miRNA: Biomarqueurs d'avenir? Application à la sarcopénie et aux pathologies osseuses.

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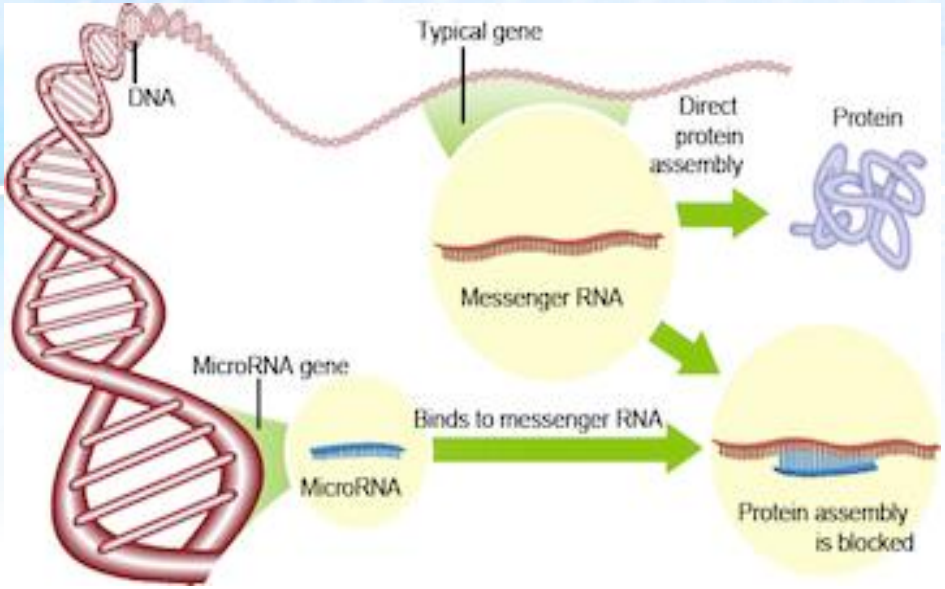
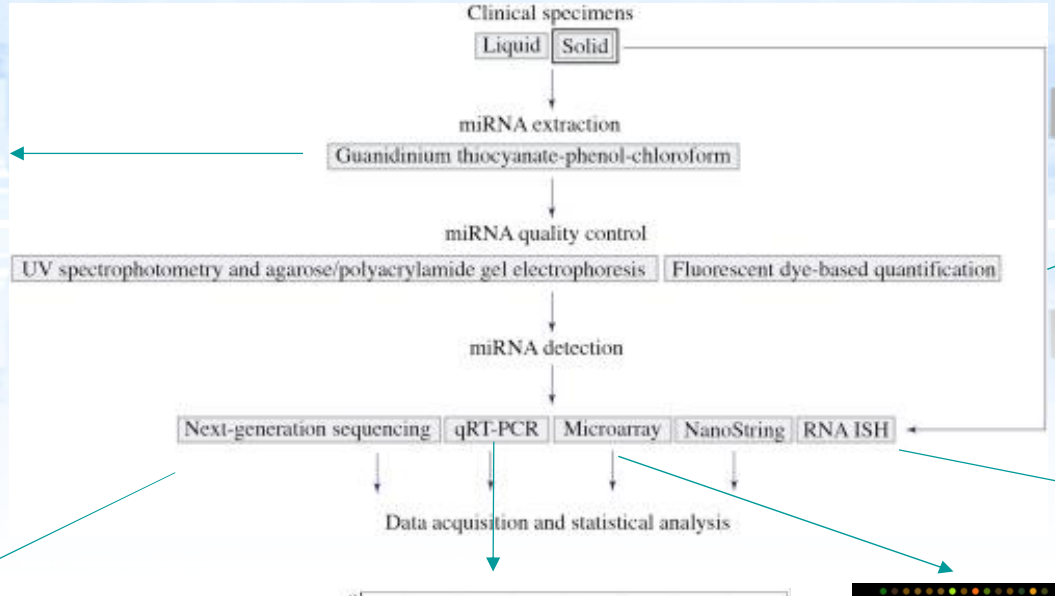
Qu’est ce que les miRNAs?

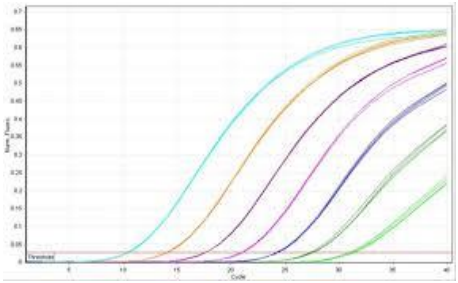


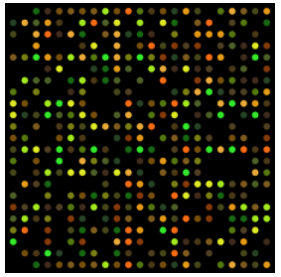
Brins D’ARN non codants
19 -24 nucleotides
Impliqués dans la répression de la traduction

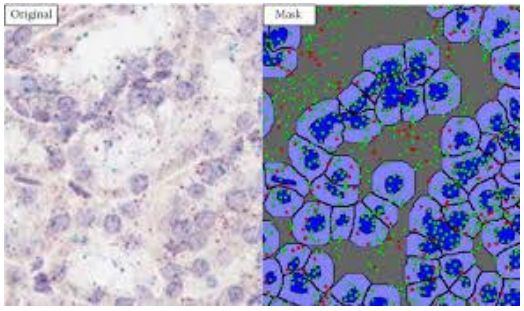
Existence de miRNAs circulants
Sous forme libre ou encapsulée dans des vésicules
Très stables dans les liquides physiologiques
Communication paracrine

Comment étudier les miRNA?

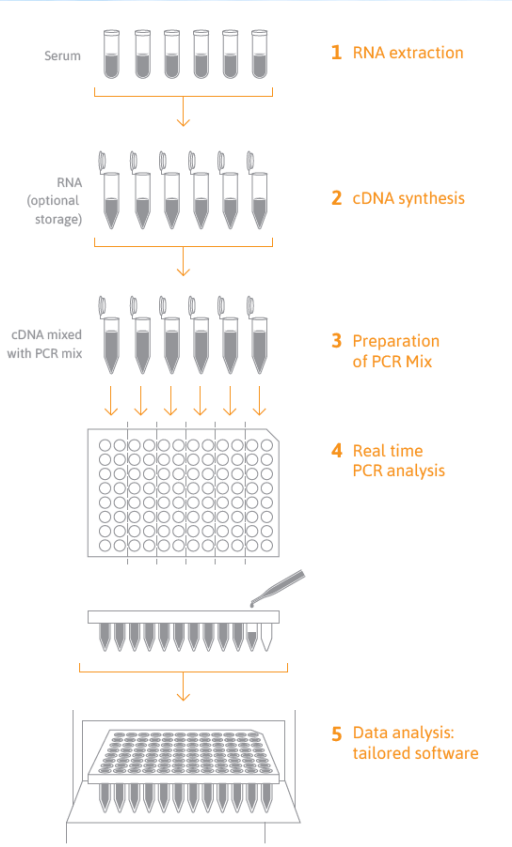








Comment étudier les miRNA par qPCR?



Considérations pré-analytiques

Serum et plasma corrélés mais pas interchangeables

EDTA: meilleur choix: enlevable du PCR mastermix
PAS d'heparine (y compris héparines thérapeutiques)!!!
    Interférences avec les Taq Polymérases
Pas de citrate!!!
    Effet inhibiteur sur la PCR
NaF: OK mais augmentation des valeurs

Besoin de standardisation pour la centrifugation
    Eviter apparition des miRNAs spécifiques des plaquettes et des GR

Précautions pour éviter les contaminations

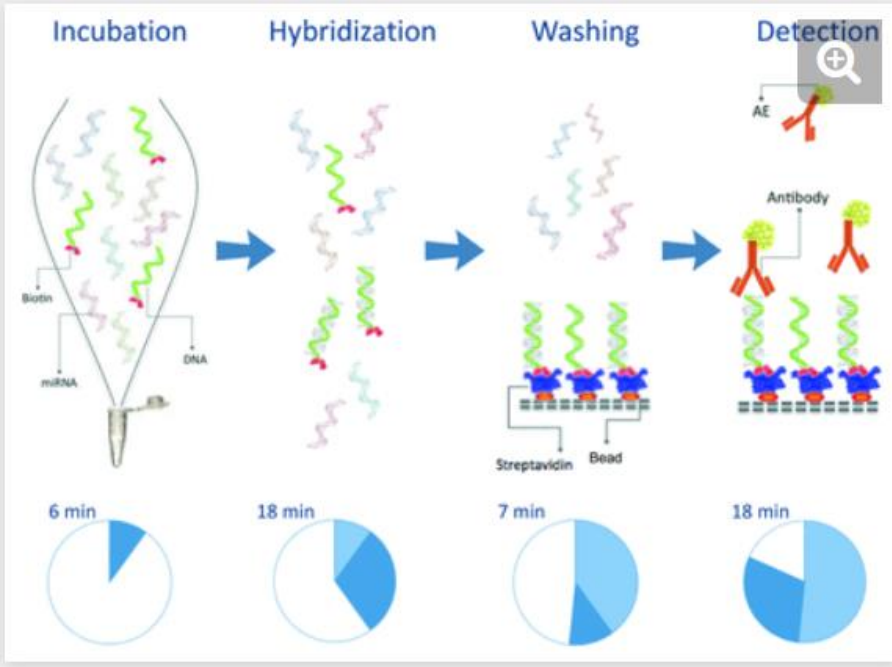
Conditions de conservations et délai de réponse

L’avenir du miRNA passera-t-il par les immunoassays?

MicroRNA In Vitro Diagnostics Using Immunoassay Analyzers

Andreas Kappel, Christina Backes, Yiwei Huang, Sachli Zafari, Petra Leidinger, Benjamin Meder, Herbert Schwarz, Walter Gumbrecht, Eckart Meese, Cord F. Staehler, Andreas Keller

DOI: 10.1373/clinchem.2014.232165 Published March 2015



Même sensibilité/spécificité
Gain de temps et d’argent
Dépend d’une extraction ARN
Difficulté de l’analyse muliplex

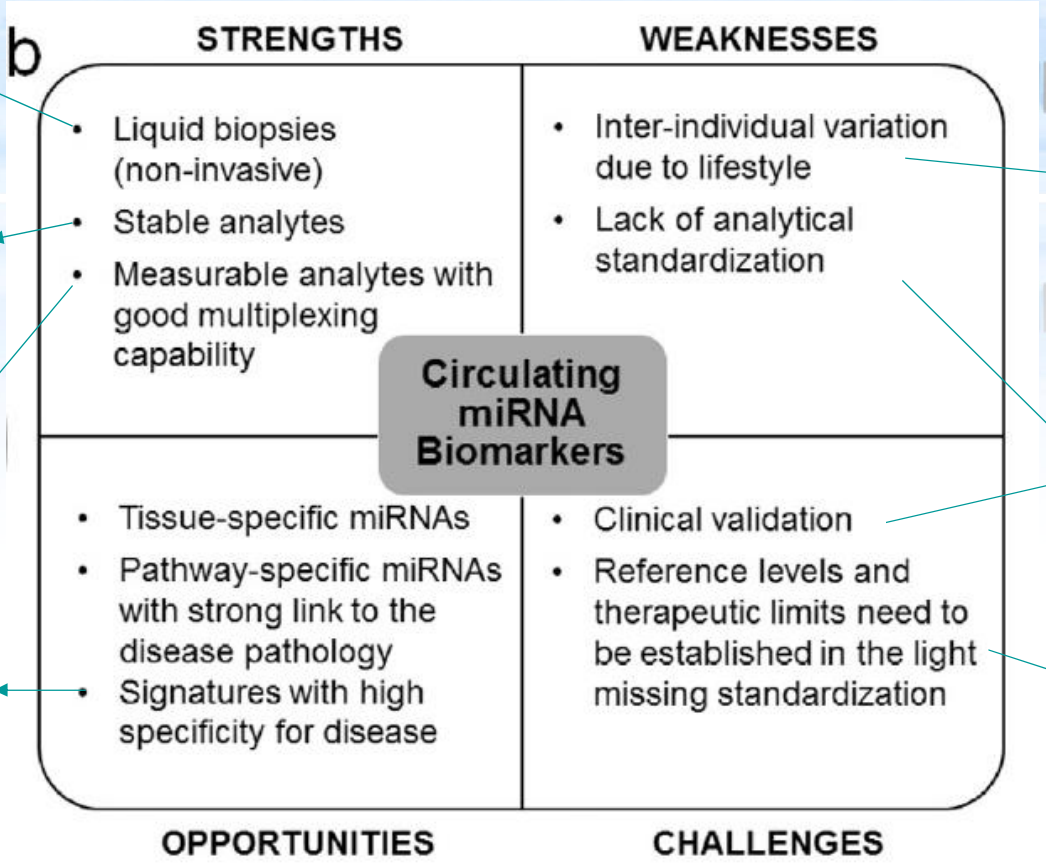
Quel est leur intérêt en biologie clinique?

Serum, plasma
Urine, LCR, Salive

Resist to freeze-thaw cycles
Long-term storage -70°C

Amplification
Sensibillté ++++
Spécificité ++++
Contamination !!!

panel vs biomarqueur unique



Impact du:
- Mode de vie (sport, diet, smoking)
- Rythme circadien
- Insuffisance rénale chronique

Indépendant du sexe

Reproductibilité inter-études limitée

Méthode de référence
QC

Quelle est la place actuelle des miRNAs en routine?

GENSIGNIA

GENSIGNIA specialises in microRNA signatures as diagnostic tools. The company has established its laboratory operations in San Diego, CA and is committed to the development and commercialization of novel diagnostic clinical tests to inform earlier and more accurate detection of lung cancer.

GENSIGNIA™ MSC LUNG CANCER TEST

GENSIGNIA's MSC ("microRNA Signature Classifier") Lung Cancer Test in development addresses the most common cancer in the world, which affects an estimated 2 million people. It is a plasma-based test aimed at decreasing the high rate of false positives from low-dose (spiral) computed tomography (LDCT).

miRview® lung – Final Approval for Rosetta Genomics' microRNA Diagnostic Allows Identification of Four Major Subtypes of Lung Cancer

Contenu des panels ???

INTERPACE DIAGNOSTICS
GUIDING TREATMENT. DELIVERING INNOVATION.

PARSIPPANY, N.J., Jan. 12, 2016 /PRNewswire/ — Interpace Diagnostics (NASDAQ: IDXG) announced today that Laboratory Corporation of America® Holdings (LabCorp®) (NYSE: LH), the world's leading health care diagnostics company, will begin offering Interpace's new ThyraMirTM microRNA classifier test. Physicians will be able to order ThyraMir through LabCorp, in addition to Interpace's ThyGenX® oncogene panel, which LabCorp already offers. These innovative assays provide enhanced options for the diagnosis of thyroid cancer in patients with indeterminate thyroid nodules.

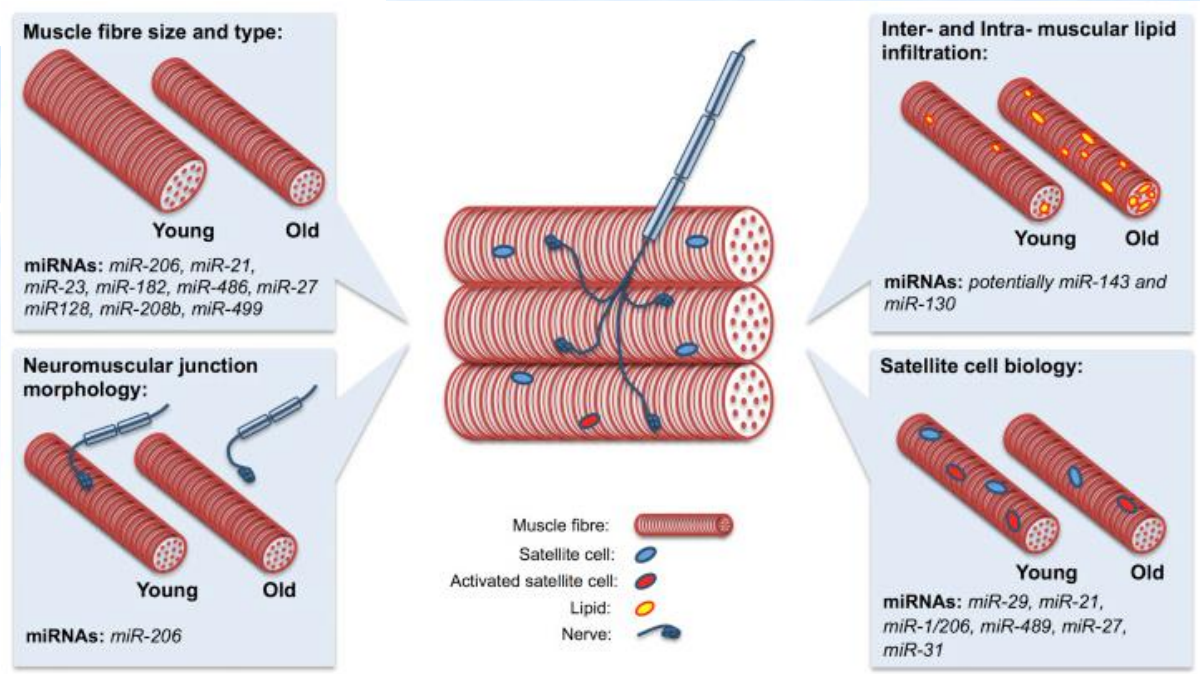
Not FDA approved

Place des miRNAs dans les pathologies musculaires

Review

microRNAs: Modulators of the underlying pathophysiology of sarcopenia?

David M. Brown, Katarzyna Goljanek-Whysall



miRNA impliqués à chaque étape de l'homéostasie musculaire
myomiR communément accéptés: **miR-1, miR-133, miR-206, miR-208 and miR-499**
Identification par screening (avant vs après exercices physiques, muscles jeunes vs muscles agés)

Place des miRNAs dans les pathologies osseuses

Table 1
Overview of studies on miRNA expression in liquid and solid biopsies in the context of human osteoporosis.

Study	Study design	Sample size	Biomarker source	Technology platform	Reported microRNA biomarkers	Potential target genes	Comments
Wang et al., 2012	Postmenopausal Caucasian women with discordant bone mineral density, i.e. low and high BMD	N = 10 per group	Cells: Human blood-circulating Monocytes	ABI Taqman RT-qPCR Cards 365 miRs tested 156 detected	miR-133a	CXCL11 CXCR3 SLC39A1	Identified the association of miR-133a levels in circulating monocytes, the osteoclast precursors, with postmenopausal osteoporosis. Limitation: small sample size
Cao et al., 2014	Postmenopausal Caucasian women with discordant bone mineral density, i.e. low and high BMD	N = 10 per group	Cells: Human blood-circulating Monocytes	ABI Taqman RT-qPCR Cards 365 miRs tested 156 detected	miR-422a	CBL CD226 IGF-1	Identified the association of miR-422a levels in circulating monocytes, the osteoclast precursors, with postmenopausal osteoporosis. Limitation: small sample size
Li et al., 2014	Postmenopausal Chinese women with normal, osteopenic or osteoporotic range of BMD	N = 40 per group	Cell-Free: Human Plasma	Exiqon LNA RT-qPCR Assays 3 miRs tested 3 detected	miR-133a, miR-21-5p	Spry1	Identified in a large cohort a clinical correlation between T-score and circulating miRNA levels Limitations: incomplete information on pre-analytical impact variables (e.g. anticoagulant)
Seeliger et al., 2014	Caucasian men and postmenopausal women with oseteoporotic and non-osteoporotic fractures Validation in bone tissue biopsies and serum samples of Caucasion postmenopausal women	Discovery: N = 10 per group (Pool) Validation: N = 30 per group (serum) and N = 20 per group (bone tissue)	Cell-Free: Human Serum	Qiagen miScript RT-qPCR Panel 83 miRs tested 51 detected	miR-21-5p, miR-23a-3p, miR-24-3p, miR-93, miR-100-5p, miR-122a, miR-124a-3p, miR-125b-5p, miR-148a	See publication for details	Comparison of serum miRNA levels against bone miRNA expression. Independent validation and assessment of diagnostic value of miRNAs using ROC analysis Limitations: Sample pools and low sample size. Patients had osteoporosis and hip arthritis
Meng et al., 2015	Discovery in postmenopausal Chinese women with BMD in osteopenic to osteoporotic range Validation in postmenopausal Chinese women in the normal to osteoporotic range	Discovery: N = 48 Validation: N = 86	Cells: Peripheral Whole Blood	Discovery: Agilent miRNA Microarray 2549 miRs tested, 331 detected Validation: ABI Taqman RT-qPCR Assays	miR-130b-3p, miR-151a-3p, miR-151b, miR-194-5p, miR-590-5p, miR-660-5p	See publication for details	Limitations No adjustment for multiple comparison. Use of pooled samples. Only miRNAs with increased expression in Osteoporosis vs. Osteopenia were reported. No information about miRNAs with decreased expression levels. No information about fracture status and fracture risk
Weilner et al., 2015	Discovery in postmenopausal Caucasian women with recent fractures and healthy controls Validation in postmenopausal Caucasion women with recent fractures and healthy controls	Discovery: N = 14 (7 CTRL, 7 FX) Validation: N = 23 (11 CTRL, 12 FX)	Cell-Free: Human Serum	Exiqon LNA RT-qPCR Panels and Assays 175 miRs tested, 165 analyzed	miR-10a-5p, miR-10b-5p, miR-133b, miR-22-3p, miR-328-3p, let-7g-5p	See publication for details	Elaborate quality control strategy. Reported changes in serum miRNA levels after osteoporotic fractures and tested osteogenic activity of the identified miRNAs. Limitations: small sample size. No in vitro experiments to test effect on osteoclast formation
Garmilla-Ezquerra et al., 2014	Discovery in Caucasian postmenopausal women undergoing replacement surgery for osteoporotic hip fractures versus non-fracture control females with hip osteoarthritis	Discovery: N = 8 per group Replication: N = 18 per group Ex Vivo: N = 8 per group	Tissue: Trabecular Bone Samples	ABI Taqman RT-qPCR Cards and Assays 768 miRs tested, 232 miRs detected in all samples	miR-187 miR-518f	IL-6, TNFα (RANKL signaling) WISP1, CTNNBIP1 (WNT signaling)	Identified and replicated differential expression of miRNAs in trabecular bone tissue biopsies in osteoporotic fracture patients and controls with osteoarthritis.

Recherche de miRNA comme potentiels biomarqueurs de la sarcopénie et des pathologies osseuses:

Etude SarcoPhage

- But: évaluer l'impact et les conséquences fonctionnelles de la sarcopénie dans une population de personnes âgées vivant à leur domicile
- Moyen: Etude prospective sur 5 ans au depart de volontaires belges de > 65 ans

  534 sujets recrutés entre Juin 2013 et Juin 2014

  Pas de critères d'exclusion sauf BMI > 50 ou amputation d'un membre
- Critères de diagnostic de la sarcopénie:

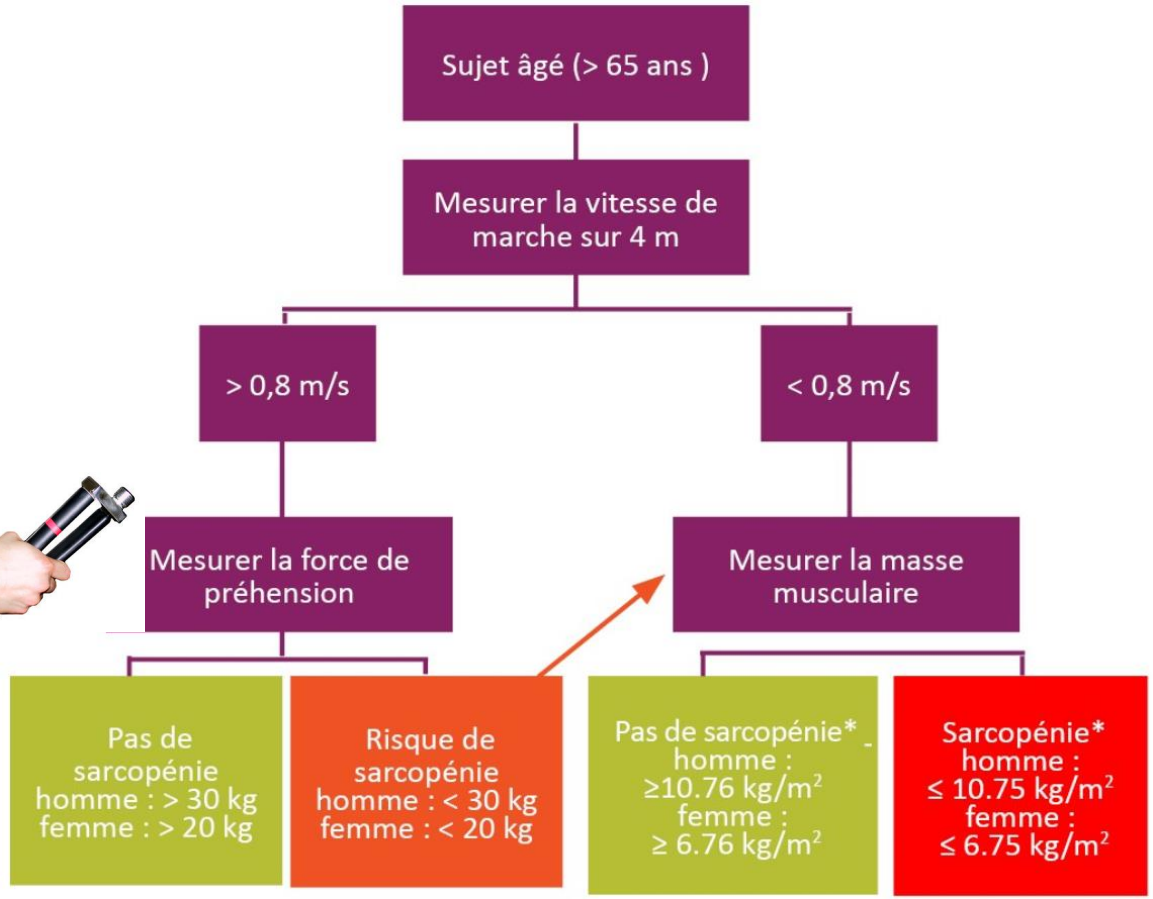
  faible masse musculaire + faible force musculaire ou score SPPB <8

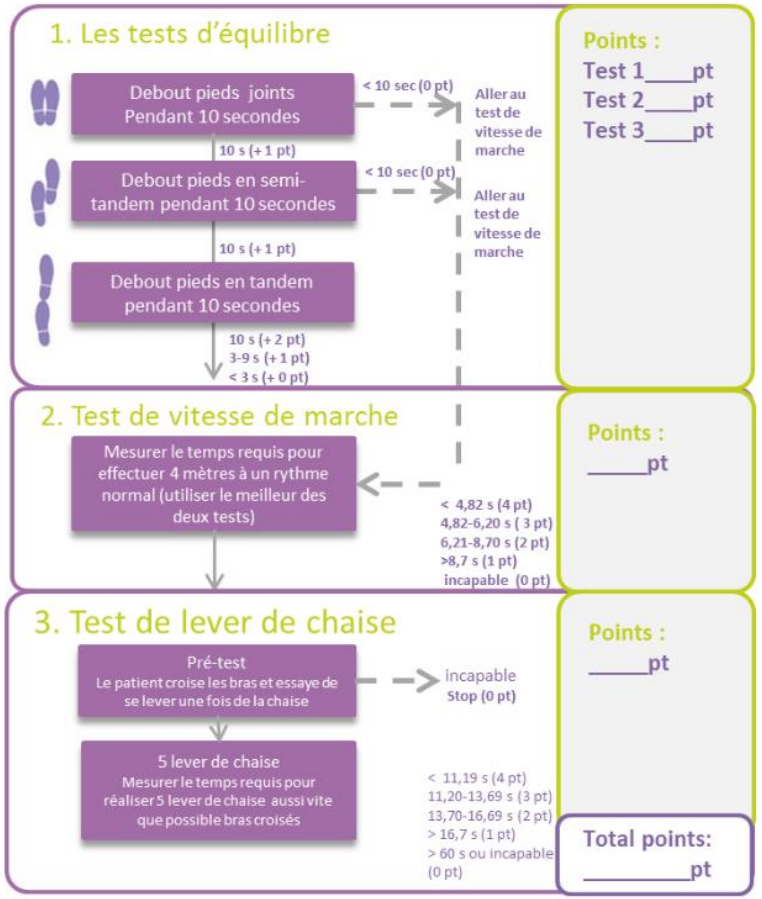
Définition de la sarcopénie

Syndrome gériatrique caractérisé par une perte progressive et généralisée de la masse musculaire et de sa capacité fonctionnelle; associée à des comorbidités telles que la réduction de la capacité physique ou la baisse de la qualité de vie ainsi qu'une augmentation de la morbi-mortalité.

Maladie d'évolution lente et multifactorielle
Impact estimé à plusieurs milliards de dollars du budget des soins de santé aux Etats-Unis
Absence de biomarqueurs diagnostics ou pronostics

Diagnostic de la sarcopénie





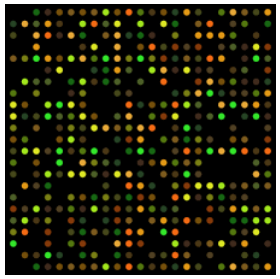
Résultats :

- **SPPB 0-6 Faible performance**
- **SPPB 7-9 Performances intermédiaires**
- **SPPB 10-12 Haute performance**

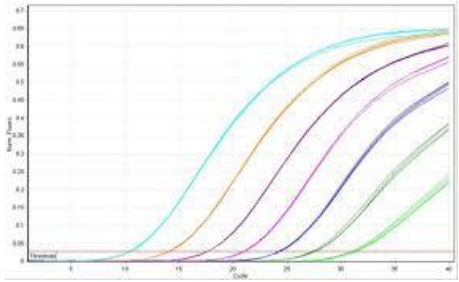
Recherche de miRNA comme potentiels biomarqueurs de la sarcopénie et des pathologies osseuses

Etude SarcoPhage

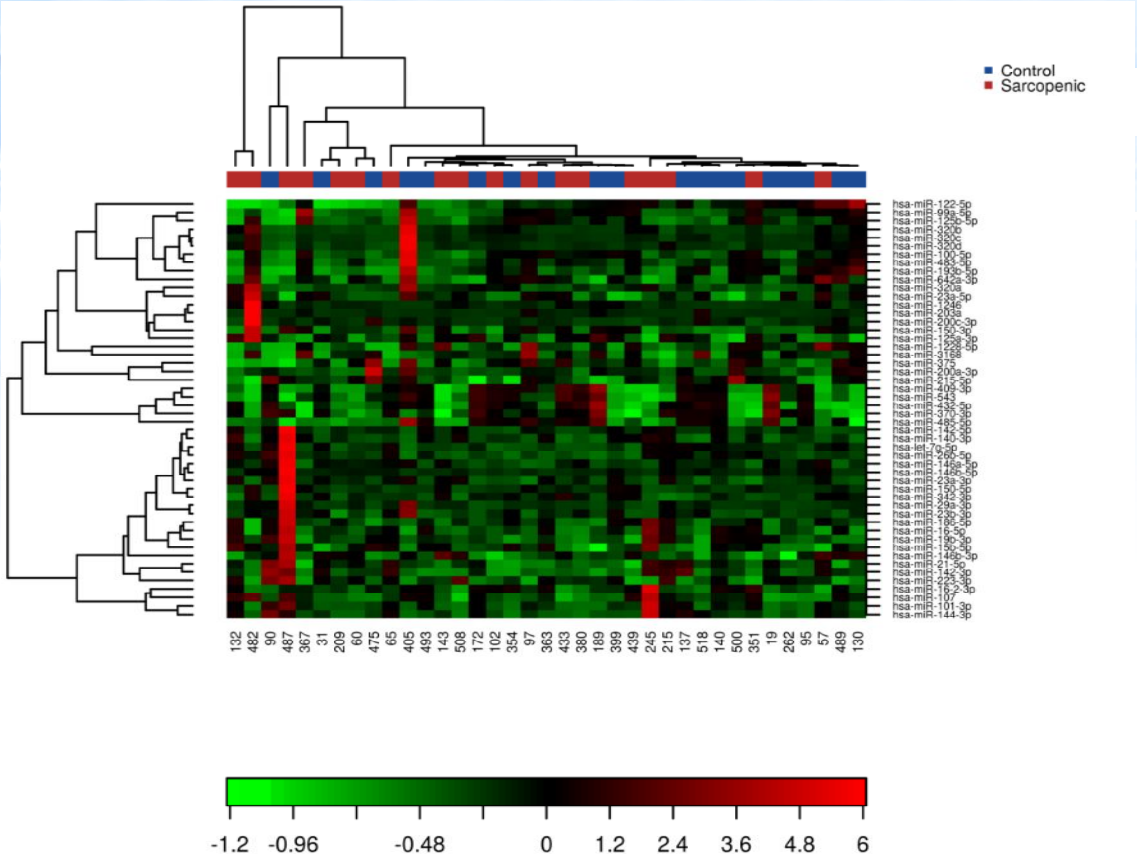
Approche de type « Omic »



Approche ciblée



Approche de type « Omic » dans la sarcopénie



Heat map and unspervised clustering

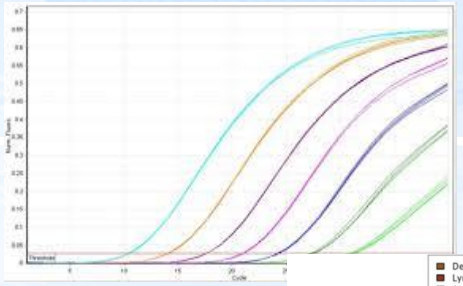
« Samples do not cluster according to their biological groups, indicating that other factors are causing the largest variation samples. »

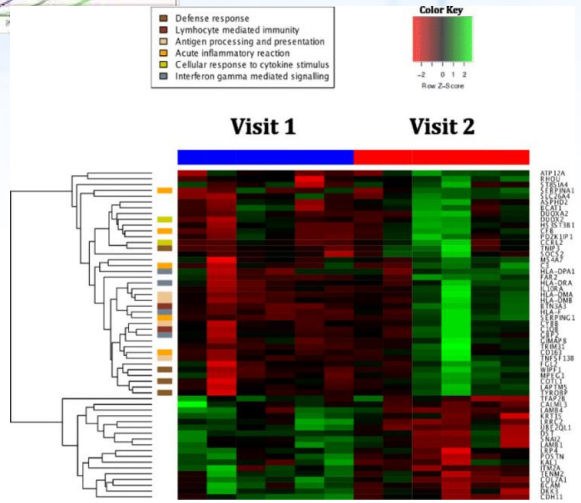
« common in bodyfluid derived samples »

Approche de type « Omic » dans la sarcopénie

Perspectives

Valider par qPCR les miRNA les plus intérressants





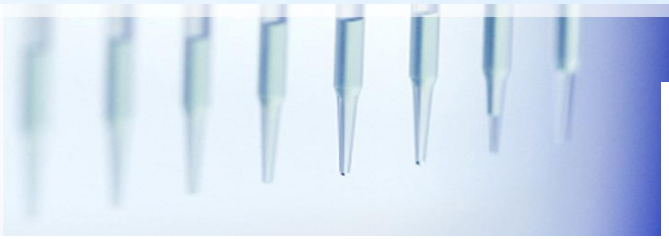
Etablir un panel de plusieurs miRNA pour augmenter le facteur discriminant
Refaire des heat map sur un nombre plus restreint de miRNA?

Approche ciblée dans les pathologies osseuses

osteomiR™

validated bone biomarkers

TAmiRNA – Introducing personalized medicine to Aging



TAmiRNA
stability for life.

Benefit:

- analyze 19 individually selected bone-related microRNAs in 48 human serum/plasma samples. Each microRNA has been characterized regarding for its function and contribution to bone disease.
- compute a novel fracture risk score using a specific combination of microRNAs that are presented on the test plates.

The osteomiR microRNA signature is intended to assess the risk of a **first fracture** in female patients of postmenopausal osteoporosis and type-2 diabetes. This information enables **timely interventions** and can help to **avoid fractures**.

Every single microRNA can be used as **individual bone-related biomarker** with distinct information content.

Our new study presents the first comprehensive attempt to model the cost-effectiveness of circulating microRNAs for bone fracture risk assessment in a central European female post menopausal population in comparison to DXA and FRAX®. The results demonstrate that the osteomiR™ kit can help reduce fracture incidence and healthcare costs.

Approche ciblée dans les pathologies osseuses

miRNA ID	Bone Turnover	Microstructure and Histomorphometry	Osteoporosis & other bone diseases	Bone Loss & Treatment Response	Calcification	Therapeutic Activity	Mechanism of action
let-7b-5p		•	•				VEGF
miR-127-3p			•				
miR-133b	•		•	•			Runx2/FOXC1[3]
miR-141-3p			•		•		
miR-143-3p			•				
miR-144-5p	•						
miR-152-3p			•				
miR-17-5p				•	•		Smad5
miR-188-5p			•	•		•	PPARγ via HDAC9/RICTOR
miR-19b-3p	•		•	•			
miR-203a			•	•	•		Runx2, Dlx5
miR-214-3p					•	•	ATF4
miR-29b-3p	•	•	•		•		
miR-31-5p			•	•	•	•	WNT via FZD3
miR-320a			•		•		HOXA10
miR-335-5p	•	•	•	•	•		WNT via DKK1
miR-375			•		•	•	WNT via LRP5 and β-catenin
miR-550a-3p	•	•	•		•		
miR-582-5p			•				

+ 5 contrôles

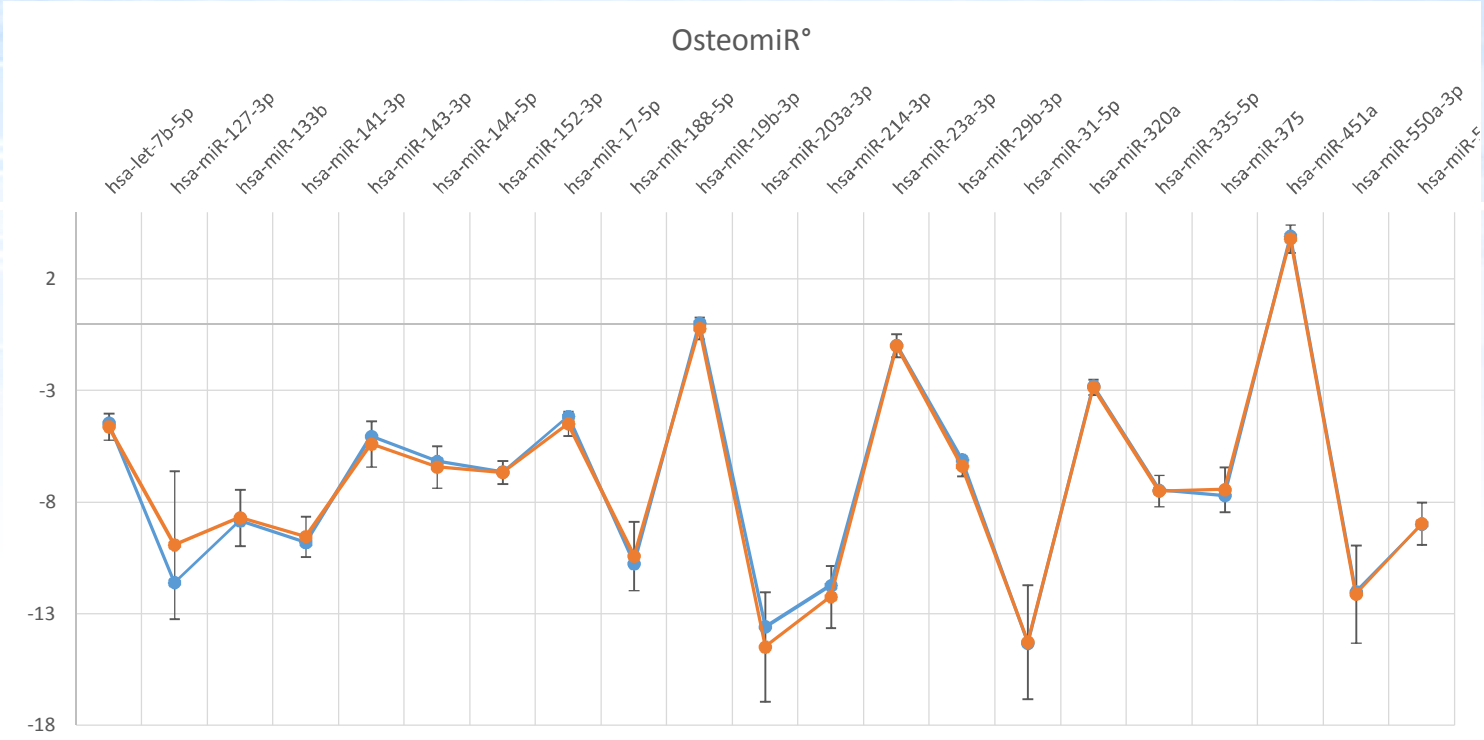
Base de donnée SarcoPhage

Critères :

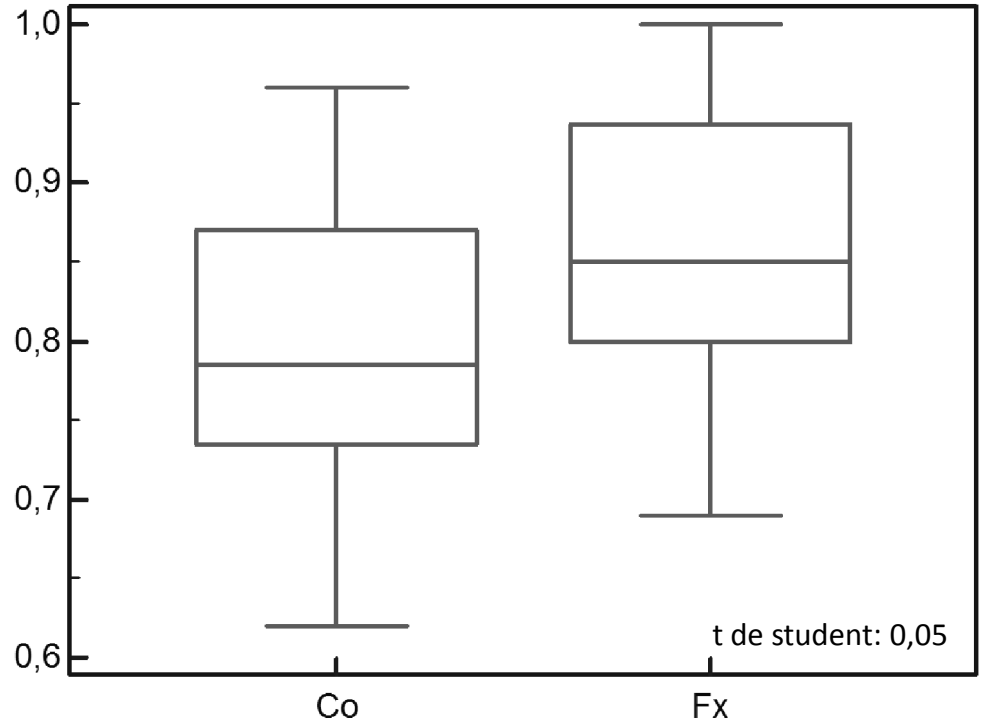
- Biologie à l'inclusion dans l'étude
- Fracture sur chute dans les 3 ans qui ont suivi
- Cystatine < 1,75 mg/L

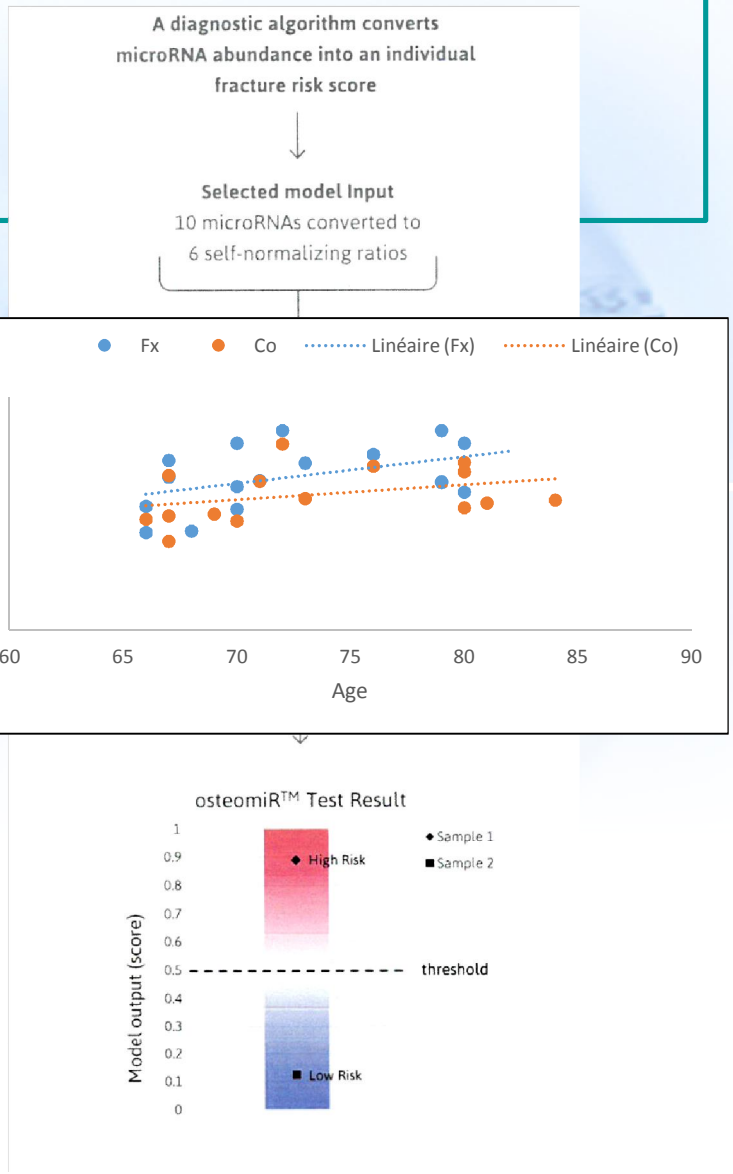
	Co (n= 16)	Fx (n= 17)
Demographics		
Sexe (F/M)	14/2	16/1
Age (years)	72,5 ± 5,2	73,8 ± 6,0
Cystatin C (mg/L)	1,1 ± 0,11	1,1 ± 0,18
Bone and muscle status		
Number of fractures	0	1,3 ± 0,6
Osteoporosis	1	1
Osteopenia	12	12
Sarcopenia	2	2

Etude de 19 biomarqueurs osseux

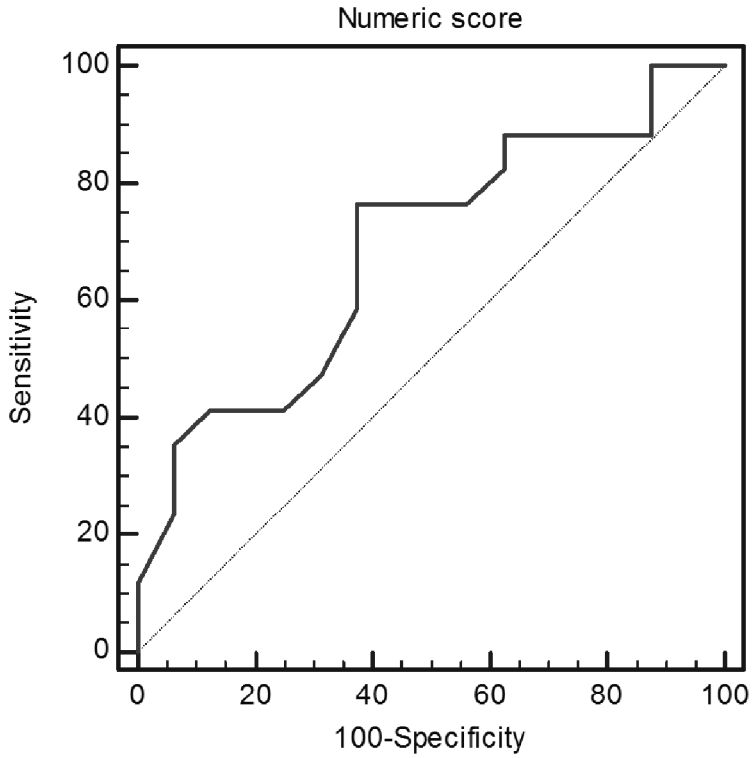


OsteomiR score





Spécifications de la méthode

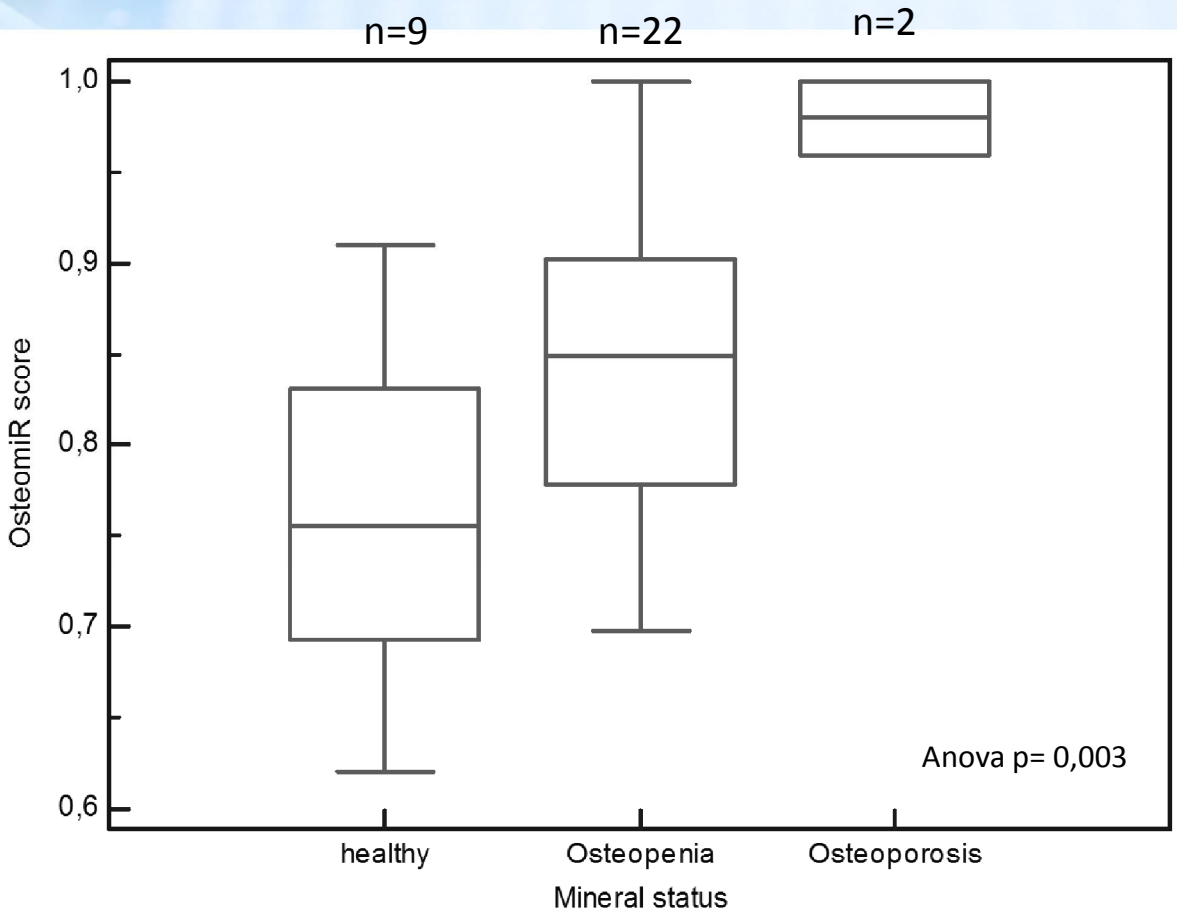


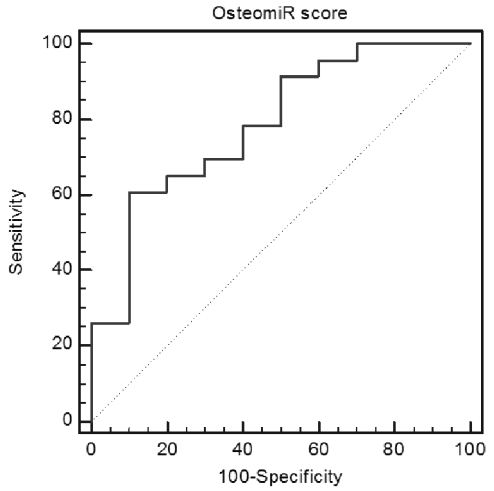
	Fractures	Controls
osteomiR positive	13	6
osteomiR negative	4	10

Sensitivity	76%
Specificity	63%
PPV	68%
NPV	71%

Test de Fisher: 0,008

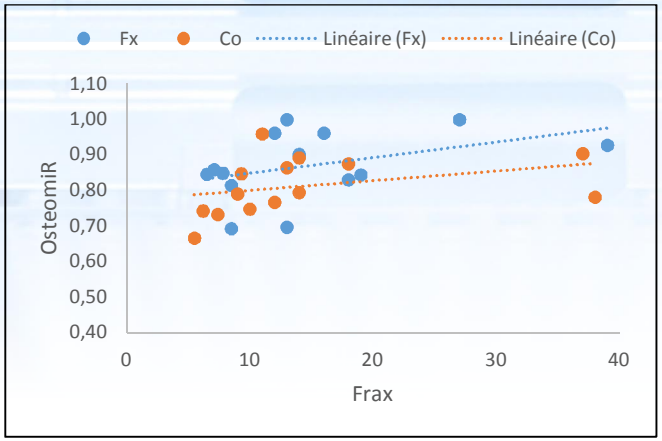
OsteomiR score





Sensitivity	60%
Specificity	90%

Comparaison avec le Frax



OsteomiR score

	Fractures	Controls
osteomiR positive	13	6
osteomiR negative	4	10

Sensitivity	76%
Specificity	63%
PPV	68%
NPV	71%

Frax score

	Fractures	Controls
Frax positive	4	3
Frax negative	11	17

Sensitivity	27%
Specificity	85%
PPV	57%
NPV	61%

Conclusions et perspectives

Tester le score OsteomiR sur une population ostéoporotique

Faisabilité de mise en routine?

- Approbation du test par l'UE
- Coût
- Intérêt médicale
- QCI, standardisation

miRNA, We are almost there!

Remerciements

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