

# Les biomarqueurs de l'arthrose

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[www.bcru.be](http://www.bcru.be)



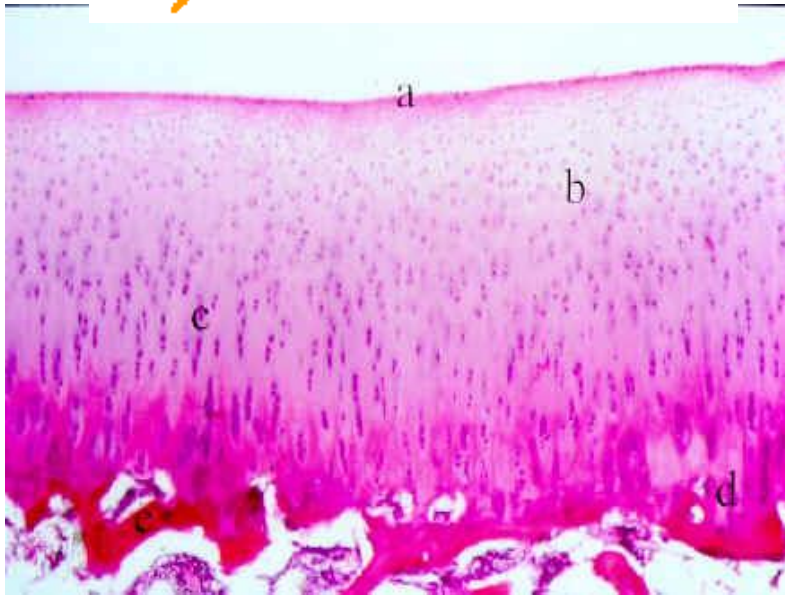
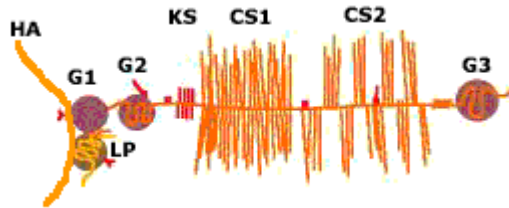
ARTHROPÔLE LIÈGE



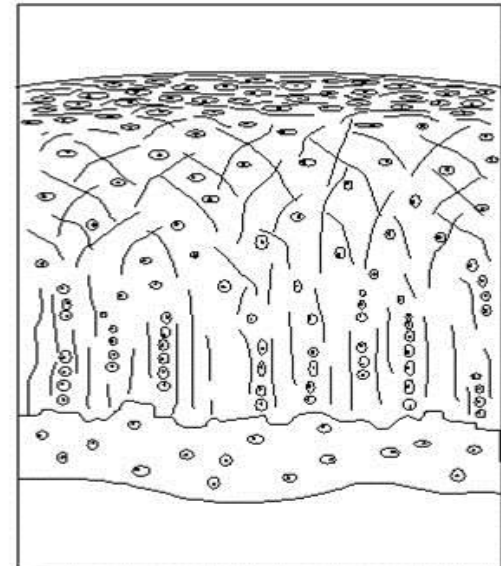
[www.artialis.com](http://www.artialis.com)



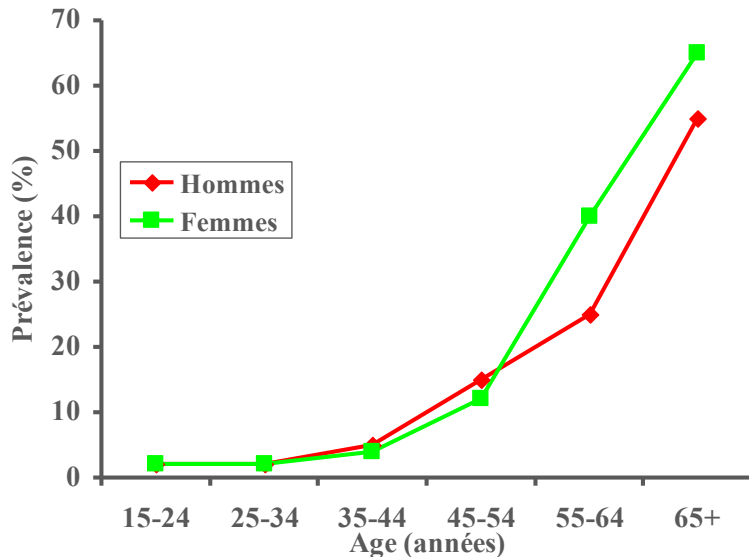
# Normal cartilage: Structure & composition



Superficial Zone  
Intermediate Zone  
Deep Zone  
Tidemark  
Calcified Zone  
Subchondral Bone



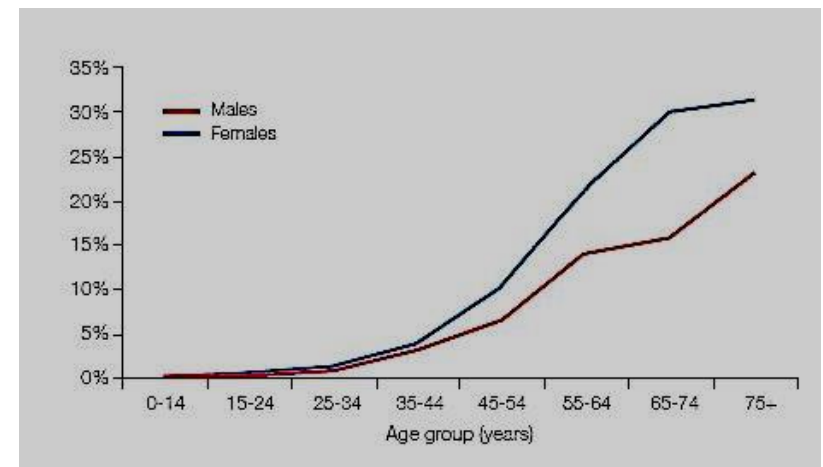
# ACTE I : Usure liée au vieillissement



A 65 ans, 70% des sujets ont des signes radiologiques d'arthrose



A 65 ans, 30% des sujets ont une arthrose invalidante



# ACTE II : Pathologie mécanique



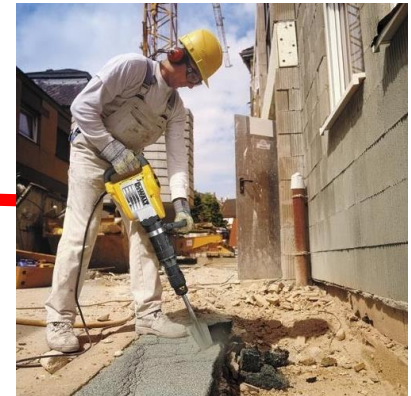
Activités sportives



Surcharge pondérale



Troubles axiaux



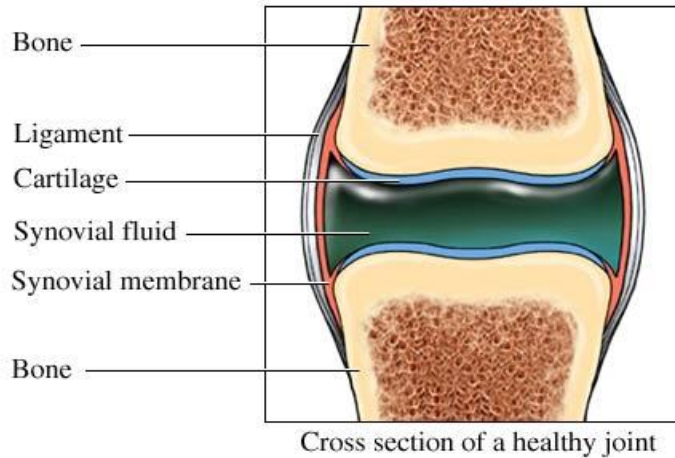
Microtraumatismes



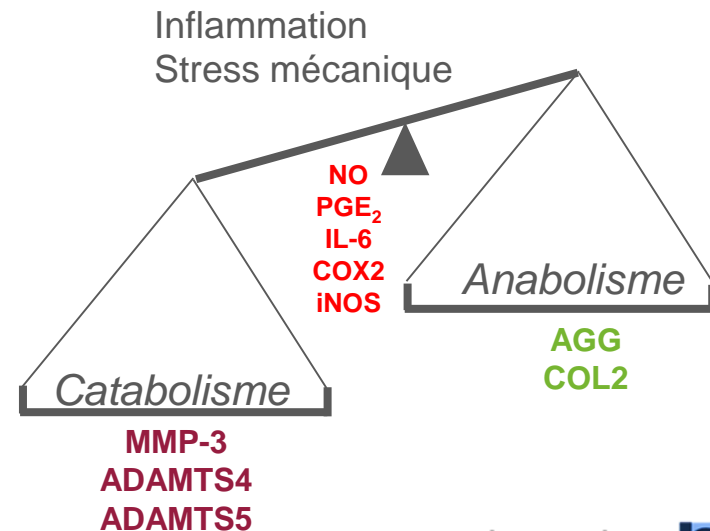
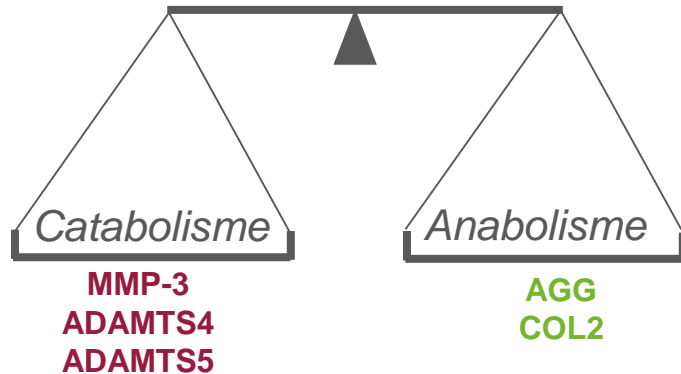
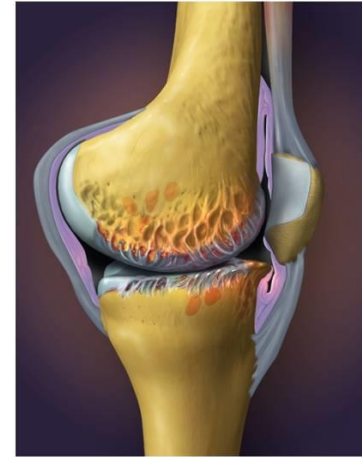
Traumatismes

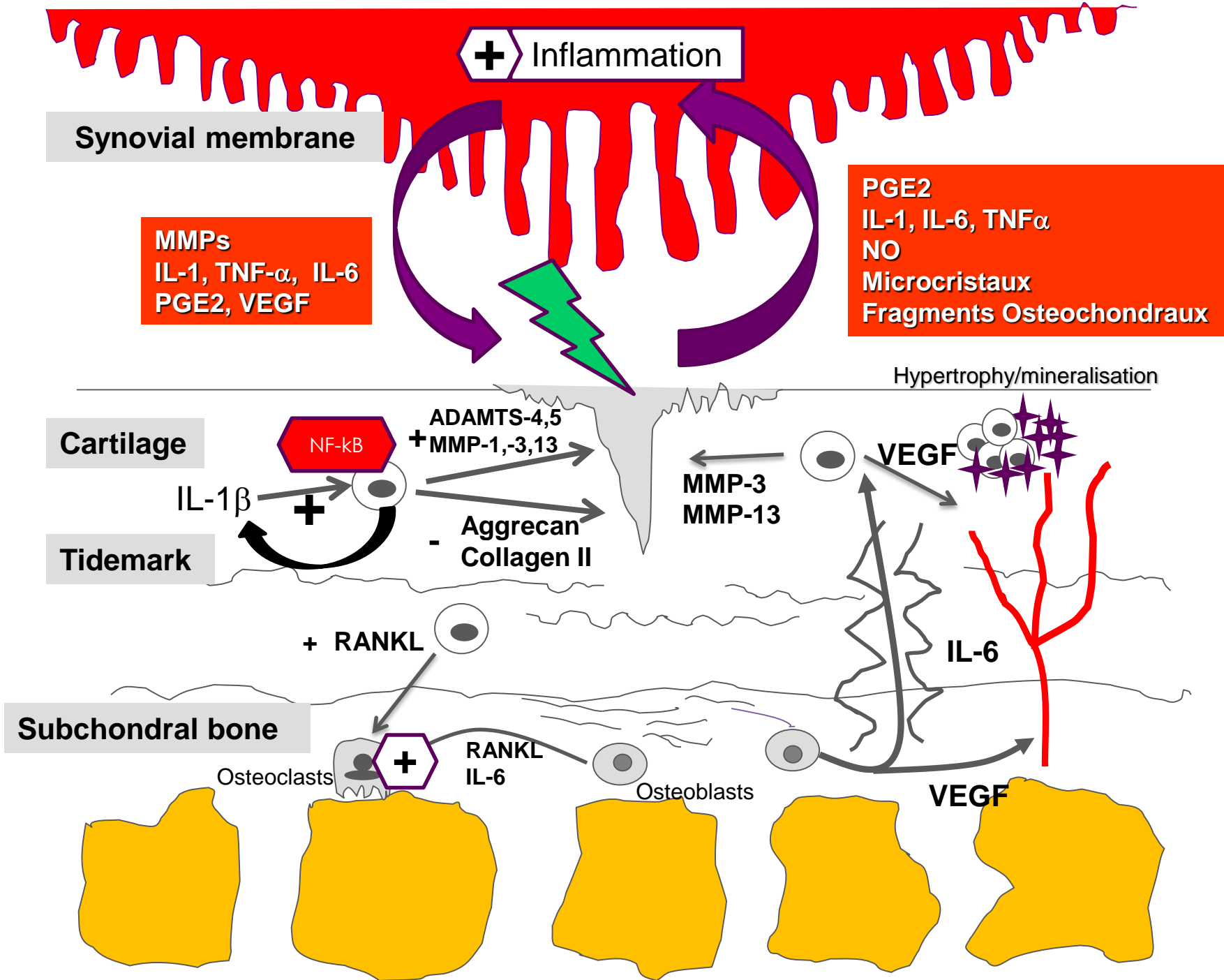
# Acte III: un déséquilibre métabolique du chondrocyte

Saine

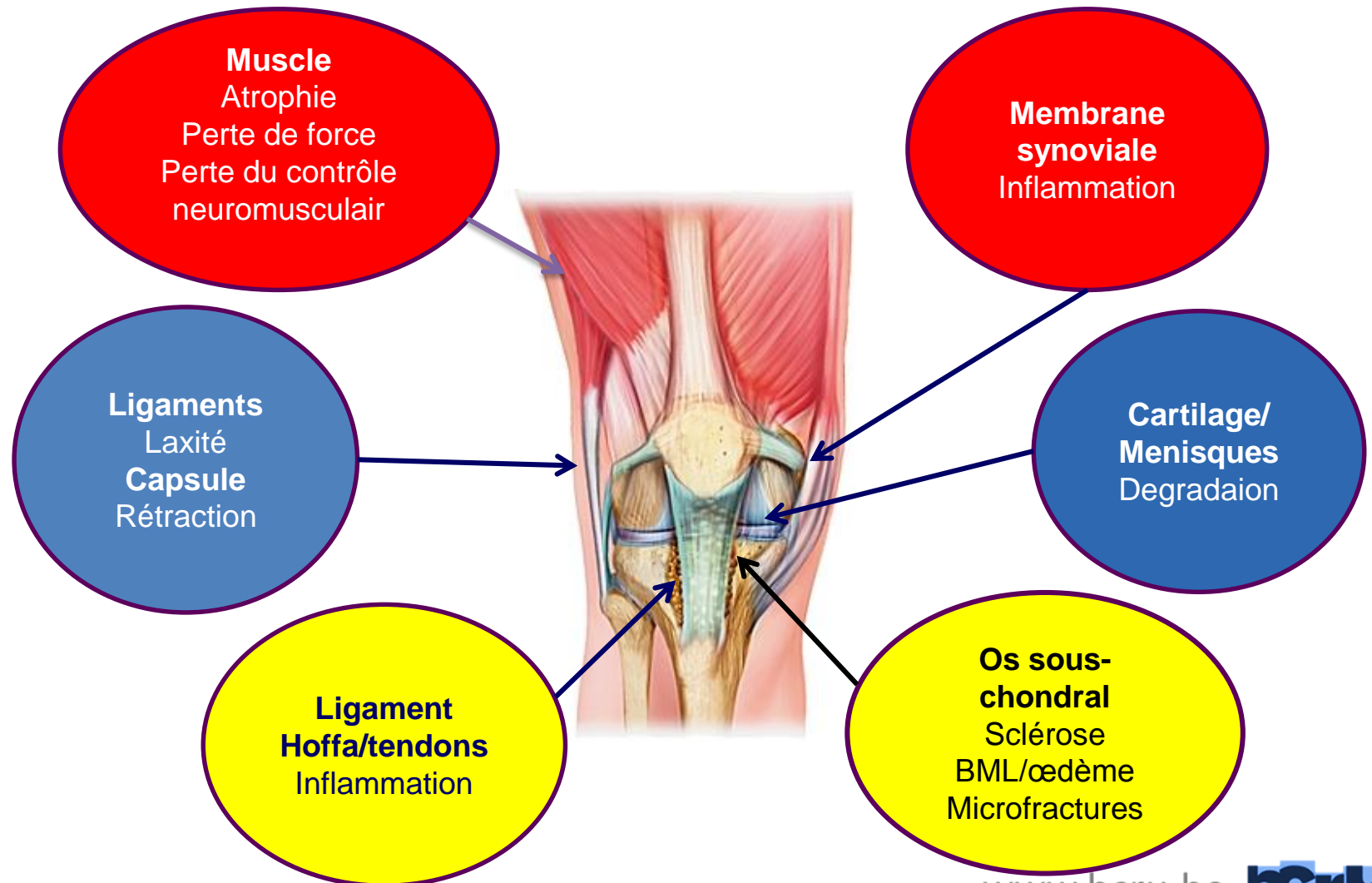


Arthrose

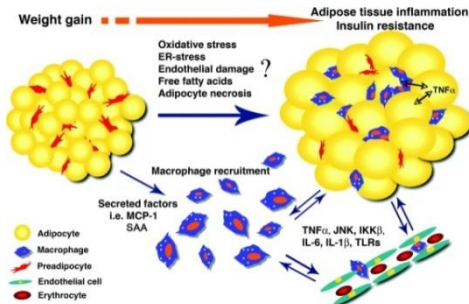




# Acte IV: une maladie globale de l'articulation



# ACT V: Une maladie métabolique et systémique



Adipokines  
Cytokines



Chronic  
Mechanical stress



Matrix peptides  
Cytokines  
Prostanoids  
Oxidized lipids

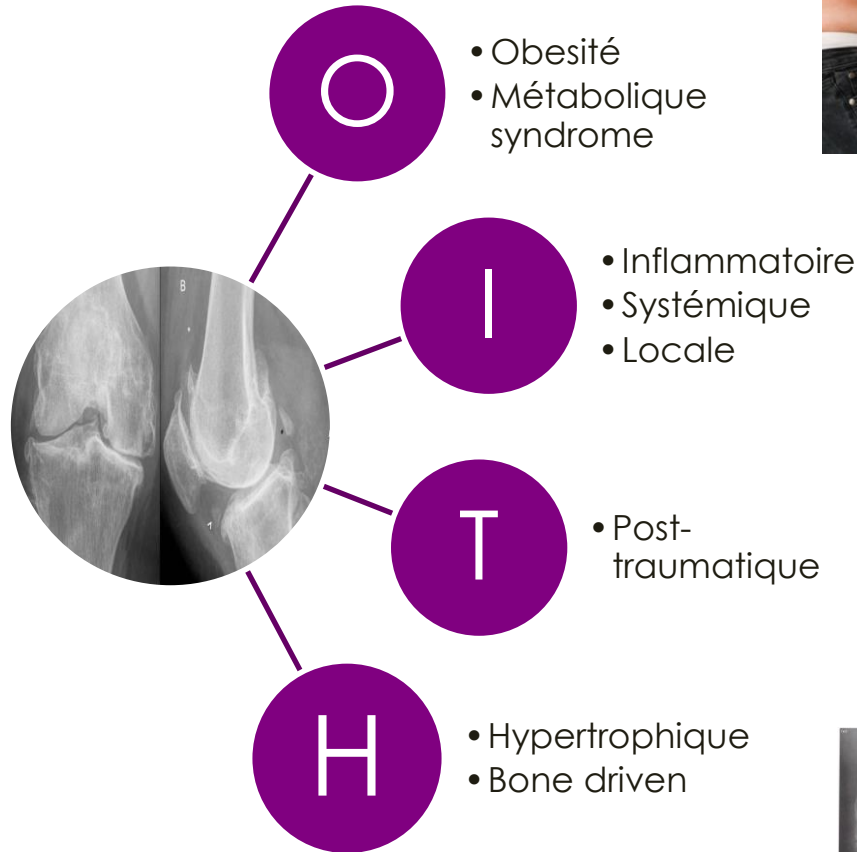


Chronic low-grade  
inflammation

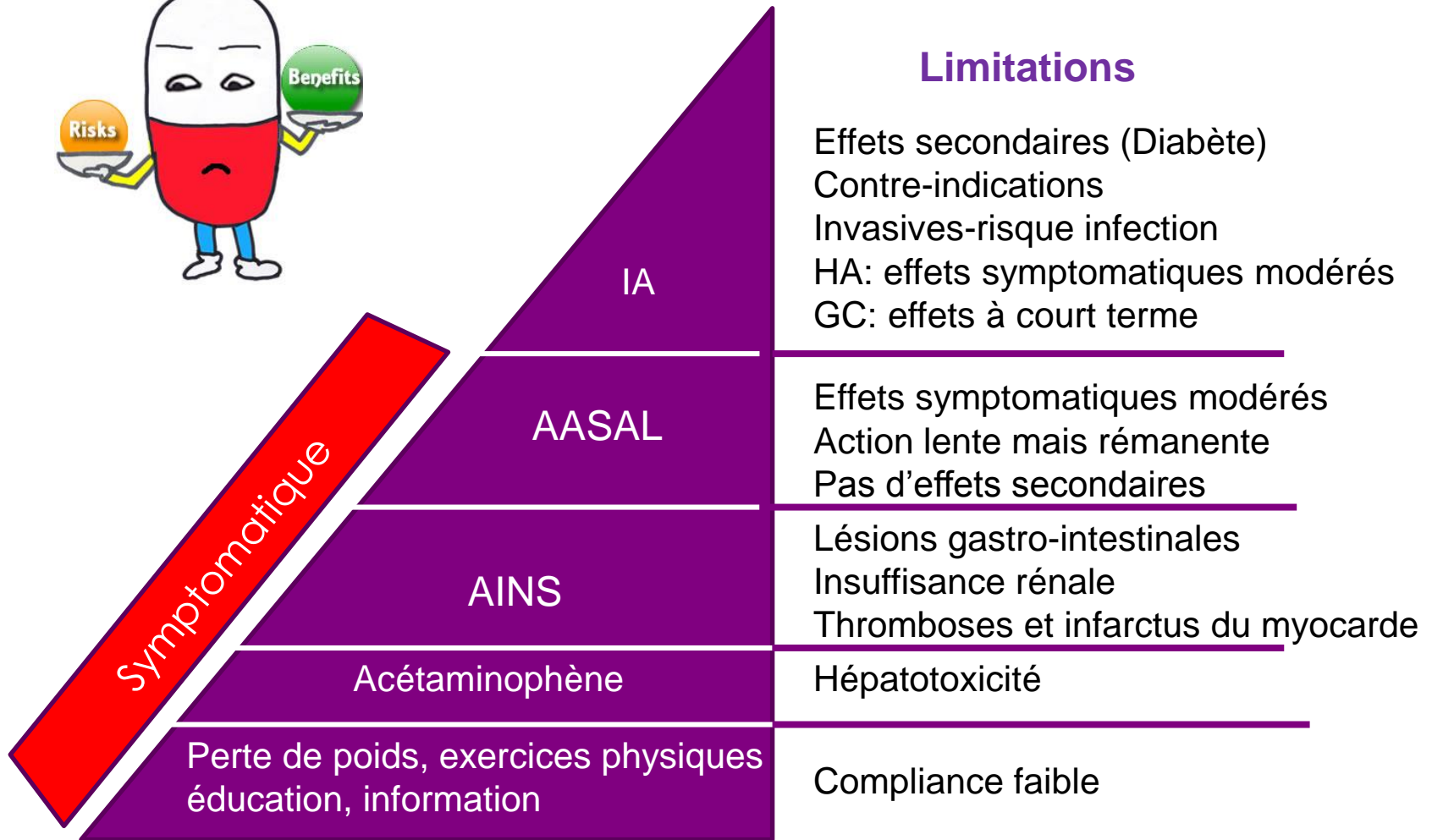
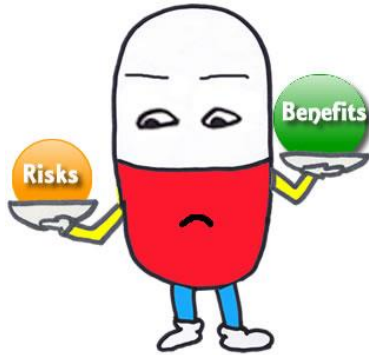
Metabolic syndrome  
-Hypertension  
-Type 2 diabetes  
-Dyslipidemia



# ACTE VI: Une maladie, plusieurs phénotypes



# Traitements non-chirurgicaux



Modified Clegg et al. Eur J Orthop Surg Traumatol, 2013

# Comment diagnostiquer l'arthrose?

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La radiographie



L'examen clinique



# Comment diagnostiquer l'arthrose?

## L'examen clinique

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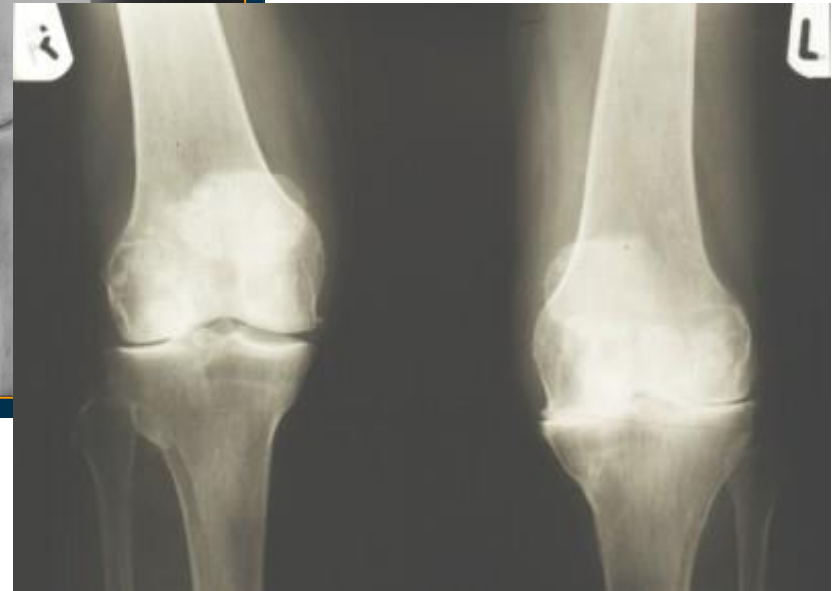
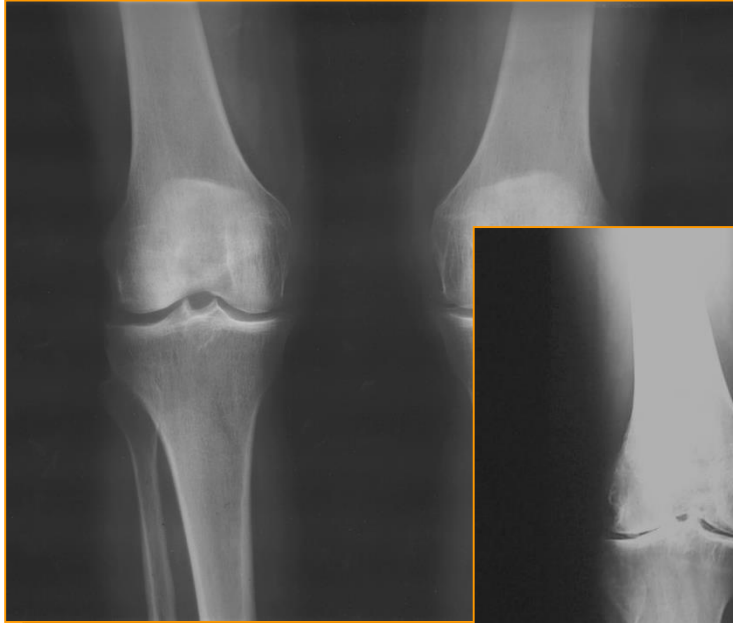


**Douleur au mouvement**  
**Raideur**  
**Gonflement**  
**Craquements**  
**Déformation**  
**Déviations**

# Comment diagnostiquer l'arthrose?

## La radiologie

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Ostéophytes  
Pincement  
Sclérose  
Géodes

# Comment diagnostiquer l'arthrose?

## Les marqueurs biologiques



Trop tard



Génétique



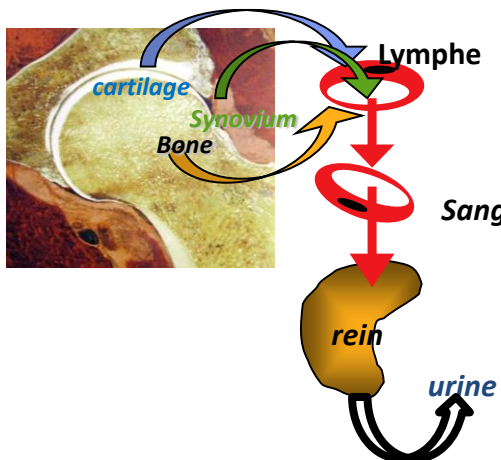
Sanguins



RMN



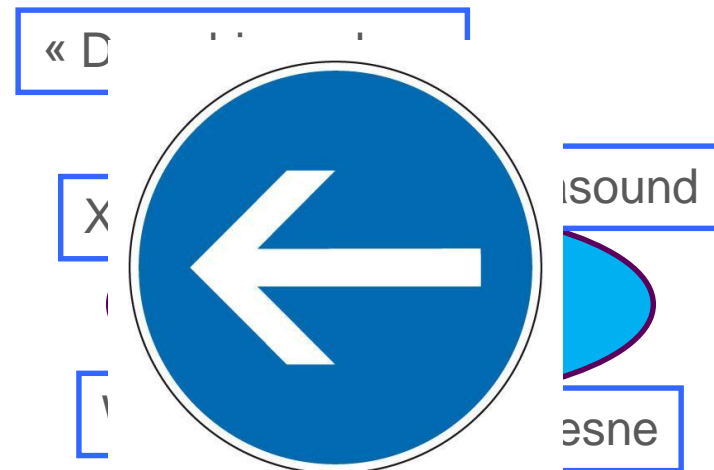
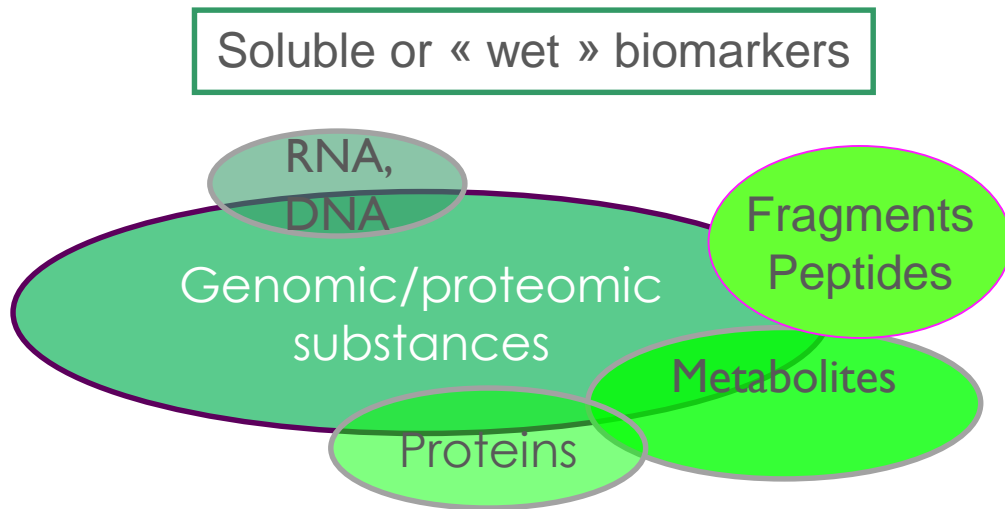
Radiographie



# Définition

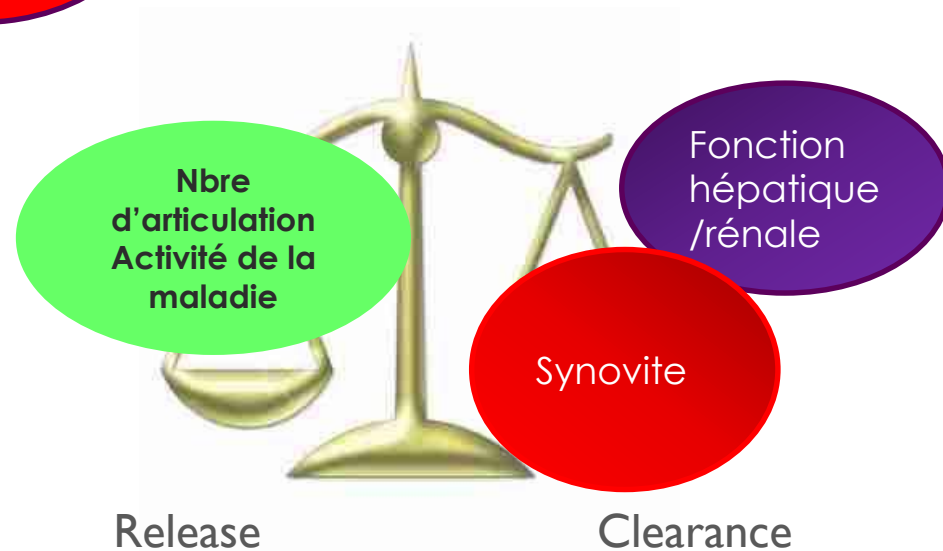
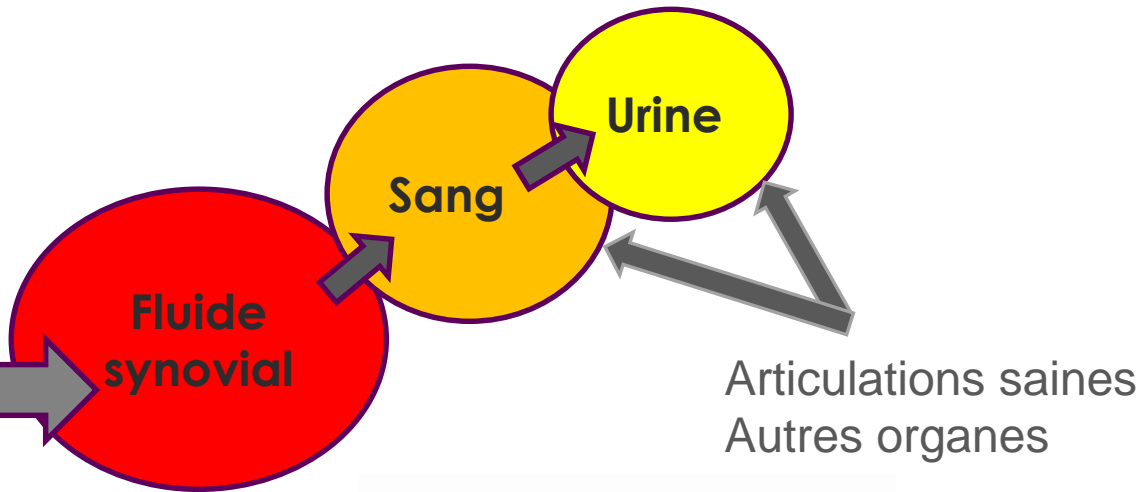
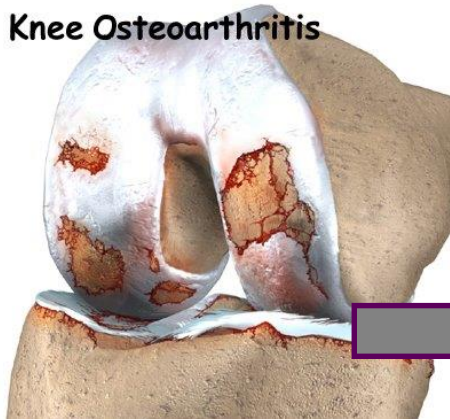
« Un biomarqueur est une caractéristique qui est mesurée et évaluée de manière objective comme un indicateur des processus physiologiques, pathologiques ou de la réponse à un traitement »

Biomarkers Definitions Working Group I. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001; 69: 89-95.



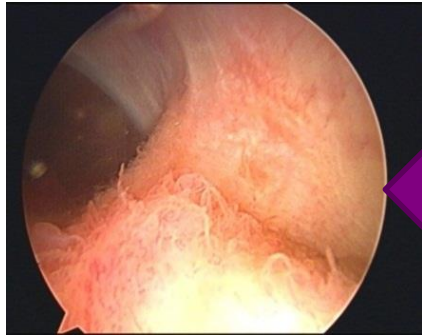
# Sources et Métabolisme

Knee Osteoarthritis

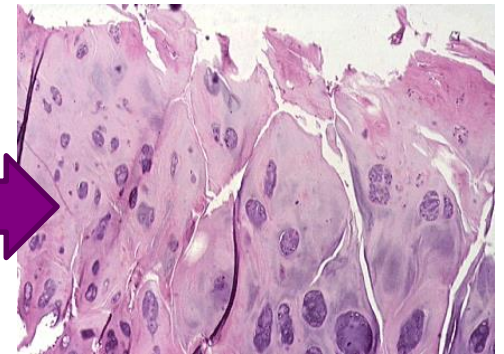
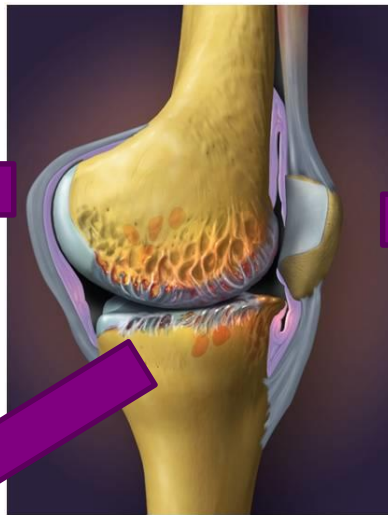




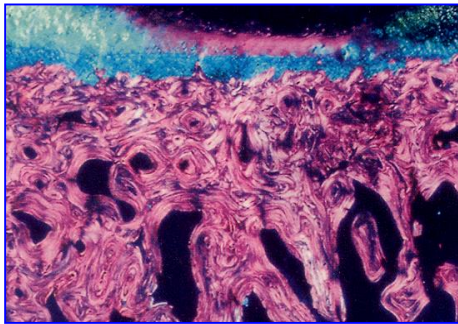
# Arthrose: une maladie de l'articulation



Membrane synoviale  
inflammation



Dégradation du cartilage  
Fibrillation/fissuration  
Minéralisation/vascularisation



Os sous-chondral  
Sclérose/BML

...Identifier les changements  
métaboliques des différents tissus

# Diagnostic: symptômes et signes radiologiques!

## Radiographie



Ostéophytes  
Pincement  
Sclérose sous chondrale  
Déformation  
Géodes

Douleur  
Raideur  
Gonflement  
Craquements  
Déformation  
Troubles axiaux

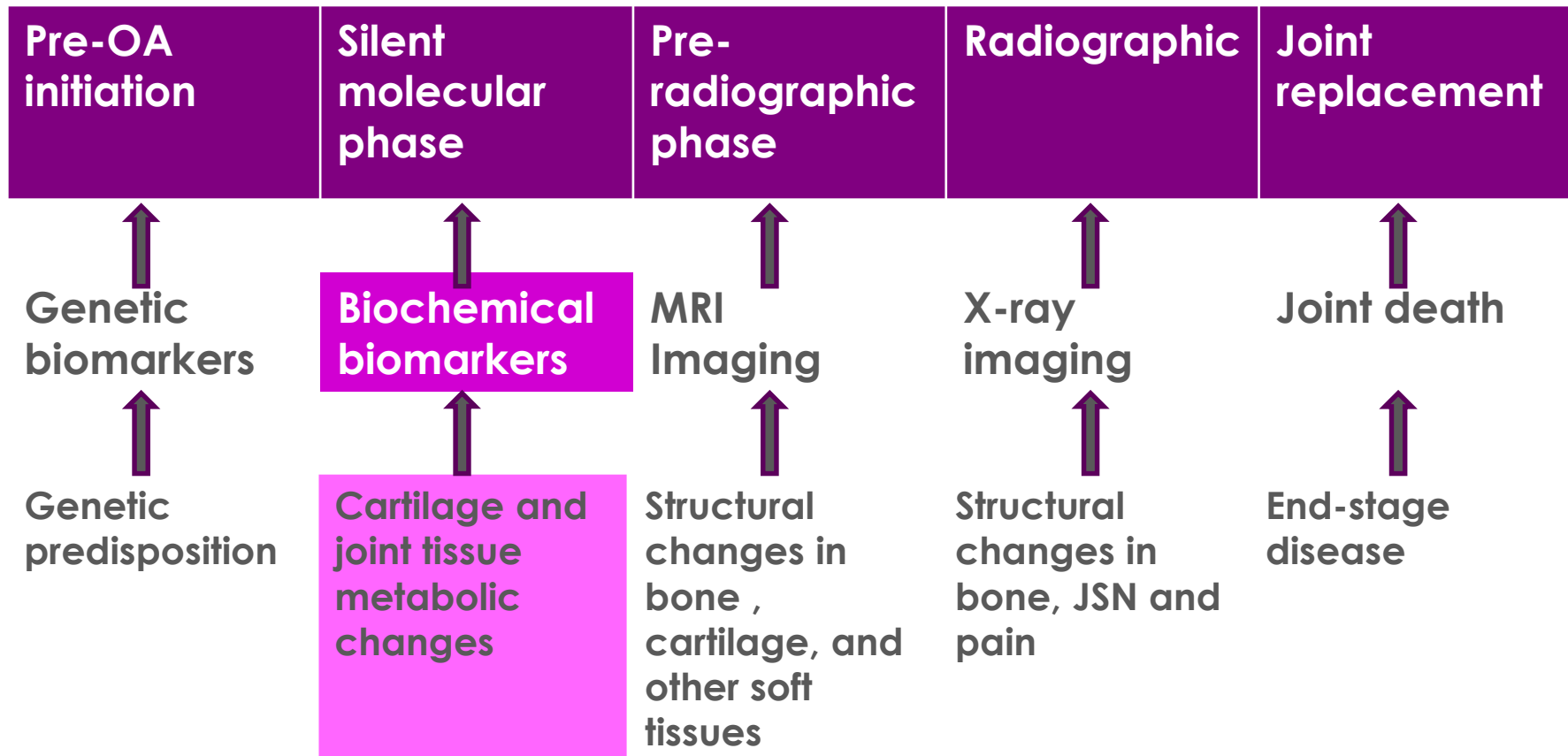
## Symptômes



Les symptômes et les signes radiologiques apparaissent trop tardivement!

# Une phase silencieuse précède l'apparition des signes radiologiques

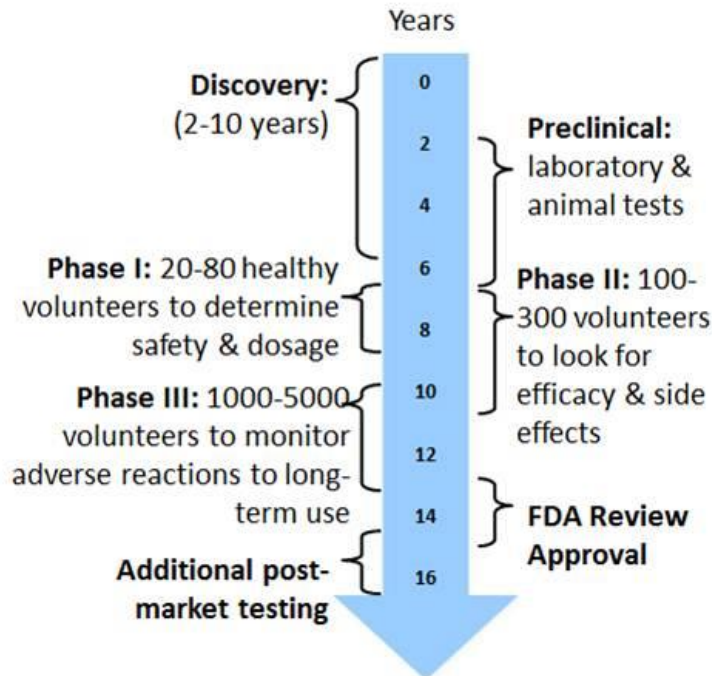
(D Patra & L Sandell, J Knee Surg, 2011)



...Diagnostiquer la maladie avant l'apparition des signes et symptômes

# Le développement des médicaments est long...trop long

**R&D is risky & costly**



**Nothing new to offer at the patients and the OA research community**



# Critères principaux d'efficacité

- **Action symptomatique** (3 à 6 mois)

Douleur (EVA)

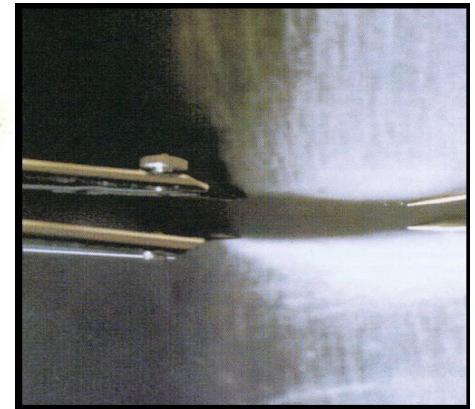
Statut-algofonctionnel (index Lequesne)

Appréciation globale du patient

- **Action structurelle** (1 à 3 ans)

Radiographie standard

Pincement minimal



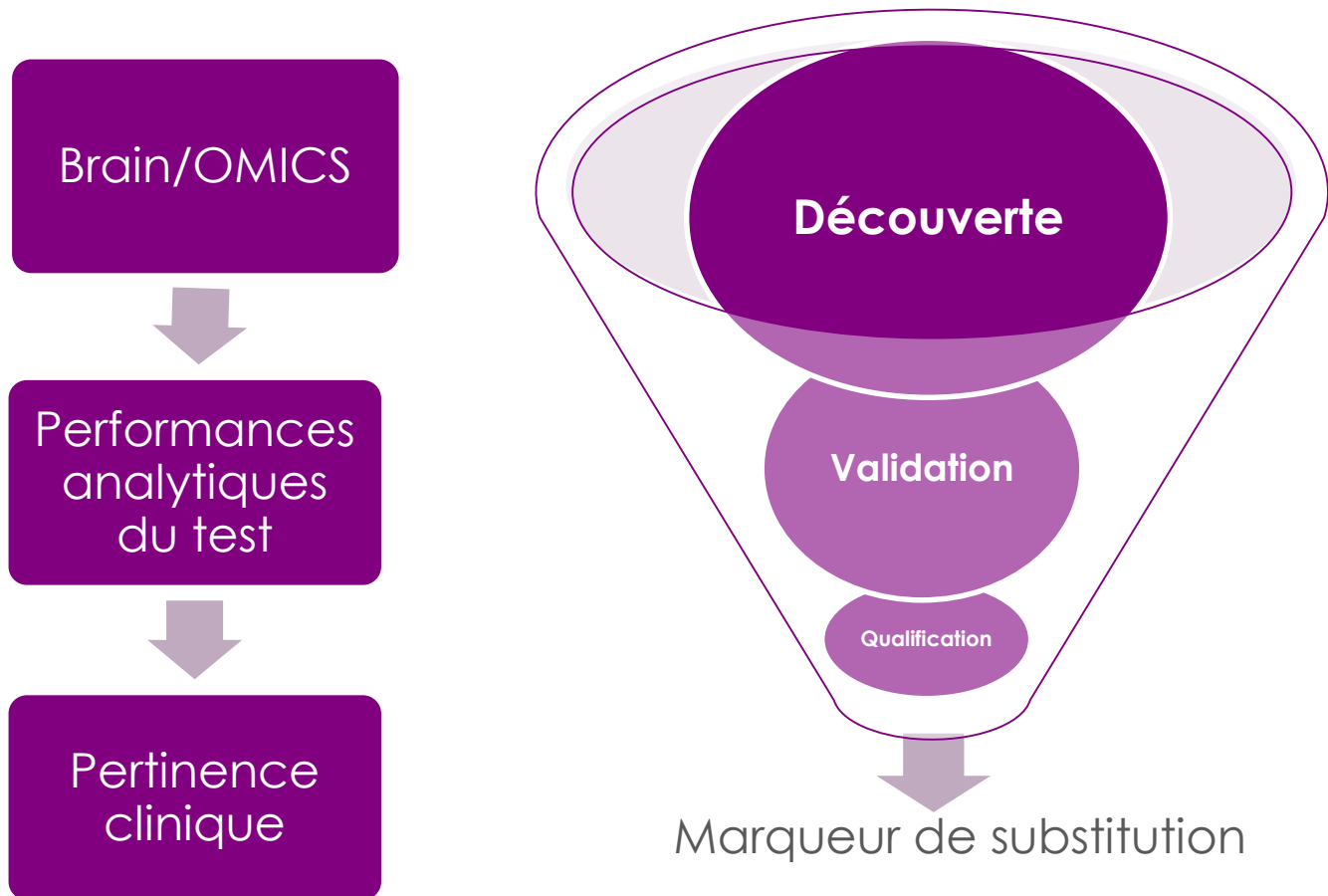
# Limites de la mesure du pincement articulaire

- Mesure indirecte de cartilage.
- Ne mesure pas un processus dynamique.
- Influencé par les lésions méniscales.
- Peu sensible.
- Peu reproductible.
- Peu ou pas corrélé avec la douleur ou la fonction.

# En résumé...

- Améliorer notre compréhension de la maladie
- Diagnostiquer précocement de la maladie, avant l'apparition des changements structuraux
- Prédire l'apparition et la progression de la maladie
- Prédire la réponse à un traitement (Théranostic)
- Réduire la durée et les coûts des essais cliniques

# Développement





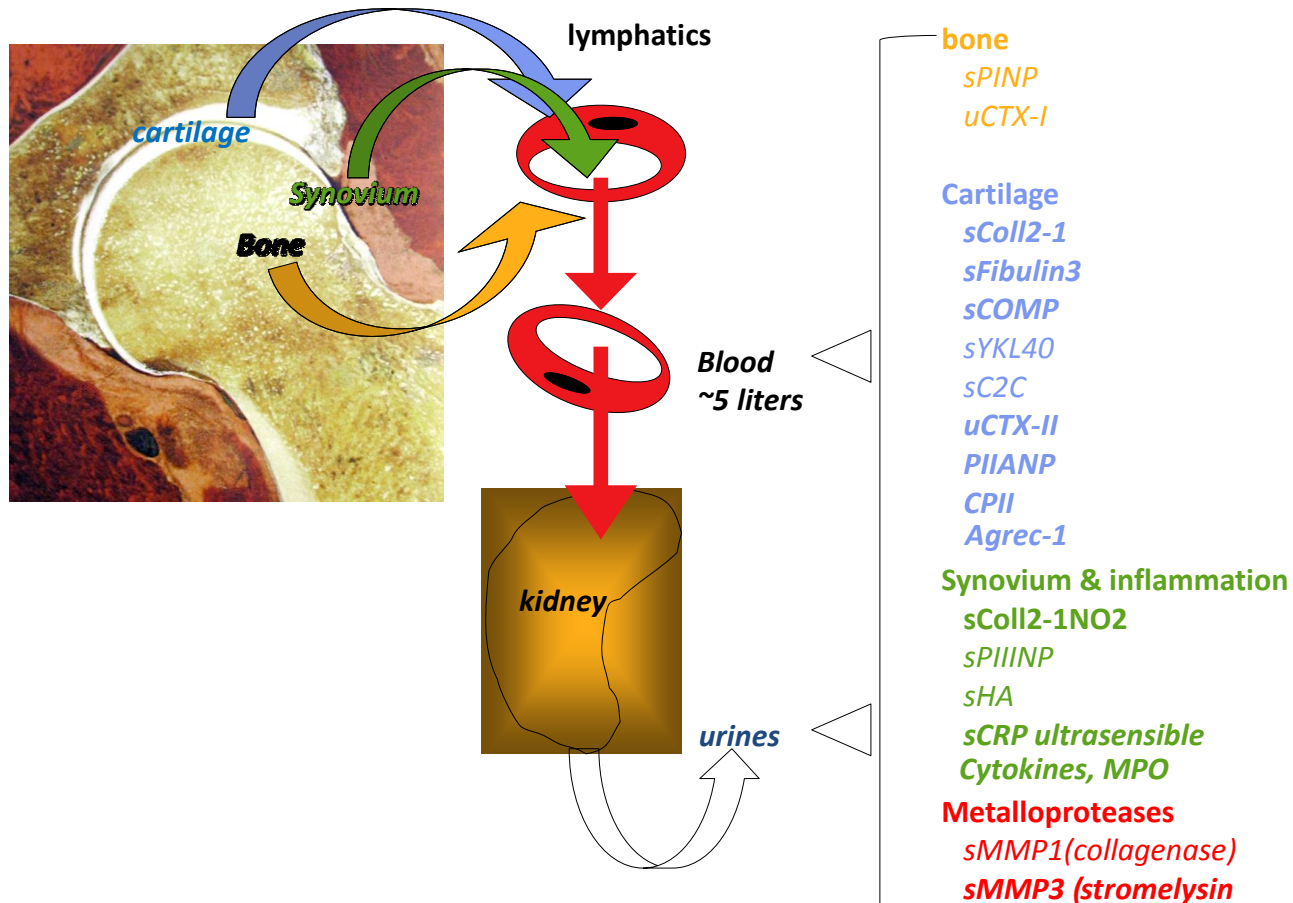
# THE PRESENT

**28 identified biochemical makers<sup>1</sup>**



<sup>1</sup> WE van Spil et al. Osteoarthritis cart, 2010; 18: 605-12

# OA Biomarkers



# Sont-ils spécifiques d'un tissu?

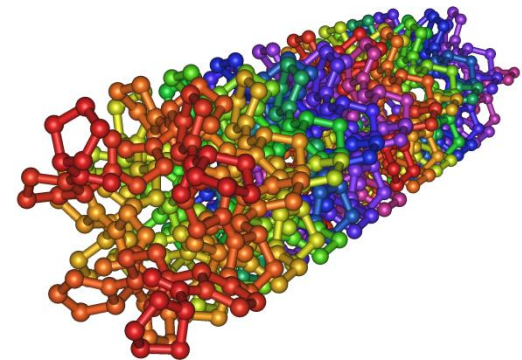
Cartilage	Coll2-I, Coll2-I NO2, CPI, TIINE, PIIANP, C2C, CI,2C, CTX-II, HA, KS, CS-846, COMP, YKL-40
Cartilage de croissance	CTX-II, CI,2C
Os	CTX-II, COMP, CI,2C, NTX-I, CTX-I, PYD, DPD, OC
Ménisque	COMP, HA, CI,2C
Tendon	COMP,
Membrane synoviale	COMP, HA, CI,2C, YKL-40, Glc-Gal-PYD

# Quel processus évaluent-ils?

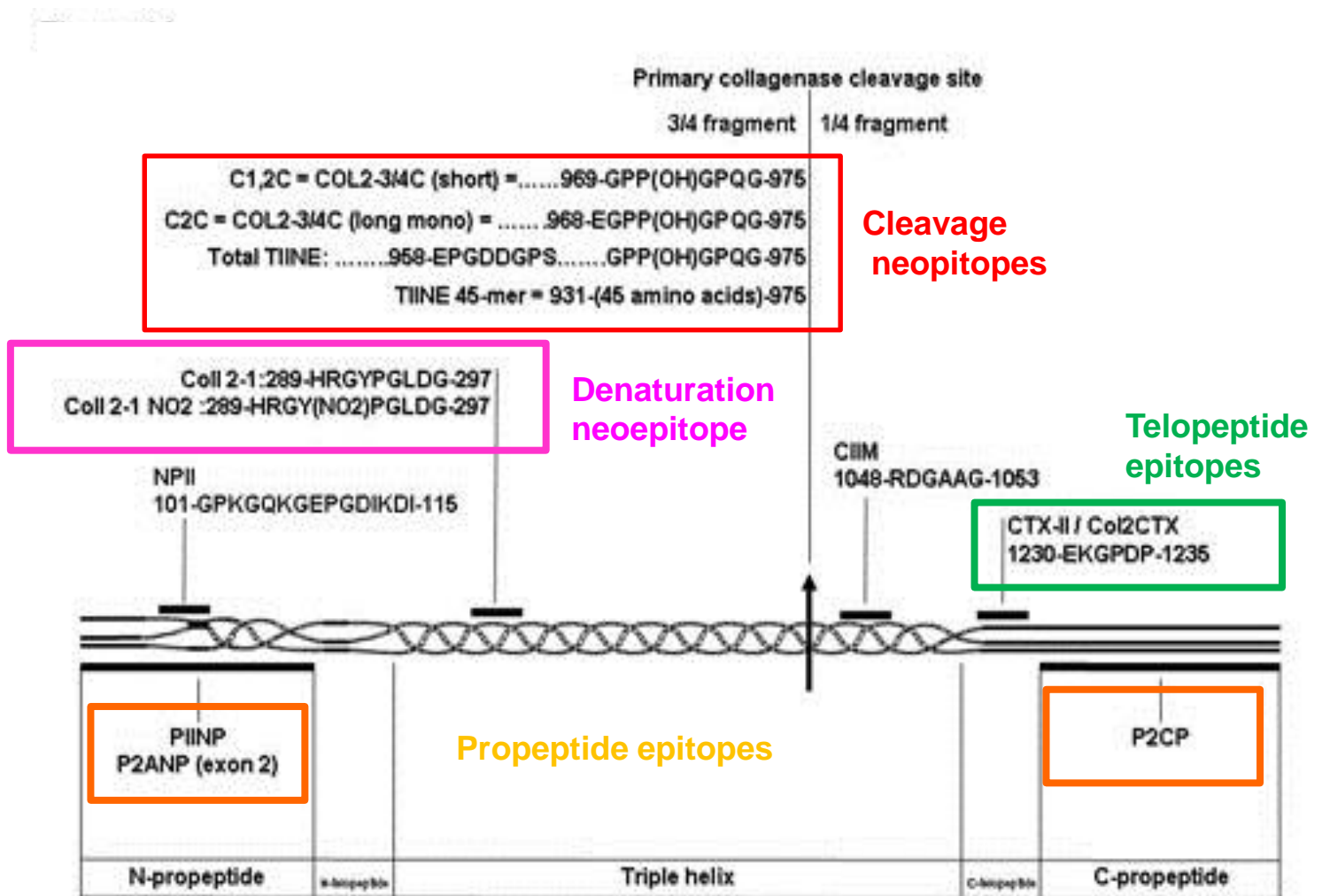
Cartilage/dégradation	Coll2-I, Coll2-I NO2, CTX-II, COMP, C2C, CI, 2C, TIINE, KS, CS-846, YKL-40
Cartilage/Synthèse	CPII, PIIANP
Ostéophytes	CTX-I, COMP, HA
Synovite	HA, Glc-Gal-PYD, PIINP
Remodelage osseux	CTX-II, NTX-I, CTX-I, PICP, PINP, PYD, DPD, OC, ICTP

# Pourquoi la recherche de biomarqueurs s'est-elle focalisée sur le collagène de type II?

- La protéine la plus abondante du cartilage
- Spécifique du cartilage articulaire
- Représente seulement 1% des collagènes
- La dégradation du collagène de type II est une étape clé de la pathogénie de l'arthrose

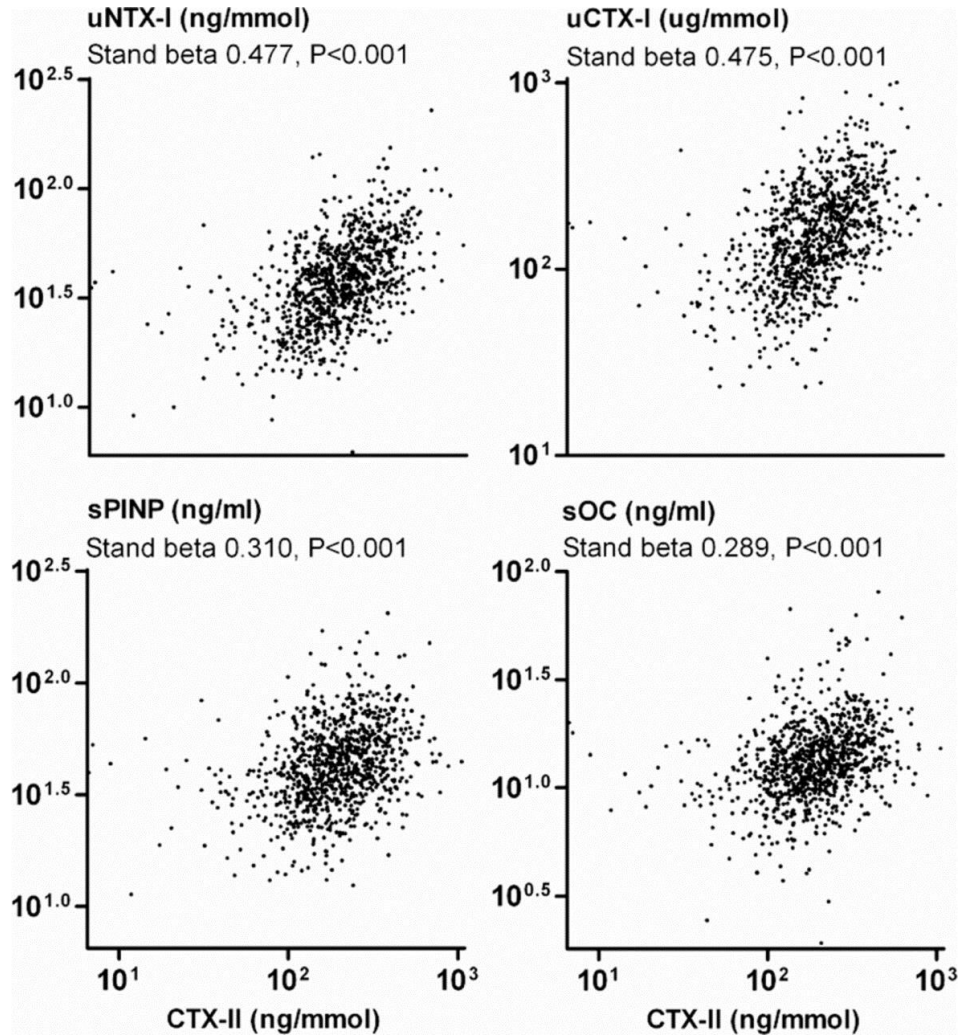


# Type II collagen biomarkers



# CTX-II biomarkers is not cartilage specific

u CTX-II reflects rather bone than cartilage metabolism



van Spil W E et al. Ann Rheum Dis 2013;72:29-36



# BIPEDS classification

*Bauer et al. Osteoarthritis Cart 2006*

## Burden of disease

- Biomarker associated with extent of severity of OA

## Investigative

- Biomarker not yet meeting criteria for another category

## Prognostic

- Predicts incidence of progression of disease or likelihood of response to a treatment

## Efficacy of treatment

- Indicative of treatment efficacy and for which the magnitude of the change is considered pertinent to the response.

## Diagnostic

- Dissociate diseased from non-diseased.

## Safety

- Identify adverse effects and provide means of safety.



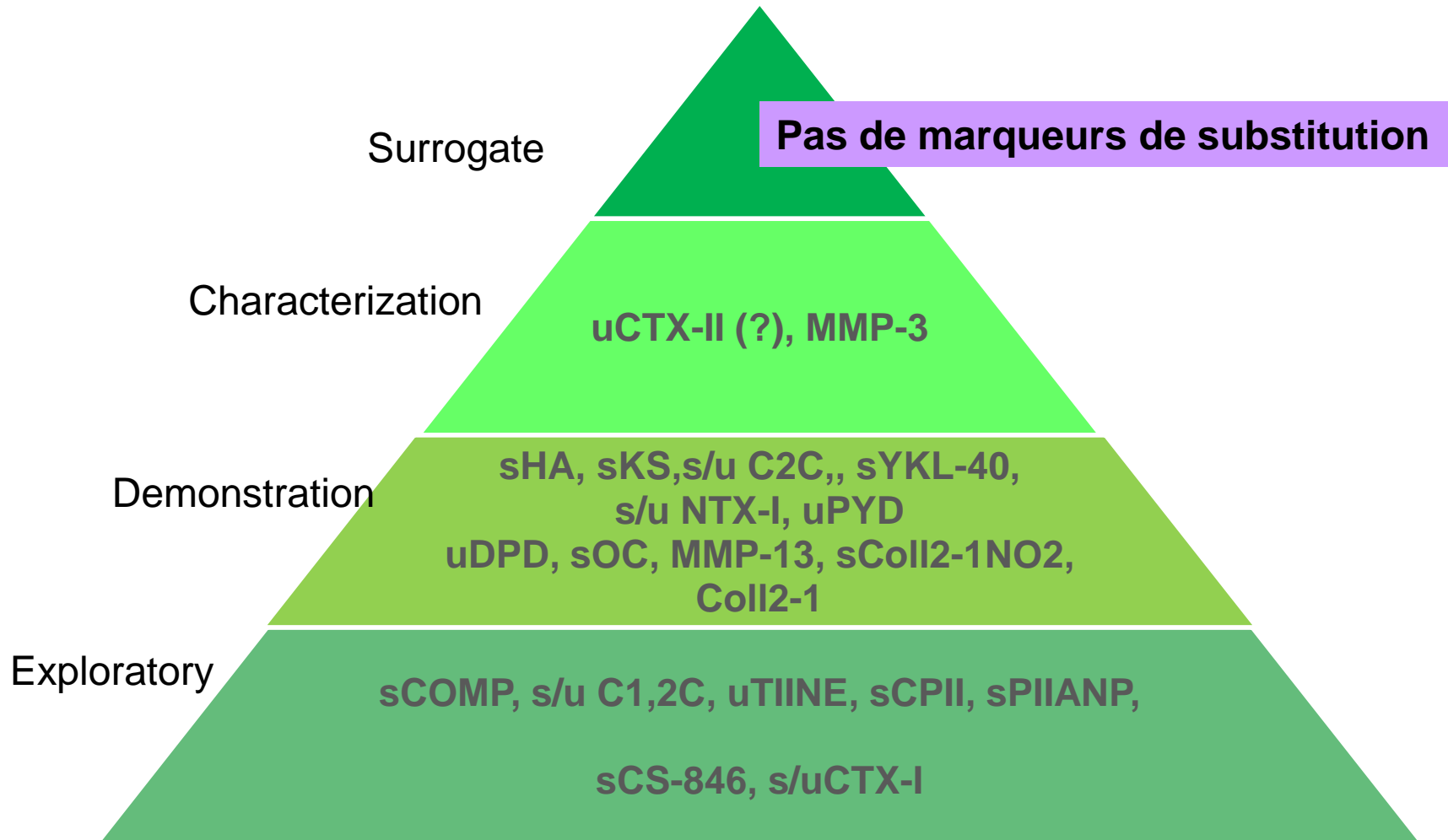


# Quatre niveaux de qualification

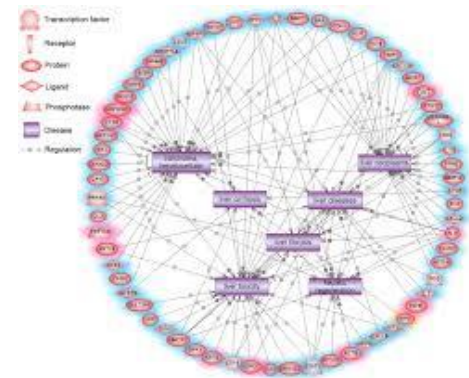
Exploratory biomarker	R&D tool In vitro/preclinical evidence No linked to clinical outcomes in humans
Demonstration biomarker	Adequate preclinical sensitivity/specificity Linked with clinical outcomes No reproducibility demonstrated
Characterization biomarker	Reproducibility linked to clinical outcome in more than one prospective human study Used in decision making and dose finding Secondary/tertiary claims
Surrogate Biomarker	Substitute for a clinical end point Requires agreement with regulatory authorities Used for drug registration



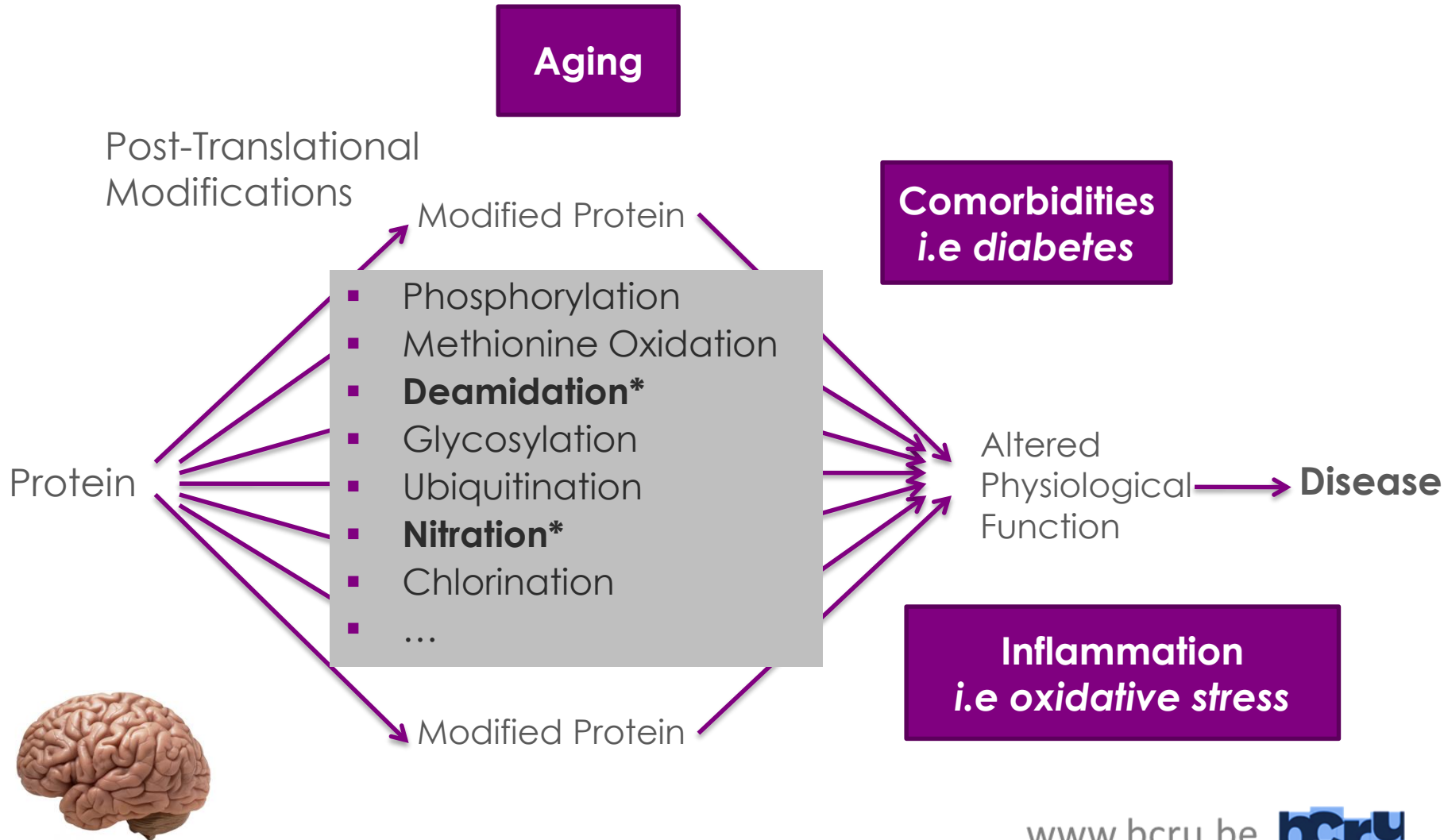
# Niveau de qualification des biomarqueurs



# COMMENT DÉVELOPPER UN NOUVEAU BIOMARQUEUR?



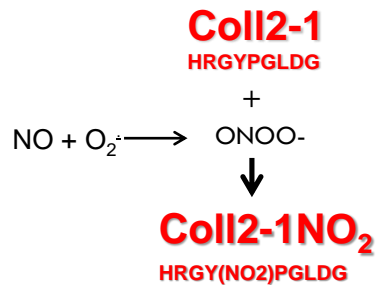
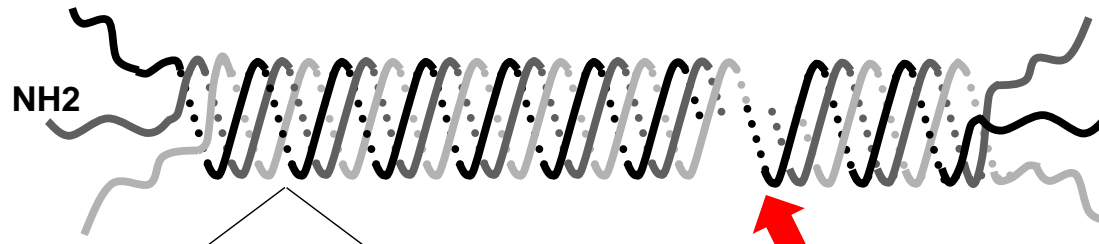
# Post-translational modifications





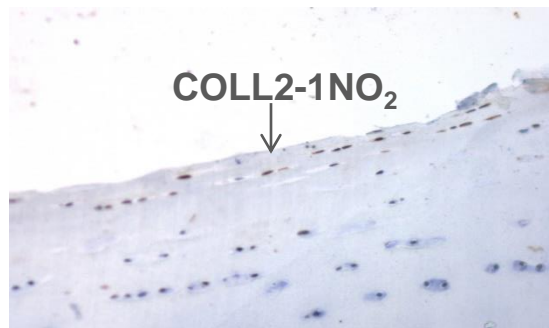
# Coll2-1NO<sub>2</sub>: a joint inflammation related biomarkers

Deberg et al. O&C 2008



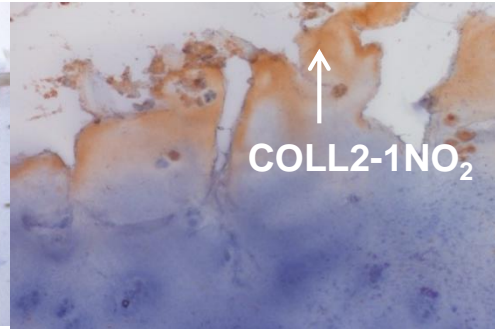
Cleavage site of MMP-1, MMP-8 and MMP-13 of *type II Collagen* molecule

## HEALTHY CARTILAGE

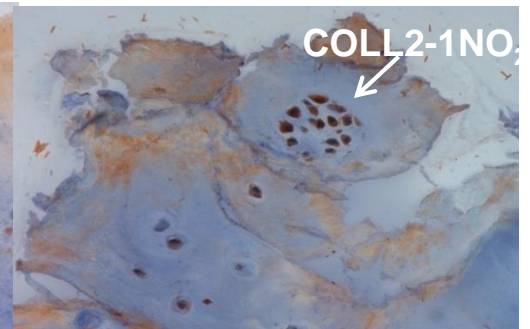


## DAMAGED CARTILAGE

### FIBRILLATION ZONE

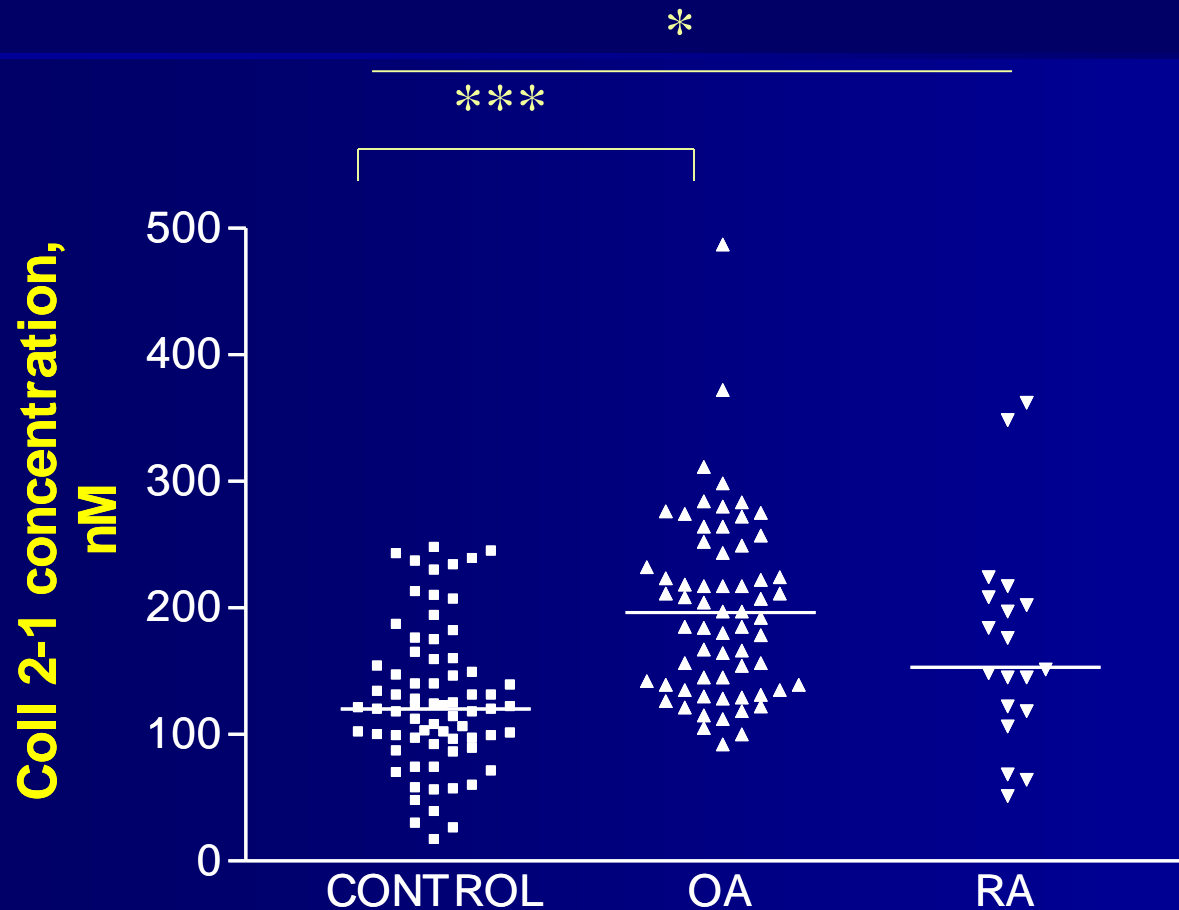


### CELL CLUSTER ZONE



# COLL 2-1 IN OA AND RA

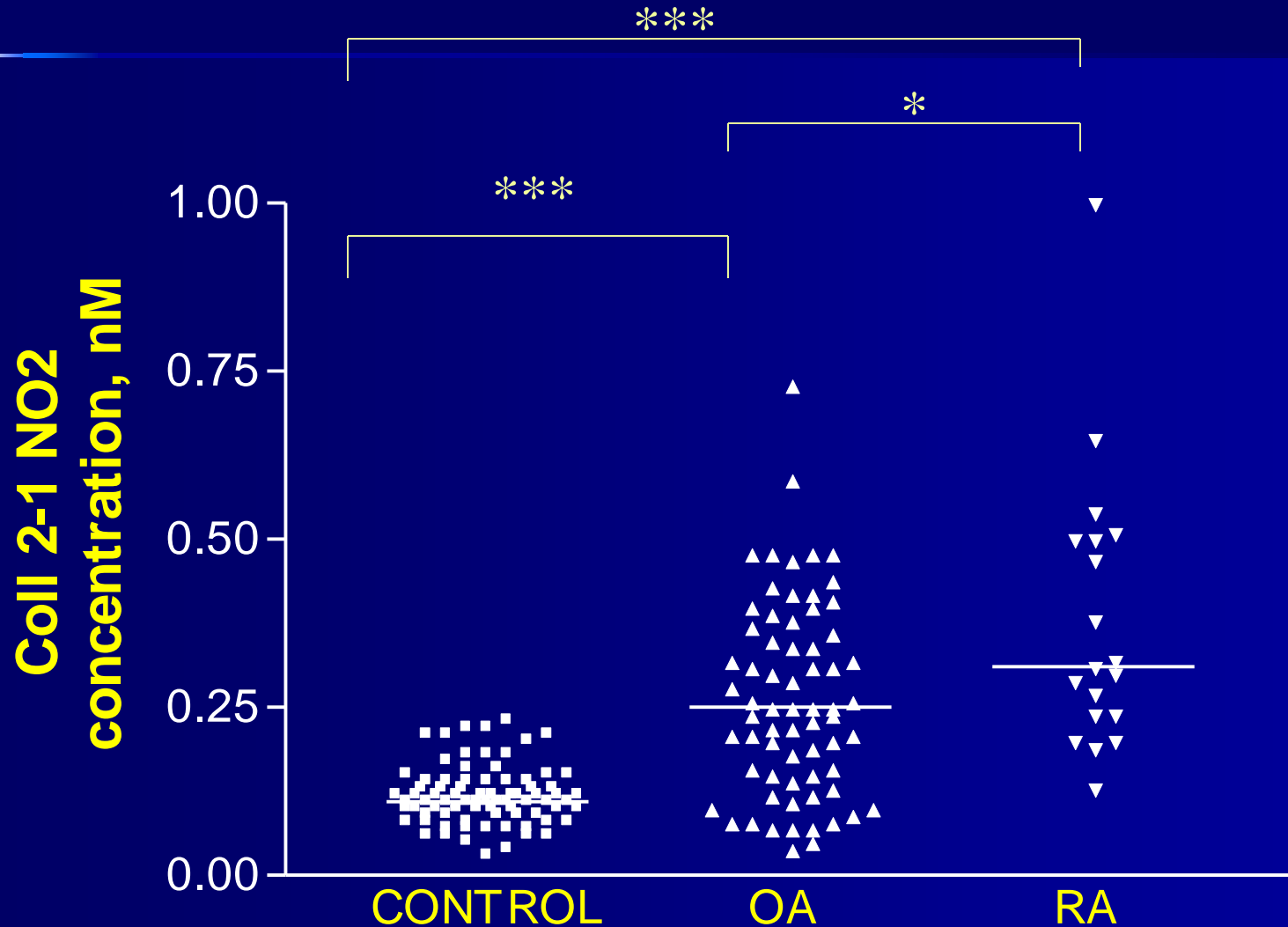
## Serum levels



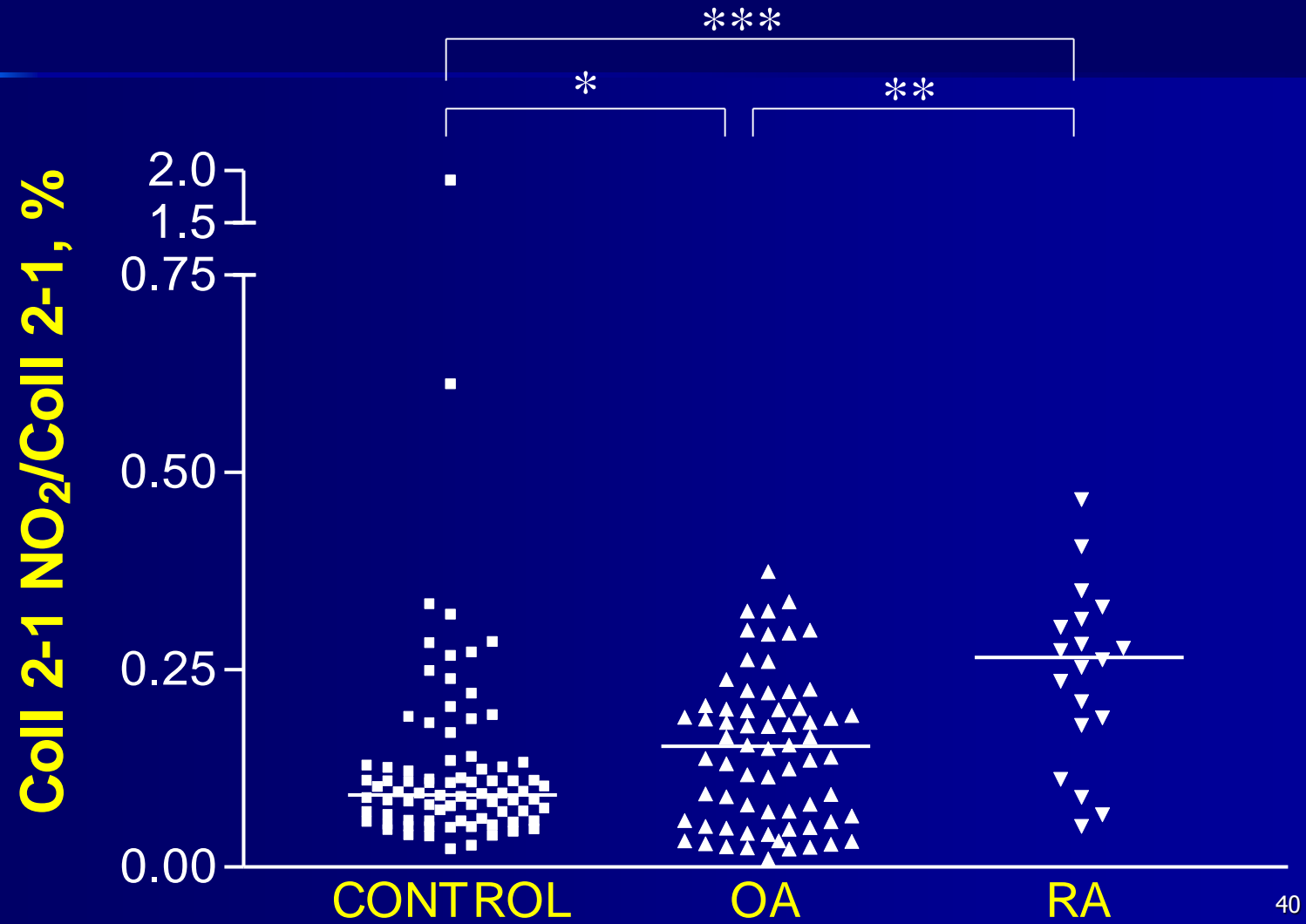
Deberg M et al. New serum biochemical markers (Coll2-1 and Coll2-1NO2) for studying oxidative-related type II collagen network degradation in patients with OA and RA. *Osteoarthritis Cart* 2005; 13: 258-65.

# COLL 2-1NO<sub>2</sub> IN OA AND RA

## Serum levels



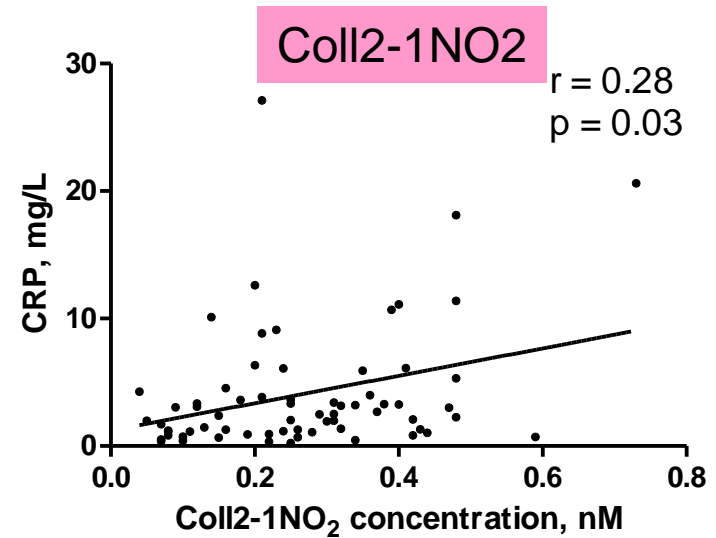
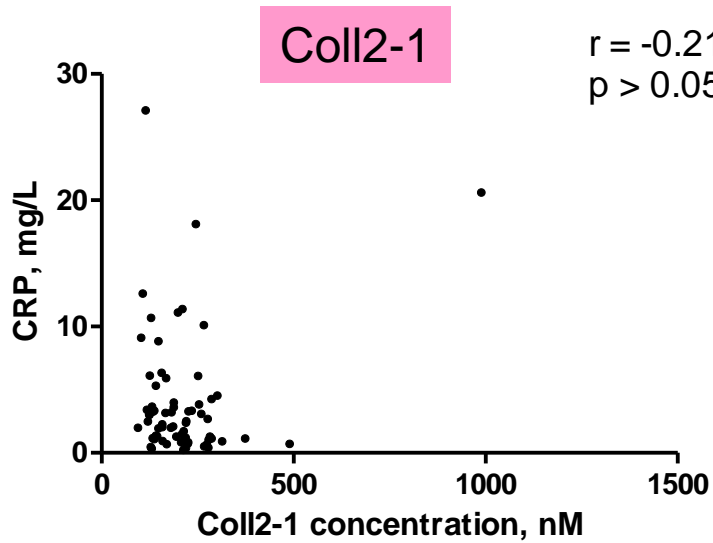
# COLL 2-1 NO<sub>2</sub> / COLL 2-1 RATIO Serum levels





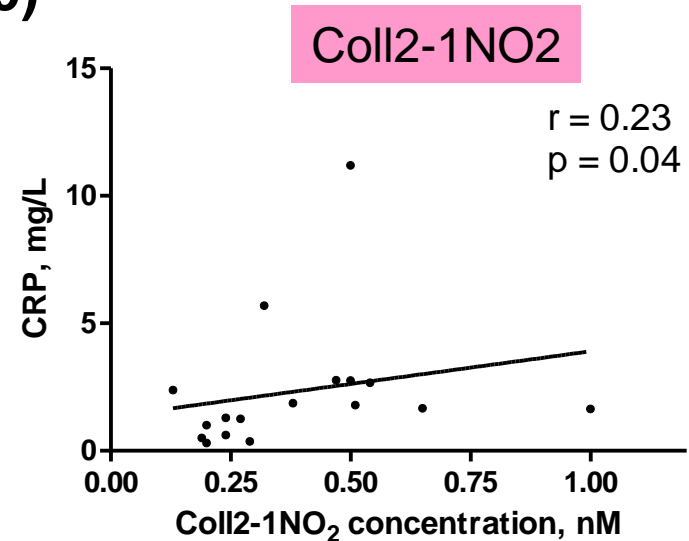
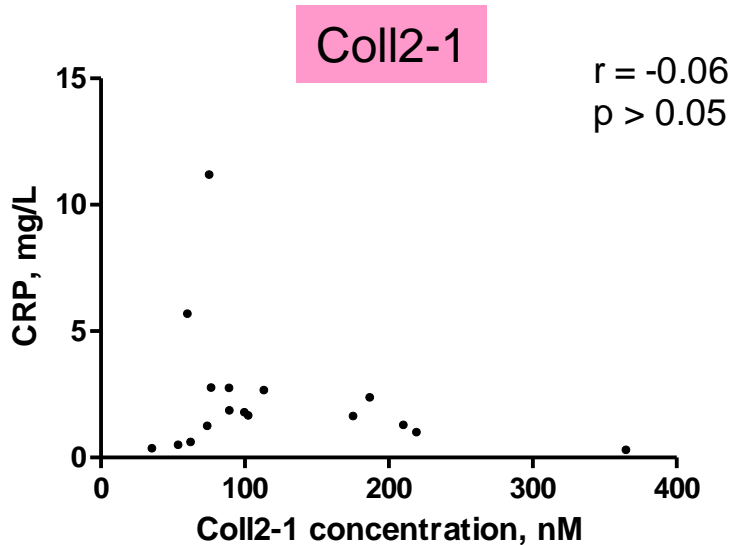
# OA PATIENTS

(n=67)

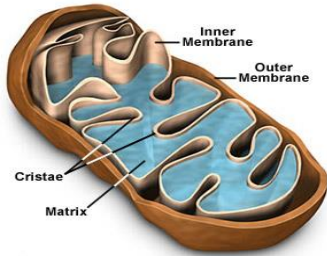


# RA PATIENTS

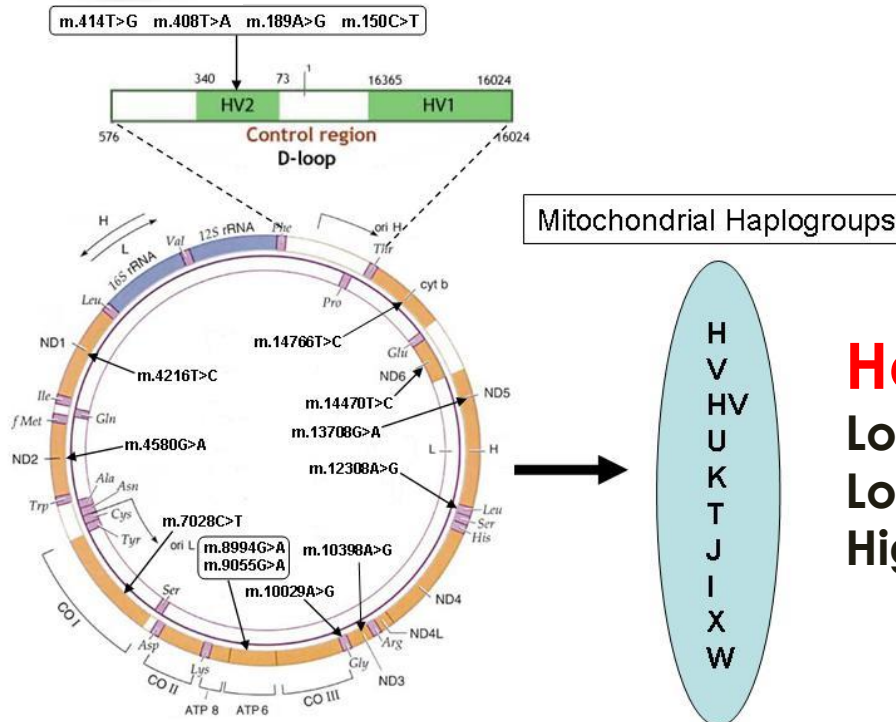
(n=19)



# Diagnosis: Coll2-1NO2 discriminates Mitochondrial Haplogroups



Comparison of biomarkers serum levels in haplogroups H and J

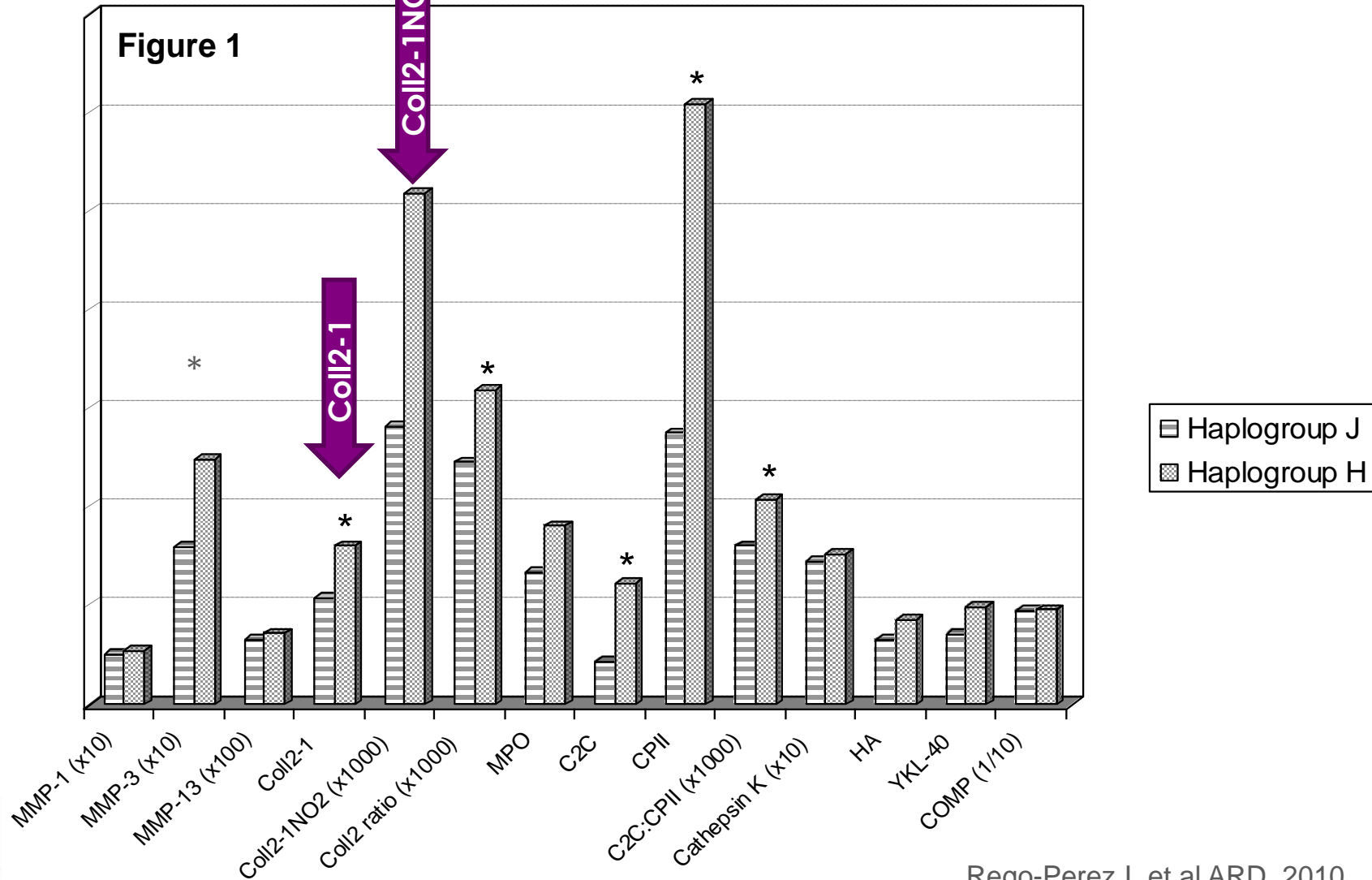


**Haplogroups J**

Lower probability to develop OA  
Lower X-ray OA severity  
Higher time to prosthesis

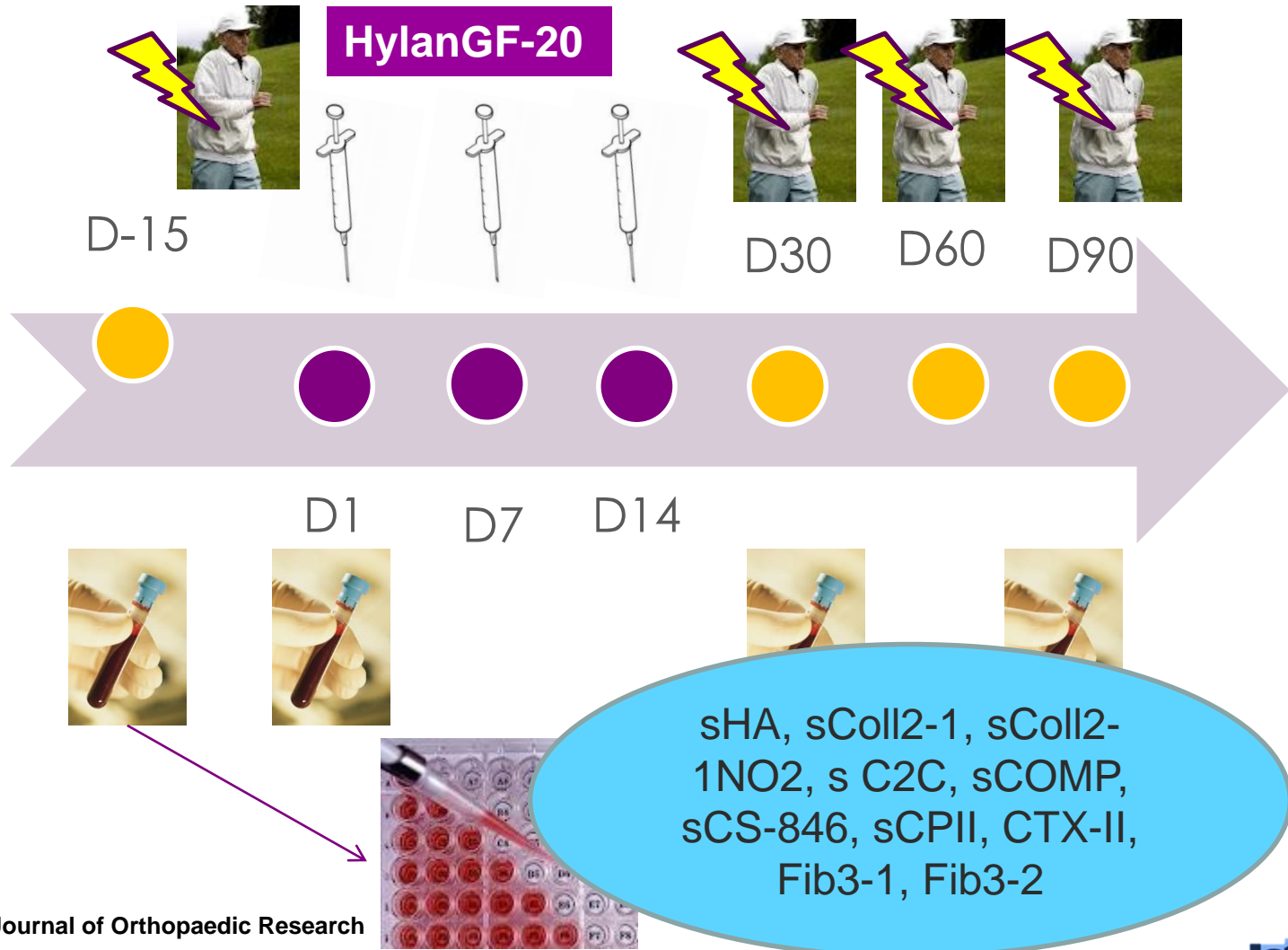


# Diagnosis: OA patients with haplogroup J have lower levels of Coll2-1NO2



# BIOVISCO study: Study design

Open-label, observational prospective study





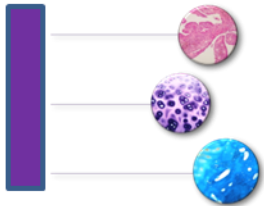
# BIOVISCO study

An open label observational prospective study

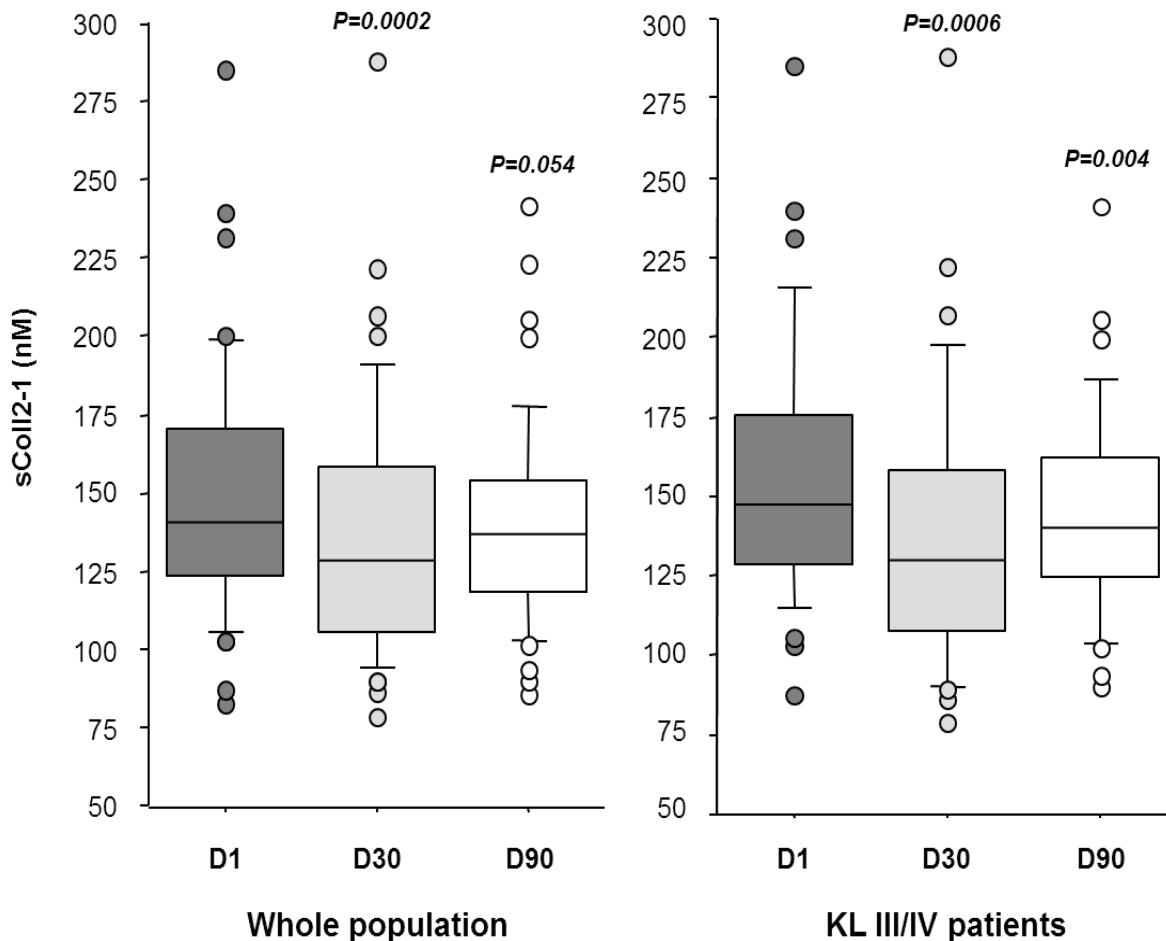
*Conrozier et al, J Orthp Res, 2012; Henrotin et al, J Orthp Res, 2013.*

- ✓ 45 patients with unilateral symptomatic tibiofemoral and/or patellofemoral OA
- ✓ 3-weekly intraarticular injection of hyalan G20 (Synvisc®)
- ✓ Follow-up D1, D30 and D90 after the last injection

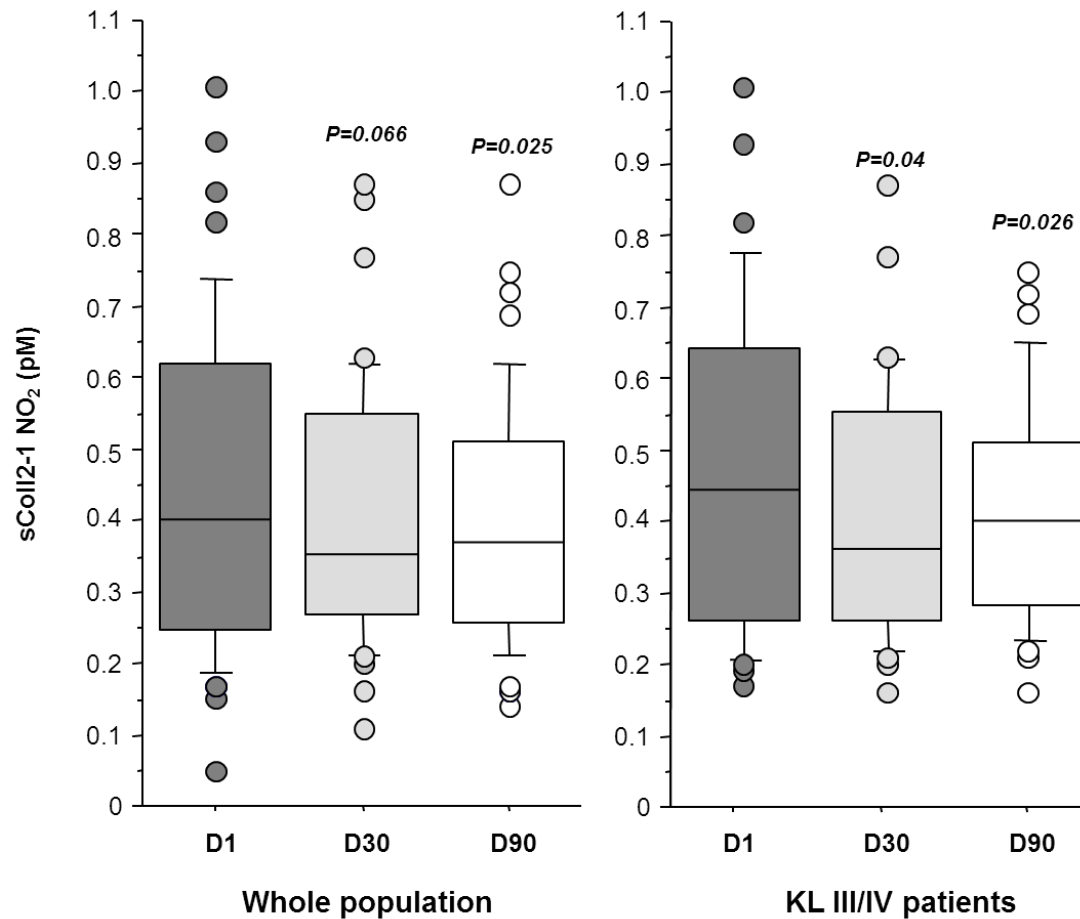
	<b>D1</b> (after the last injection)	<b>90 days</b> (after the last injection)	<b>p-Value</b> D1 vs D90
sColl2-1 (nM)	140.34(882.44-285.32)	128.41 (85.6-241.34)	<b>0.05*</b>
sColl2-1NO2 (nM)	0.400 (0.050-1.010)	0.370 (0.14-0.870)	<b>0.025*</b>
uCTX-II (ng/nmolcreat)	392.7 (90.0-816.4)	306.0 (90-1123.9)	<b>0.02*</b>
sPIICP (ng/ml)	817.9 (131.4-1848.6)	874.8.3 (326.4-1435.0)	0.41
sC2C (ng/ml)	223.6 (99.4-329)	209.5 (135.9-291.7)	0.11
sCOMP (U/L)	10.9 (6.0-20.2)	10.5 (6.0-20.0)	0.82
sCS846 (ng/ml)	99.8 (45.9-172.3)	102.2 (53.0-190)	0.38
sHA (ng/ml)	34.1 (15.4-211)	33.3 (9.5-230.1)	0.38



# Effets de HA sur les taux sériques de Coll2-1

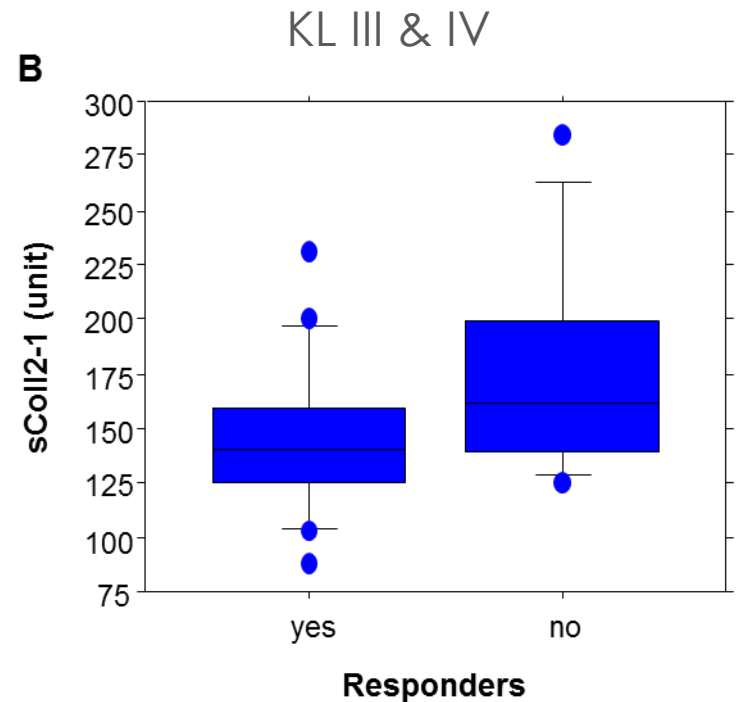
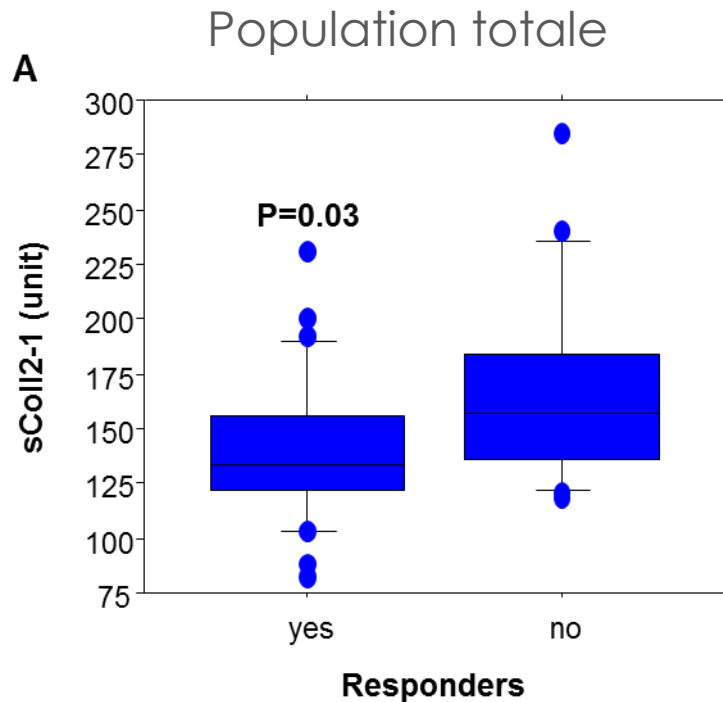


# Effets de HA sur les taux sériques de Coll2-1NO2



# Coll2-1 a l'inclusion: un marqueur de prédiction d'efficacité du traitement

Critère réponse: diminution de la douleur à la marche J1 – J90  $\geq 50\%$







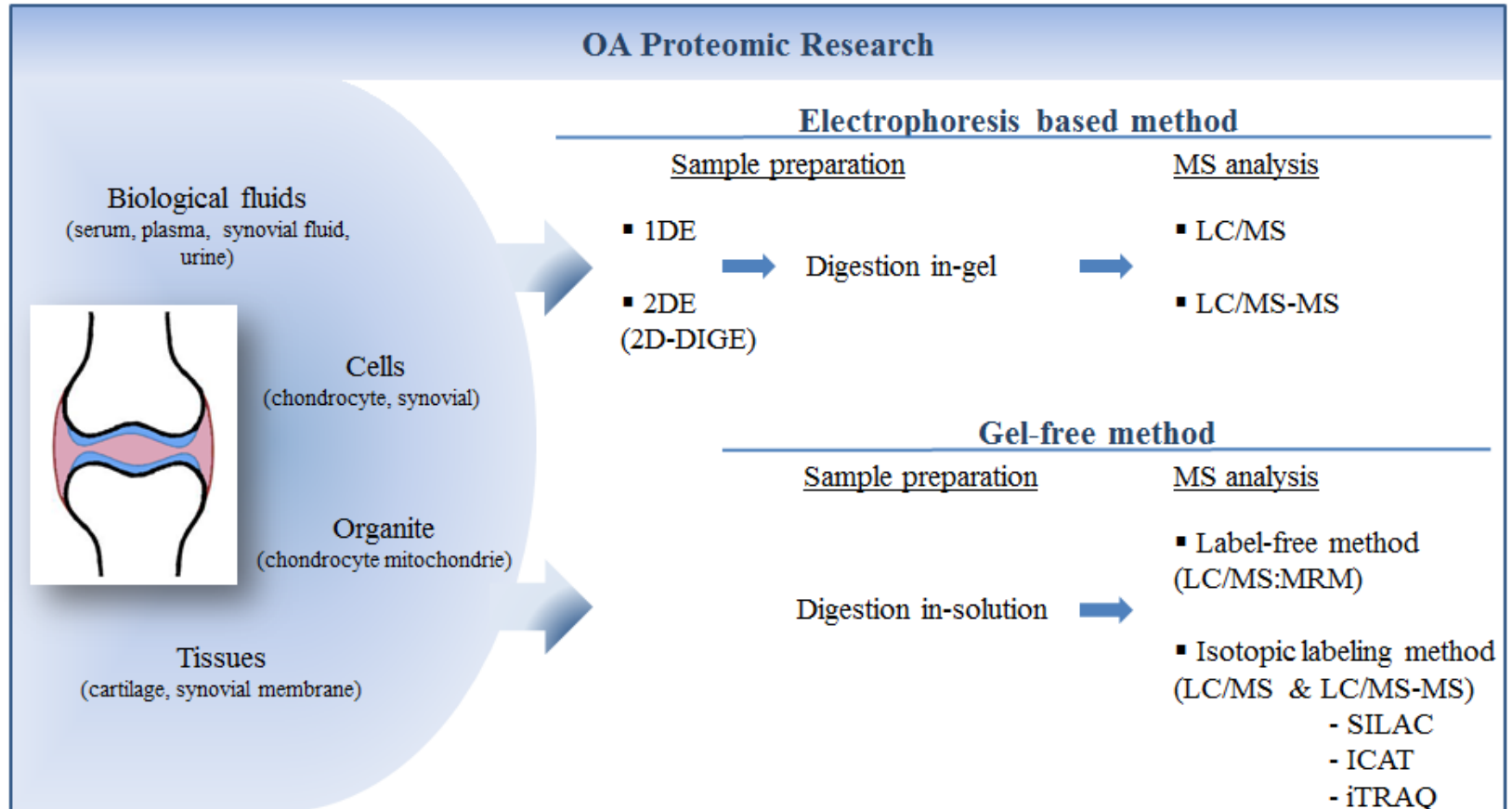
# Conclusions

- Coll2-1 et Coll2-1NO2 marqueurs d'efficacité du traitement par viscosupplémentation (Hylan GF-20)
- Le taux basal sérique de Coll2-1 est prédictif de la réponse au traitement
- La viscosupplémentation avec l' AH diminue in vivo le catabolisme du collagène II



# Proteomic research

Gharbi et al. *Frontiers in physiology*, 2011



# Comment rechercher un nouveau biomarqueur?

## 1. Identification

- Protéomique (gel + Chromato + Swissprot)

## 2. Selection

- Pouvoir discriminant
- Littérature

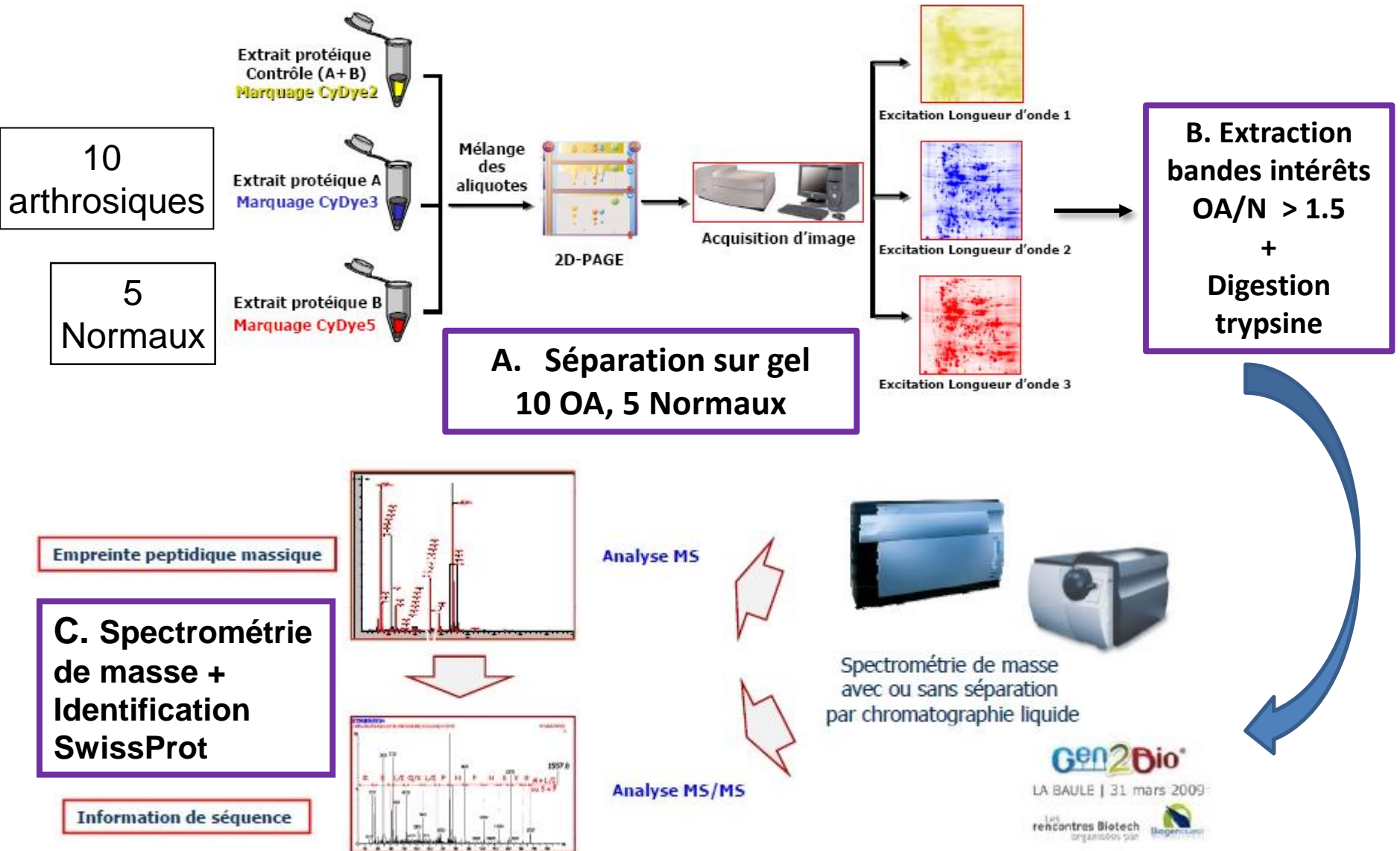
## 3. Dosage (Validation)

- Production d'anticorps polyclonaux
- Dosage immunologique

## 4. Qualification

- Etude transversale (diagnostic)
- Critères BIPEDS

# 1. IDENTIFICATION: Analyses protéomiques



# Identification des protéines d'intérêt

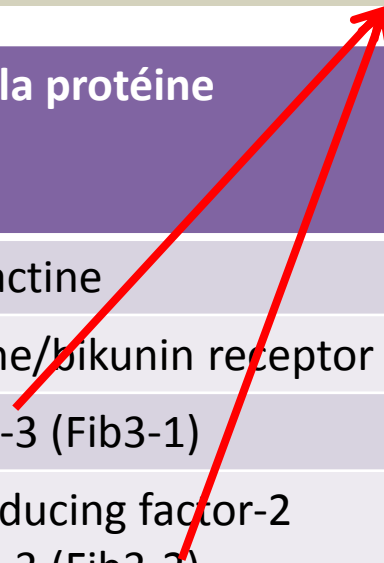
40 protéines avec un rapport OA/N > 1,5

Top 5

Fib3-1: **TCQDINECETTNECR**

Fib3-2: **CVCPVSNAMCR**

Rapport d'abondance OA/N	Nom de la protéine	Signification statistique Valeur de p
4,1	$\beta$ -actine	P < 0,0001
2,2	$\alpha$ 1-microglobuline/bikunin receptor	P<0,001
2,2	Fibuline-3 (Fib3-1)	P< 0,001
2,2	Apoptosis-inducing factor-2 Fibuline-3 (Fib3-2)	P<0,001
2,0	Zn- $\alpha$ -2-glycoprotein precursor	P<0,001



## 2. Selection: Fragments Fibuline-3

- Rapport OA/N > 2

### Littérature

- Identifiée dans le cartilage arthrosique
- Associée à TIMP-3
- Inhibiteur de la différenciation hypertrophique des chondrocytes
- Inhibiteur de l'angiogénèse (néovascularisation)

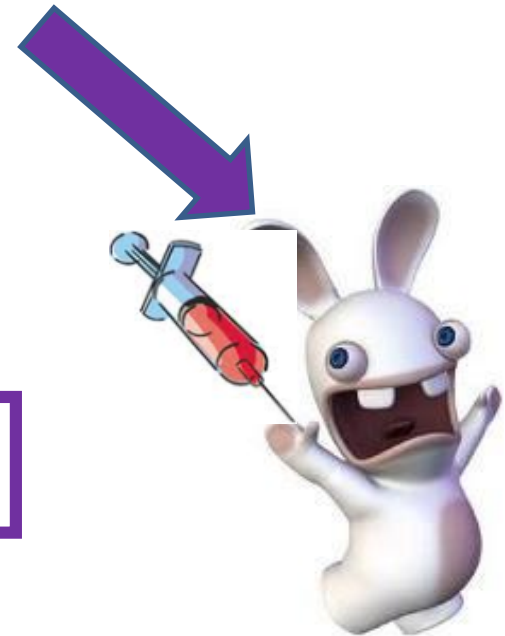
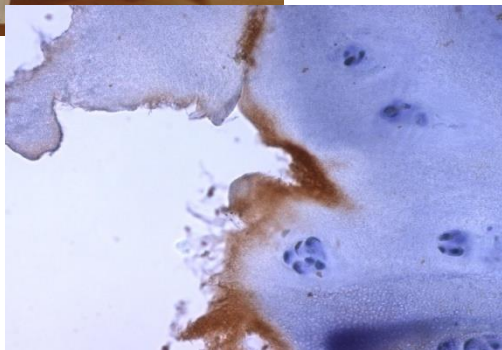
# 3. Validation des immunodosages

Fib3-1: **TCQDINECETTNECR**

Fib3-2: **CVCPVSNAMCR**



**Immunodosage  
Immunohistochimie**



**Deux antiséra:  
AS88: Fib3-1  
AS94: Fib3-2**

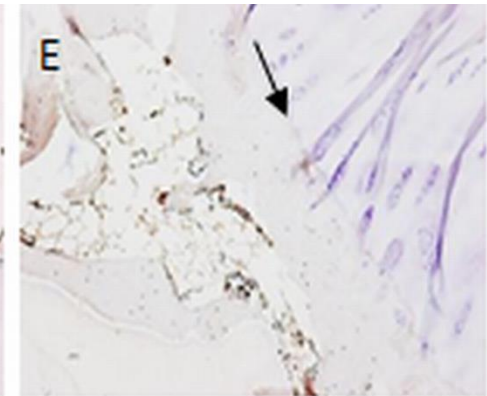
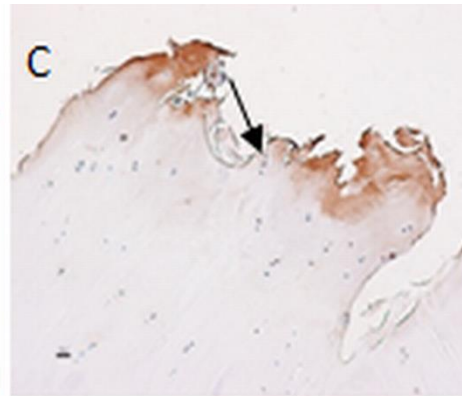
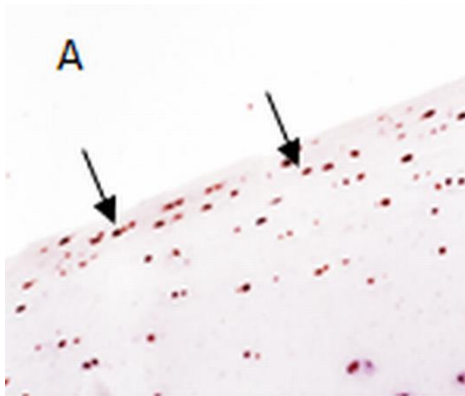
# Localisation de Fib3-1 and Fib3-2 par immunohistochimie

Normal

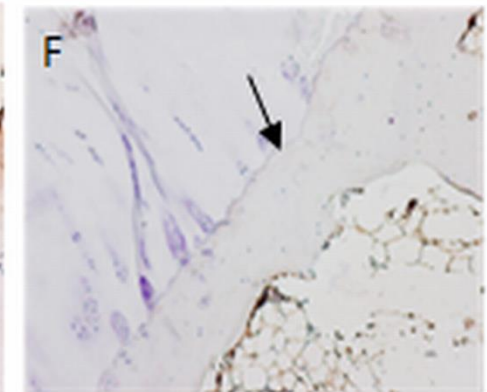
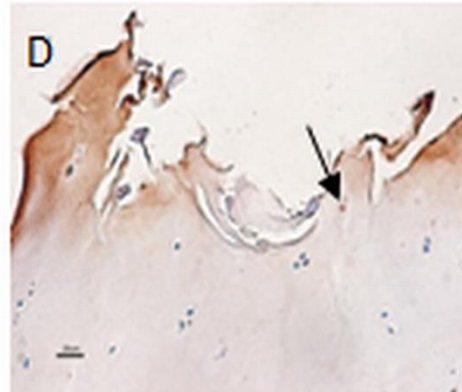
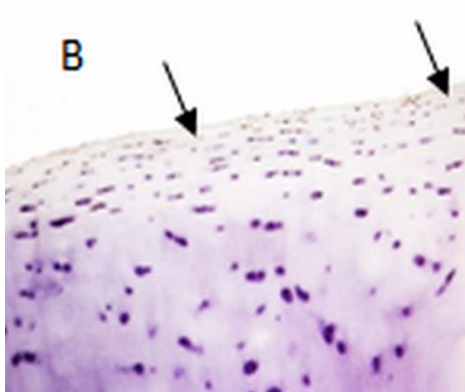
Arthrosique  
surface

Arthrosique  
Zone profonde

Fib3-1



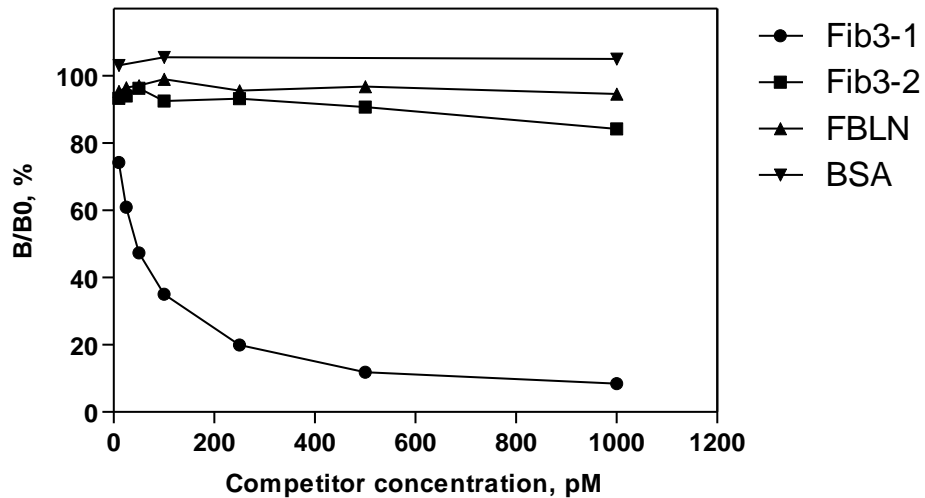
Fib3-2



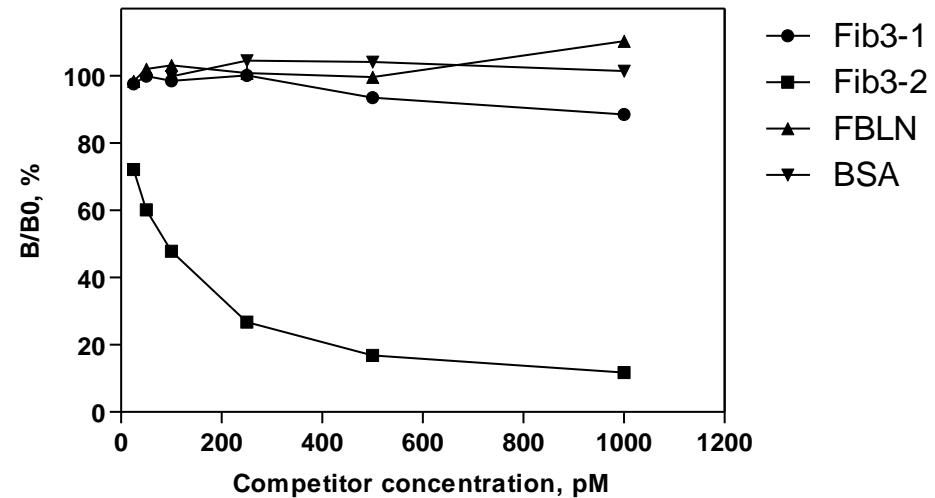


# Performances analytiques du dosage immunologique

Fib3-1



Fib3-2

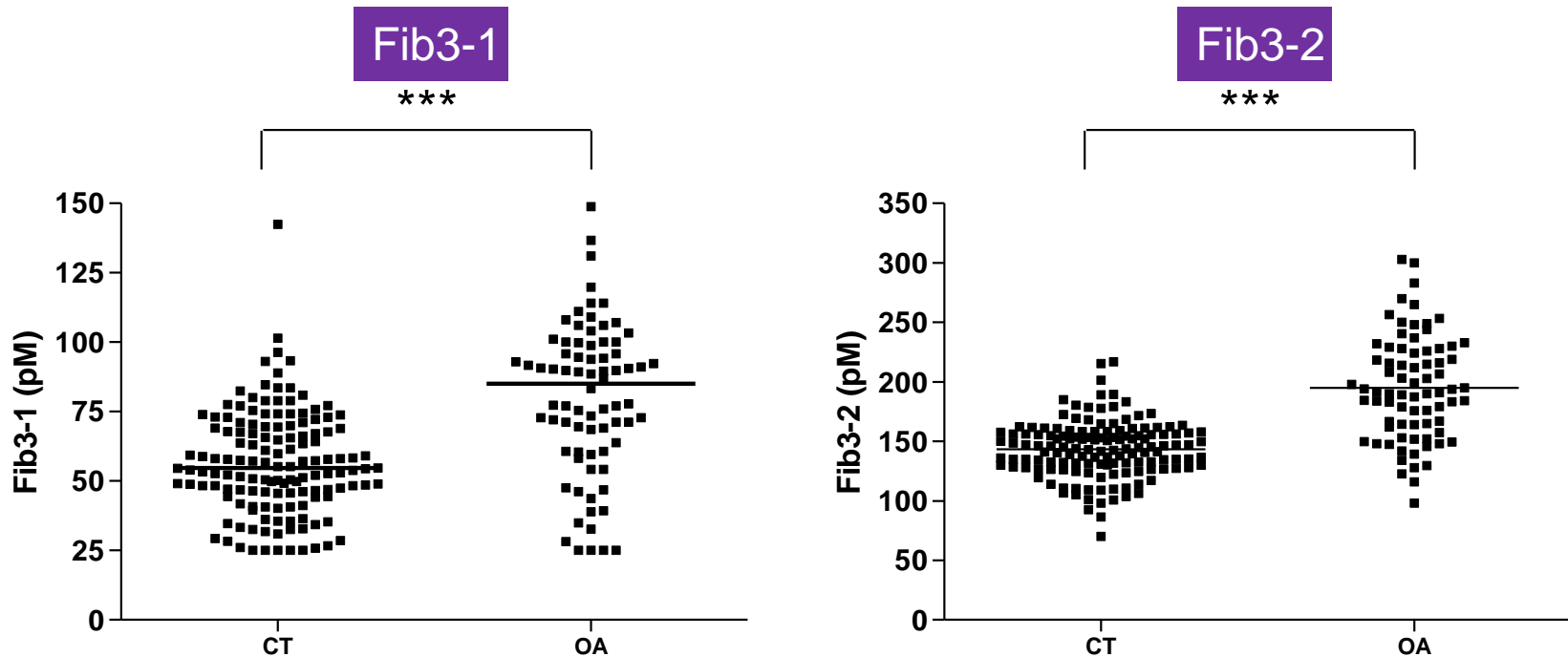


	Fib3-1	Fib3-2
LIMIT OF DETECTION (pM)	5.2	8.0
CVs INTRA-ASSAY (%)	< 10	
CVs INTER-ASSAY (%)	<11	
LINEARITY (%)	85.8-104.0	80.5-105.1
ANALYTICAL RECOVERY (%)	92.3-103.2	101.3-112.4

# 4. Qualification

- 236 sujets normaux
- 76 patients arthrose fémoro-tibiale
  - 54 femmes; 22 hommes
  - 67,3 +/- 12,5
  - 55 bilatérale, 21 unilatérale
  - Critères ACR
  - RX : K&L III & IV
  - BMI: 28,4 +/- 5,2

# Les concentrations de Fib3-1 et Fib3-2 sont augmentées chez les arthrosiques

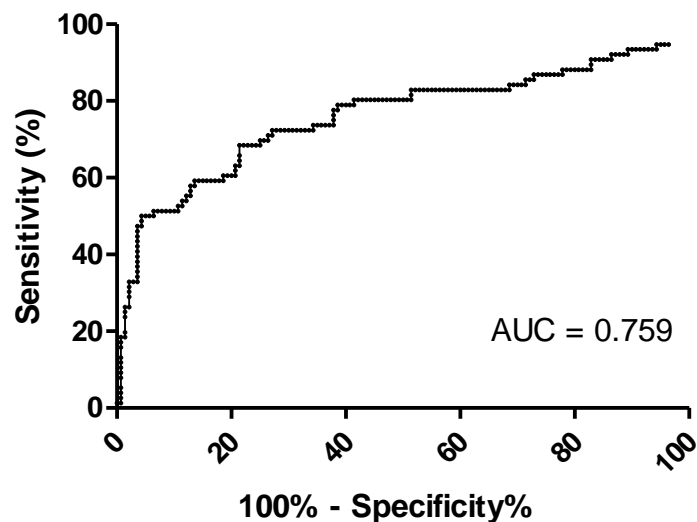


	OA (n=76)	CTRL (n=140)
Fib3-1 (median, 25%>75% range , pM)	85.10, 60.7 > 99.5	54.63, 45.59 > 69.56
Fib3-2 (median, 25%>75% range , pM)	191.4, 162.5 > 227.5	144.4, 128.7 > 157.7

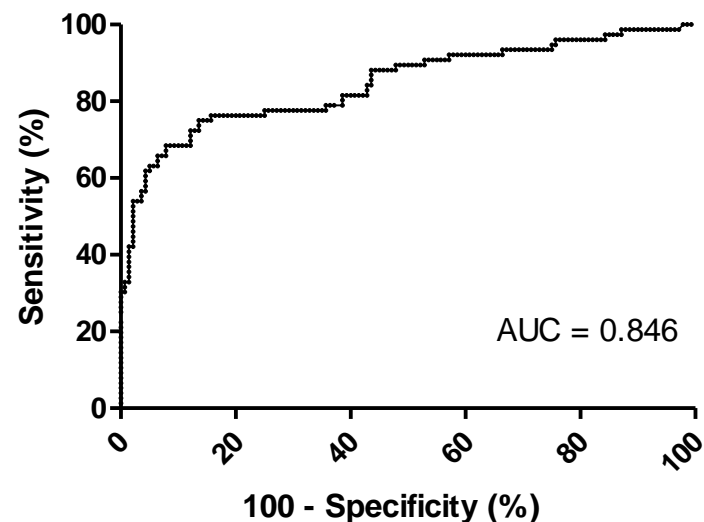
**Fib3-1 est influencé par le sexe et l'âge**  
**Fib3-1 et Fib3-2 ne sont pas corrélés**

# Fib3-2: un marqueur diagnostique de l'arthrose du genou

Fib3-1: ROC curve



Fib 3-2: ROC curve



	Sensibility	Specificity
Fib3-1 (cut-off: 71.1 pM)	78.5%	68.4%
Fib3-2 (cut-off: 163.7 pM)	75.0%	86.4%

# Conclusions

- **Fib3-2 est un marqueur potentiel de diagnostic de la gonarthrose:**
  - Exprimé au niveau des lésions du cartilage (peu au niveau du cartilage sain)
  - Pas influencé par le sexe, l'âge et l'IMC
  - Bonne valeur diagnostic (bonne sensibilité, bonne spécificité)

Back to the  
future.....to transform  
the dream in reality  
What do we need?



DeLorean sport car

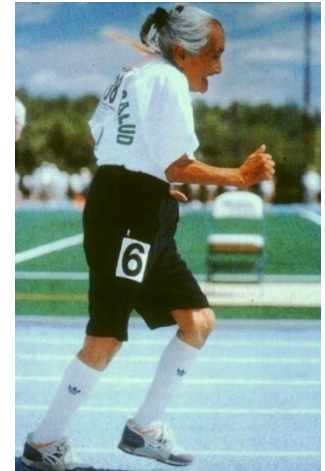
# Critical needs

- Reliable structural endpoints (MRI? Scintigraphy? Ultrasound?)
- Prospective clinical trials designed for biomarkers qualification and representative of the general population
- Therapies with structure/disease modifying activities
- To develop biomarkers fit-to-purpose and for various stage of the disease



# Short-term Perspectives

- New biomarkers « fit-for-purpose » developed (proteomics, metabolomics)
- Combination of biomarkers in multiplex tests
- Agregate score including clinical, imaging and biological parameters







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**THANK YOU !**



# Thank you for your attention !

## International collaborations:

- F Blanco (La coruna, Spain)
- T Conrozier (CHU Lyon, France)
- V Kraus (Duke University, USA)
- L Punzi (University of Padova, Italy)
- A Mobasher (University of Nottingham, UK)
- J Monfort (Hospital del mare (Spain)
- P Richette (Lariboisiere, France)
- J Runhaar (Erasmus MC, Rotterdam)



# Liège: a belgium city



Thank you...