clutterbuck, Julia R. Smith, David Allaway, Pat Harris, Susan Liddell, Ali Mobasheri

Applications of proteomics in cartilage biology and osteoarthritis research

Adam Williams, Julia R. Smith, David Allaway, Pat Harris, Susan Liddell, Ali Mobasheri

Applications of proteomics to osteoarthritis, a musculoskeletal disease characterized by aging

Ali Mobasheri

Musculoskeletal Research Group, Division of Veterinary Medicine, School of Veterinary Medicine and Science, University of Nottingham, Nottingham, UK
Affinity purification coupled with mass spectrometry (AP-MS) is a widely used approach for the identification of protein-protein interactions. However, for any given protein of interest, determining which of the identified polypeptides represent bona fide interactors versus those that are background contaminants (for example, proteins that interact with the solid-phase support, affinity reagent or epitope tag) is a challenging task.
Using Matrix Metalloproteinases (MMPs) as biomarkers of responses to NSAIDs
Cartilage shavings collected from equine metacarpophalangeal joints

Five 3 mm diameter explant discs cut and placed in 1 ml serum free DMEM to establish explant cultures.

- Untreated (DMEM alone)
- IL-1β (10 ng/ml)
- Carprofen (100 µg/ml)
- Carprofen + IL-1β

Trypsin protein digestion

High throughput shotgun MS/MS

Protein identification via MASCOT database search

DMMB assays to assess GAG release

Quantitative western blots of selected proteins
Carprofen inhibits the release of matrix metalloproteinases 1, 3, and 13 in the secretome of an explant model of articular cartilage stimulated with interleukin 1β

Williams et al.

Analysis of mass spectrometry data from the secretome of an explant model of articular cartilage exposed to pro-inflammatory and anti-inflammatory stimuli using machine learning

Anna L Swan¹, Kirsty L Hillier², Julia R Smith³, David Allaway⁴, Susan Liddell¹,⁵,⁷, Jaume Bacardit⁶,⁷,⁸† and Ali Mobasher²,⁷,⁹,¹⁰,¹¹,¹²,¹³,¹⁴,¹⁵††
MMP-1

(A) Untreated

(B) MMP-1 release IL-1beta Vs Carprofen + IL-1beta

P value < 0.0001
MMP-3

(A) M  Untreated  IL-1β  Carprofen + IL-1β  Carprofen

(B) MMP3 Release IL-1β vs Carprofen + IL-1β

P value < 0.0001

Graph showing relative intensity to IL-1β for IL-1β and Carprofen + IL-1β treatments.
MMP-13

(A) Untreated 1 2 3 Carprofen + IL-1beta 1 2 3 Carprofen

(B) MMP-13 release IL-1beta Vs Carprofen + IL-1beta

P value < 0.0001
**Fibronectin Fragmentation – FN1**

A

B

Release of 60 kDa Fibronectin

- **Relative intensity to IL-1β**
  - IL-1β
  - Carprofen + IL-1β

**Treatment**

***
Bioinformatics and Data Mining
Bruker mass spectrometry data

**Mascot** database search using **UniprotKB**

- Extract **emPAI** scores
- Extract **ProteinProphet scores**

**ProteinProphet [TPP]**

**Machine learning**

- Classification using range of machine learning methods **[WEKA]**
- Classification & top ranking proteins **[BioHEL]**
Methods that used lots of different machine learning methods to build a model:

- Stacking
- Vote

Methods that used the same machine learning method, lots of different times:

- Bagging
- Boosting
Ensemble methods identify proteins associated with OA

- Proteins known to be associated with OA and the extracellular matrix of cartilage:

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<tr>
<th>Metalloproteinase inhibitor 1 (TIMP1)</th>
<th>Decorin (DCN)</th>
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<tr>
<td>Cartilage intermediate layer protein 2 (CILP2)</td>
<td>Chondroadherin (CHAD)</td>
</tr>
<tr>
<td>Fibronectin (FN1)</td>
<td>Aggreecan core protein (ACAN)</td>
</tr>
<tr>
<td>Lumican (LUM)</td>
<td>Alpha-1-antitrypsin (A1AT)</td>
</tr>
<tr>
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<td>Fibromodulin (FMOD)</td>
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RESEARCH

A machine learning heuristic to identify biologically relevant and minimal biomarker panels from omics data

Anna L. Swan¹, Dov J. Stekel¹, Charlie Hodgman¹,², David Allaway², Mohammed H. Alqahtani⁵, Ali Mobasheri²,³,⁵,⁶, Jaume Bacardit²,⁷
• Biomarkers are important for OA diagnosis/prognosis and drug development
• OMICs approaches can be used to identify new biomarkers and validate existing biomarkers
• However, we need to be clear about what biomarkers will be markers of:
  – radiographic progression
  – symptomatic disease
  – cartilage loss
  – progression to surgery
  – prognosis following injury
  – potential for intrinsic repair
  – suitability for extrinsic repair strategies
Each OA phenotype may need to be treated differently
A case for personalized medicine?

Safety and Cost of New and Existing Treatments - Relevance for Stakeholders – Patients and Healthcare Providers

Analgesics

Anti-resorptives?

Biologics?
Anti-TNF-α? Anti-IL-1β?
Non-pharmacological therapies? Exercise?

Karsdal et al, Osteoarthritis and cartilage, 2013

Slide courtesy of Dr. Anne-Christine Bay-Jensen, Nordic Bioscience Company
Need for a Better “Gold Standard” in Diagnosing Arthritis

Predictive biomarkers could provide an early warning of joint degeneration which could prompt earlier, more targeted and personalized treatment and facilitate drug discovery.

The ideal biomarker for lameness is likely to be a “combination biomarker” consisting of:

1. Changes in behaviour and activity (detected using an activity monitor)
2. Body score (adiposity?)
3. Inflammatory status (innate immunity?)
4. Changes in gait and posture
5. Temperature and blood flow in the hoof (thermography?)
6. Biochemical markers measured non-invasively in milk?

Challenges: is milk a suitable body fluid for monitoring changes in the hoof and the joints?
Acknowledgements: Funding

D-BOARD
European partnership for biomarker discovery

SEVENTH FRAMEWORK PROGRAMME

MARIE CURIE ACTIONS

BBSRC
bioscience for the future

EPSRC
Engineering and Physical Sciences Research Council

wellcome trust

Bridging the Gaps
infinite possibilities
Thank You