UPDATE ON THE FNIH OSTEOARTHRITIS BIOMARKERS CONSORTIUM PROJECT

THURSDAY, APRIL 30, 2015

9:00 AM – 12:00 PM

Welcome and Opening Remarks

David J. Hunter, MBBS, PhD

University of Sydney

Virginia Byers Kraus, MD, PhD

Duke University Medical Center

OARSI World Congress Workshop

AGENDA

9:00 AM – 9:10 AM	**Welcome and Opening Remarks** *David J. Hunter (USydney), Virginia Byers Kraus (Duke)*
9:10 AM – 9:30 AM	**Study Design and Case/Control Selection** *Michael C. Nevitt (UCSF)*
9:30 AM – 10:45 AM	**Imaging Biomarkers** *David J. Hunter (USydney), Jamie E. Collins (BWH), Virginia Byers Kraus (Duke)*
10:45 AM – 11:45 AM	**Serum & Urine Biomarkers** *Virginia Byers Kraus (Duke), Elena Losina (BWH)*
11:45 AM – 12:00 PM	**Next Steps and Closing Remarks** *David J. Hunter (USydney); Virginia Byers Kraus (Duke)*
12:00 PM	**Adjournment**

Video recording of this workshop will be available in late May

www.biomarkersconsortium.org

Osteoarthritis (OA) Biomarkers Project

2½ year, $3.014M study; nested case-cohort (200 cases & 400 controls)

- ***Contributions***

  Abbott Labs; Amgen; Arthritis Foundation; Bioiberica S.A.; DePuy Mitek; Flexion Therapeutics; GlaxoSmithKline; Merck Serono; Rottapharm Madaus; Sanofi

- ***Principal Investigators:***

  - David J. Hunter, MD, PhD, University of Sydney
  - Virginia Byers Kraus, MD, PhD, Duke University

- ***Specific aims:***

  - To examine the relationship between putative efficacy of intervention markers (biochemical markers, imaging features on x-ray and MRI and their progression) and clinically relevant outcome over a 4-year follow-up period

  - To identify the most responsive marker(s) of OA progression

  - To develop a risk score based on baseline values of several biomarkers including JSN, BTI/FSA, knee alignment, quantitative and semi-q-MRI measures and biochemical biomarkers that would determine those who progress rapidly to case status

Image Analysis

	Imaging Biomarker	Analytic Group	Parameter(s) Measured
Radiography			
	Minimum joint space width (JSW) & joint space area (JSA) and bone trabecular integrity (BTI) by fractal signature analysis (FSA)	Duke Image Analysis Lab (DIAL)	Medial and lateral & minimum JSW and JSA; medial & lateral BTI/FSA
MRI			
	Quantitative cartilage morphometry	Chondrometrics	Cartilage volume, thickness, denuded surface area
	Quantitative bone morphometry	Qmetrics	Bone area, bone curvature, bone/cartilage interface signal contrast
	Quantitative bone morphometry	Imorphics	Area of bone covered by cartilage (tAB) & volume of osteophytes
	Semi-quantitative whole joint scoring	Boston Image Core Lab (BICL)	Assessment of the joint organ morphology using the MRI OA Knee Score (MOAKS) system
	Quantitative cartilage and meniscus morphometry	Biomediq	Cartilage and meniscus volume

Panel of OA-Related Biomarkers

Biomarker	Process (preliminary)	BIPEDS Classifications	Surrogacy Based on Human Clinical Trials (preliminary)	ELISA assay type
urinary CTX-II	type II collagen degradation	Knee: BPED Hip: BPD	characterization: changed significantly in 3 pharmacologic trials that met primary clinical endpoints (Christgau 2004, Gineyts 2004, Manicourt 2006)	competitive-inhibition
serum COMP	cartilage degeneration	Knee: BPD Hip: BPD	exploration: not used to date in pharmacologic trial	competitive-inhibition & sandwich
serum HA	osteophyte burden, synovitis	Knee: BPED Hip: P	demonstration: changed significantly in one pharmacologic trial that met primary clinical endpoints (Manicourt 2006)	sandwich protein binding assay
serum and urine C1,2C	Types I and II collagen degradation	Knee: D(u) Hip: none	exploration: nonsignificant change in one pharmacologic trial that met primary clinical endpoint (Mazzuca 2006)	competitive-inhibition
serum and urine C2C	type II collagen degradation	Knee: E(s), D(u) Hip: B(s)	demonstration: nonsignificant change in one pharmacologic trial that met primary clinical endpoint (Mazzuca 2006)	competitive-inhibition
serum and urine Coll2-1NO2	type II collagen degradation	Knee: D(s),B(u),P(u) Hip: D(s)	exploration: not used to date in pharmacologic trial	competitive-inhibition
serum CPII	type II collagen synthesis	Knee: D(s) Hip: B(s)	exploration: nonsignificant change in one pharmacologic trial that met primary clinical endpoint (Mazzuca 2006)	competitive-inhibition
Serum PIIANP	Type II collagen synthesis	Knee: BPD Hip: none	exploration: not used to date in pharmacologic trial	competitive-inhibition
urine/serum NTX-1	bone resorption	Knee: P(u),E(u) Hip: P(s)	demonstration: changed significantly in one pharmacologic trial that met primary clinical (WOMAC) endpoint (Spector 2005)	competitive-inhibition
Urine CTXI alpha and beta/serum CTX-1	bone resorption	Knee: B(u), D(s/u), P(u) Hip: none	exploration: not used to date in pharmacologic trial	competitive-inhibition
serum CS846	cartilage aggrecan synthesis/turnover	Knee: P Hip: none	exploration: nonsignificant change in one pharmacologic trial that met primary clinical endpoint (Mazzuca 2006) but changed associated with concurrent JSN	competitive-inhibition
serum MMP-3	protease involved with joint tissue degradation	Knee: E Hip: none	characterization: changed significantly in two pharmacologic trials that met primary clinical endpoints (Lohmander 2005, Manicourt 2006)	sandwich for total MMP-3 assay

OA Biomarkers Project Team

- **Neil Bodick, MD, PhD** (Flexion Therapeutics)
- **Jamie Collins, PhD** (Brigham and Woman's Hospital)
- **Sahar Dawisha, MD** (FDA/CDRH)
- **Klaus Flechsenhar, MD** (Sanofi)
- **Fiona Germaschewski (GSK)**
- **Ali Guermazi, MD** (Boston University Medical Center)
- **Yves Henrotin, PhD** (Univ. of Liege)
- **Steve Hoffmann, MS** (FNIH)
- **David J. Hunter, MBBS, PhD** (Univ. of Sydney) ⭐
- **Joanne Jordan, MD** (Univ. of North Carolina at Chapel Hill)
- **Jeffrey Katz, MD, MS** (Brigham and Woman's Hospital)
- **Virginia Byers Kraus, MD, PhD** (Duke University) ⭐
- **Kent Kwoh, MD** (Univ. of Arizona)
- **Christoph Ladel, PhD (Merck Serono)**
- **Jonathan Larkin, PhD (GSK)**
- **Gayle Lester, PhD** (NIH/NIAMS)
- **Elena Losina, PhD** (Brigham and Women's Hospital)
- **John Lynch, PhD (**Univ. of Calif, San Fran)
- **Helena Martinez, MSc** (Bioiberica S.A.)
- **Gloria Matthews, PhD** (Genzyme/Sanofi)

- **Janet Maynard, MD, MHS** (FDA/CDER)
- **Charles McCulloch, PhD** Univ. of Calif, San Fran)
- **Michael Nevitt, MD, PhD** (Univ. of Calif, San Fran)
- **Nikolay Nikolov, MD** (FDA/CDER)
- **Amanda Niskar, DrPH, MPH, BSN** (Arthritis Foundation)
- **Bill Parrish, PhD** (DePuy Mitek)
- **Stefano Persiani, PhD** (Rottapharm Madaus)
- **Frank Roemer, MD** (Klinikum Augsburg)
- **Lucio Rovati, MD** (Rottapharm Madaus)
- **Roger Sabata** (Bioiberica S.A.)
- **Linda Sandell, PhD** (Washington University, St.L)
- **Csaba Siffel, MD, PhD** (Arthritis Foundation)
- **Valorie Thompson, PhD** (OARSI)
- **Wayne Tsuji, MD** (Amgen)
- **Josep Vergés, MD, PhD** (Bioiberica S.A.)
- **Susanne Wang, MD, PhD** (AbbVie)
- **Yingtao Zhou, MS** (Arthritis Foundation)

⭐ **Co-Chairs**

Statistical Analysis Center

- **Analytic Group serves as an independent statistical center**
  - Dr. Elena Losina, PhD (Center Director)
  - Dr. Jamie Collins, PhD (Principal Statistician)
  - Dr. Jeffrey N. Katz, MD, MSc (Clinical Epidemiologist)

  BWH **BRIGHAM AND WOMEN'S HOSPITAL**

- **Statistical Analysis Plan (SAP) Development:**
  1. Conceptual SAP based on original OA Biomarkers Project Plan
  2. Specific biomarker SAPs incorporate:
     - Draft analysis plans proposed through collaborative efforts of Statistical Center and Project Team Core Group
     - Vendors provide assay kit information or prepare brief presentation(s) of methodologies and analytical systems tailored for specific sets of biomarkers
  3. Following consensus Core Group approval, final SAP shared with the entire OA Biomarkers Project Team
     - Monthly meetings to monitor analytical progress and review results

ALL STATISTICAL ANALYSES ARE PERFORMED INDEPENDENTLY FROM VENDORS

Acknowledgements

Scientific and Financial Support

abbvie · AMGEN · Arthritis Foundation · BIOIBERICA · DePuy Synthes · flexion Advancing Pain Management

gsk · Merck KGaA · OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL · ROTTAPHARM | MADAUS · SANOFI · stryker

NIH Osteoarthritis Initiative

NIH National Institutes of Health — Turning Discovery Into Health

OAI osteoarthritis initiative — a knee health study

NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases · NOVARTIS · MERCK Be well

NIH National Institute on Aging · gsk · Pfizer · FNIH Foundation for the National Institutes of Health

In-Kind Project Support

artialis Passion for joint health · IBEX · QUIDEL · DIAL · **Pivotal OAI MRI Analysis (POMA)**

Alere · BioVendor Research and Diagnostic Products · ids immunodiagnosticsystems · LabCorp Laboratory Corporation of America

Study Design and Case Control Selection
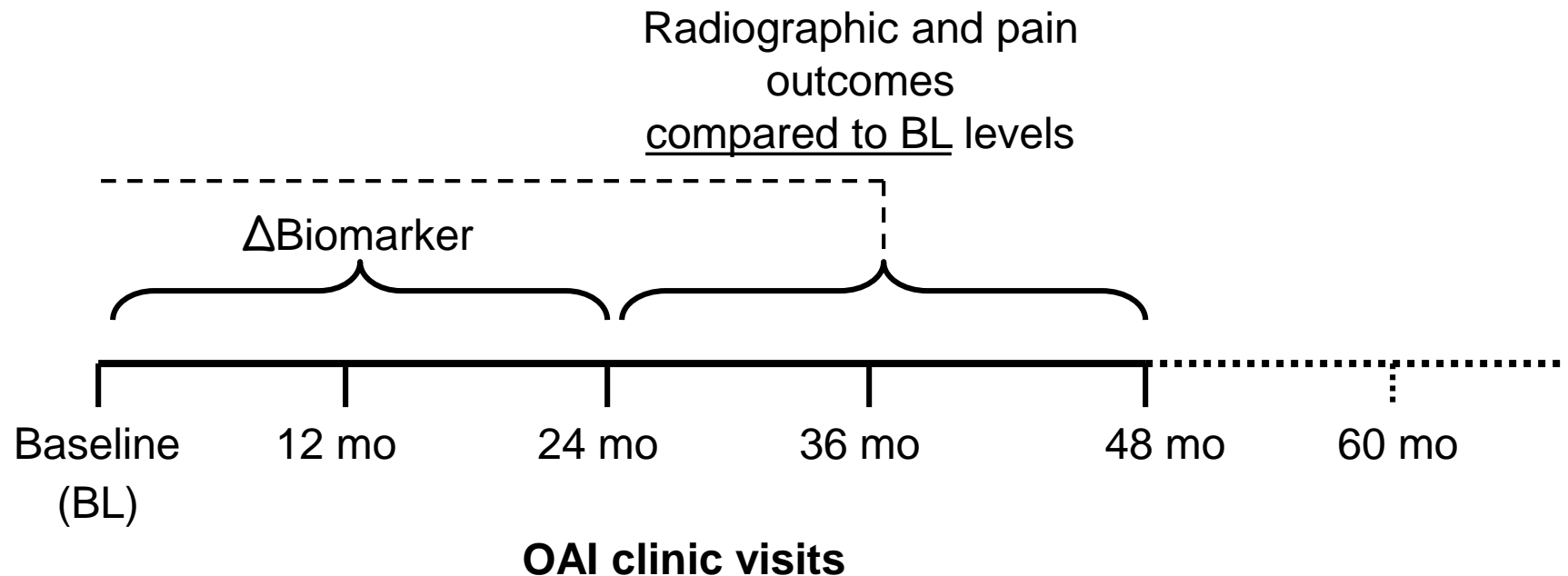
Michael C. Nevitt

Overview

- Using data from the first four years of the Osteoarthritis Initiative (OAI), perform a <u>nested case-control</u> study to determine the predictive and concurrent validity and responsiveness of Δstructural and Δbiochemical biomarkers for radiographic and pain progression in knees with mild to moderate T-F OA.

- OAI is a longitudinal cohort study of 4,796 men and women ages 45–79 with, and at high risk for, knee OA that contains a repository of serial knee images and blood and urine biospecimens and extensive longitudinal clinical profile data.

Biomarkers and Outcomes from OAI Study Visits

- Biomarkers (imaging, biochemical) assessed using data from BL, 12 mo and 24 mo visits

- Radiographic and pain progression outcomes assessed using data from 24, 36, 48 mo (and for pain, 60 mo) compared to BL

Radiographic and pain
outcomes
compared to BL levels

ΔBiomarker

Baseline | 12 mo | 24 mo | 36 mo | 48 mo | 60 mo
(BL)

OAI clinic visits

Radiographic (X-ray) Progression

- Radiographic progression = loss of medial minJSW ≥ 0.70 mm from BL to 24, 36 or 48 mo

- Annual knee radiographs using PA "fixed-flexion" protocol
  - minJSW in medial TF compartment assessed with automated software (Duryea, 2013, Osteo Cart)

- Study-specific smallest detectable change determined from serial OAI images
  - 90 reference cohort knees, KLG = 0 and no pain BL to 24 mo
  - ΔMinJSW from BL to 12 mo (no real change expected)

$\triangle$ **minJSW (mm) BL-12 mo**

Mean -0.083
Below mean
SD 0.47
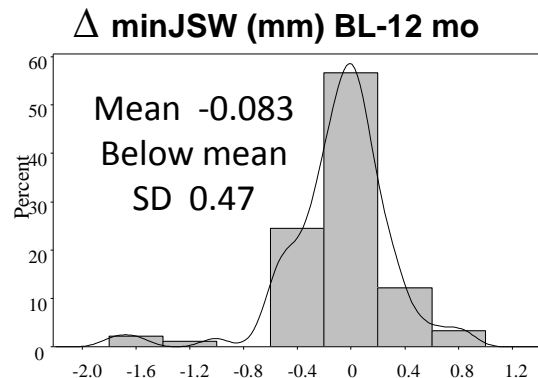
	Probability that change <X is due to measurement error				
	2.5%	**5%**	**10%**	**25%**	**50%**
ΔminJSW BL-12m (mm)	**< -1.02**	**< -0.87**	**< -0.70**	**< -0.49**	**< 0.08**

Knee Pain Progression

- Knee pain progression = <u>persistent</u> increase vs. baseline in total WOMAC pain score above MCID (≥9 pts on 0-100 scale)
  - Persistent = increase at ≥2 timepoints from 24 to 60 mo

MCID references

1. Angst F, et al. Smallest detectable and minimal clinically important differences of rehabilitation intervention with their implications for required sample sizes using WOMAC and SF-36 quality of life measurement instruments in patients with osteoarthritis of the lower extremities. *Arthritis Rheum* 2001; 45: 384-391.
2. Angst F, et al. Minimal clinically important rehabilitation effects in patients with osteoarthritis of the lower extremities. *J Rheumatol* 2002; 29: 131-138.
3. Tubach F. et al. Minimum clinically important improvement and patient acceptable symptom state in pain and function in rheumatoid arthritis, ankylosing spondylitis, chronic back pain, hand osteoarthritis, and hip and knee osteoarthritis: Results from a prospective multinational study. *Arthritis Care Res* 2012; 64:1699-707.

Case – Control Knees: Definitions

Primary <u>case</u> definition = knee having *<u>both</u>* radiographic (X-ray) and pain progression (**Progressor**)

Primary <u>control</u> definition = knee eligible for X-ray and pain progression that *<u>does not reach criteria for both</u>* endpoints

- Knee with X-ray progression but *<u>not</u>* pain progression (**X-ray only progressor<u>)</u>**

- Knee with pain progression but *<u>not</u>* X-ray progression (**Pain only progressor)**

- Knee with *<u>neither</u>* X-ray nor pain progression (**<u>Non-progressor</u>**)

Sampling Design

- **Eligible knees**
  - KLG 1-3 at BL
  - JSW and pain data at BL-48 mo
  - Knee MRI, serum and urine at BL and 24 mo

- **Eligible knees classified into four outcome groups, cases and controls**

Control

Case

1. X-ray and Pain Progressor

2. X-ray Only progressor

4. No X-ray or Pain progression (Non-progressor)

3. Pain Only progressor

Knees/subjects eligible for both radiographic (X-ray) and pain progression outcomes
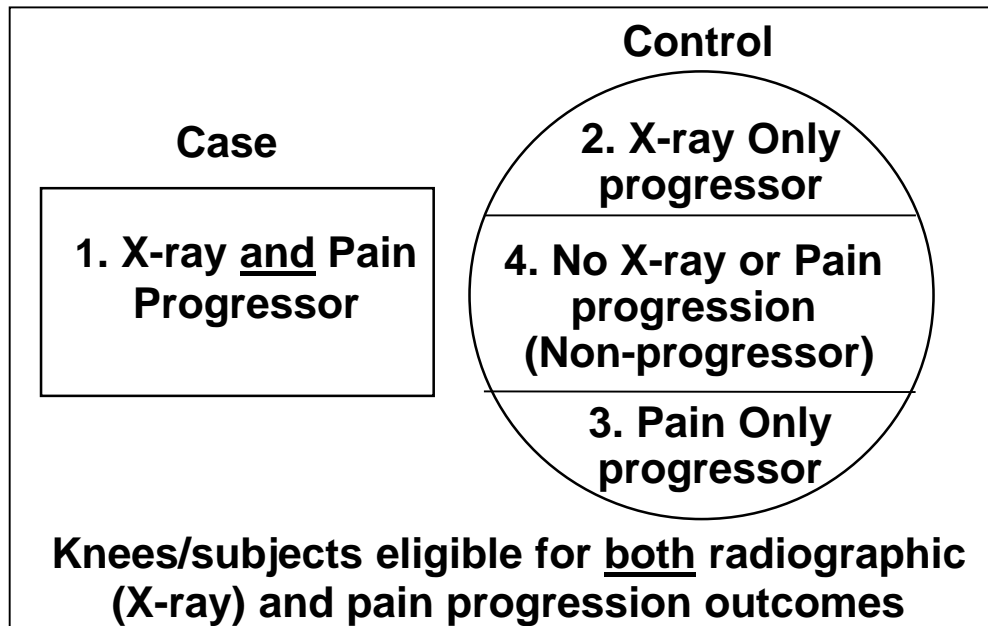
Pre-specified group sizes

1. **N = 200**
2. N = 100
3. N = 100
4. N = 200

400

Sampling Design (Cont.)

- Outcomes and imaging biomarkers are knee-specific measurements
  - One index /study knee selected per subject

- Exclusions: knees
  - Unable to progress: minJSW < 1.0mm or WOMAC pain >91 (0-100)
  - MRI artifacts likely to affect image analysis
  - Poor radiograph quality or positioning (poor or variable tibial rim alignment)
  - Controls: BL lateral JSN and/or lateral radiographic progression

- Exclusions: subjects (biochemical markers are subject-level measurements; take both knees into account)
  - Either knee meets primary case definition by 12 mo
  - TKR or THR up to 24 mo (effects on biochemical markers)

Sampling Design (Cont.)

■ Exclusions: subjects (Cont.)

- If both knees have same outcome: one randomly selected

- If outcomes in a subject's knees are discordant

  ○ E.g. one knee is a pain only progressor and the other is a X-ray only progressor

...then subject excluded

Knee (Subject) Selection Flow Diagram

BL KLG 1-3 and biomarker data
3,481 (2,246)

Exclusions or don't meet criteria for any outcome group based on both knees

1,908 (1,519)

252 (234)	444 (377)	269 (236)	943 (672)	Eligible subjects (knees)
*	* **	* **	* **	
194 (194)	103 (103)	103 (103)	200 (200)	Selected sample

X-ray and Pain progressor (Case)   **X-ray only progressor**   **Pain only progressor**   **Neither X-ray nor pain progression (Non-progressor)**

* MRI artifact, knee positioning exclusions
** **Frequency matching** for 15 KLG by BMI strata, with random selection

Frequency Matching

- Frequency matching of control BMI and KLG to radiographic and pain progressors (composite cases)
  - Cases: greater % in high BMI groups and KLG 3

- Goal: better balance among groups for covariate adjustment

- 15 BMI by KLG strata
  - BMI <25; 25 to <27.5; 27.5 to <30; 30 to <35; ≥35
  - KLG 1; KLG 2; KLG 3

- Difficult strata to match e.g.
  - Pain only progressor and nonprogressors with KLG = 3

Frequency Matching: Baseline BMI

BL BMI, by outcome group

Legend:
- BMI <25
- BMI 25-30
- BMI 30-35
- BMI >35
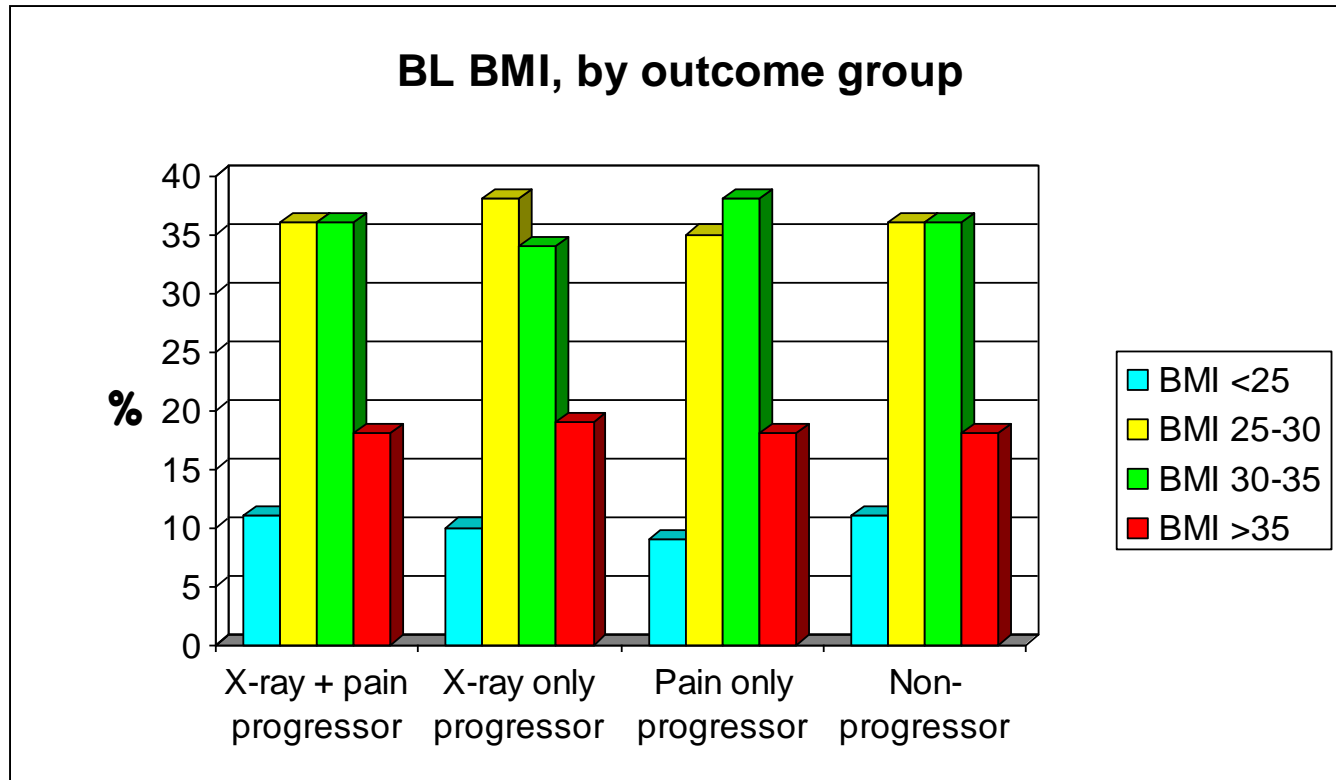
- Good balance achieved for BMI groups

Frequency Matching: Baseline KL grade

Index knee BL KLG, by outcome group

Before matching

Legend: KLG 1, KLG 2, KLG 3
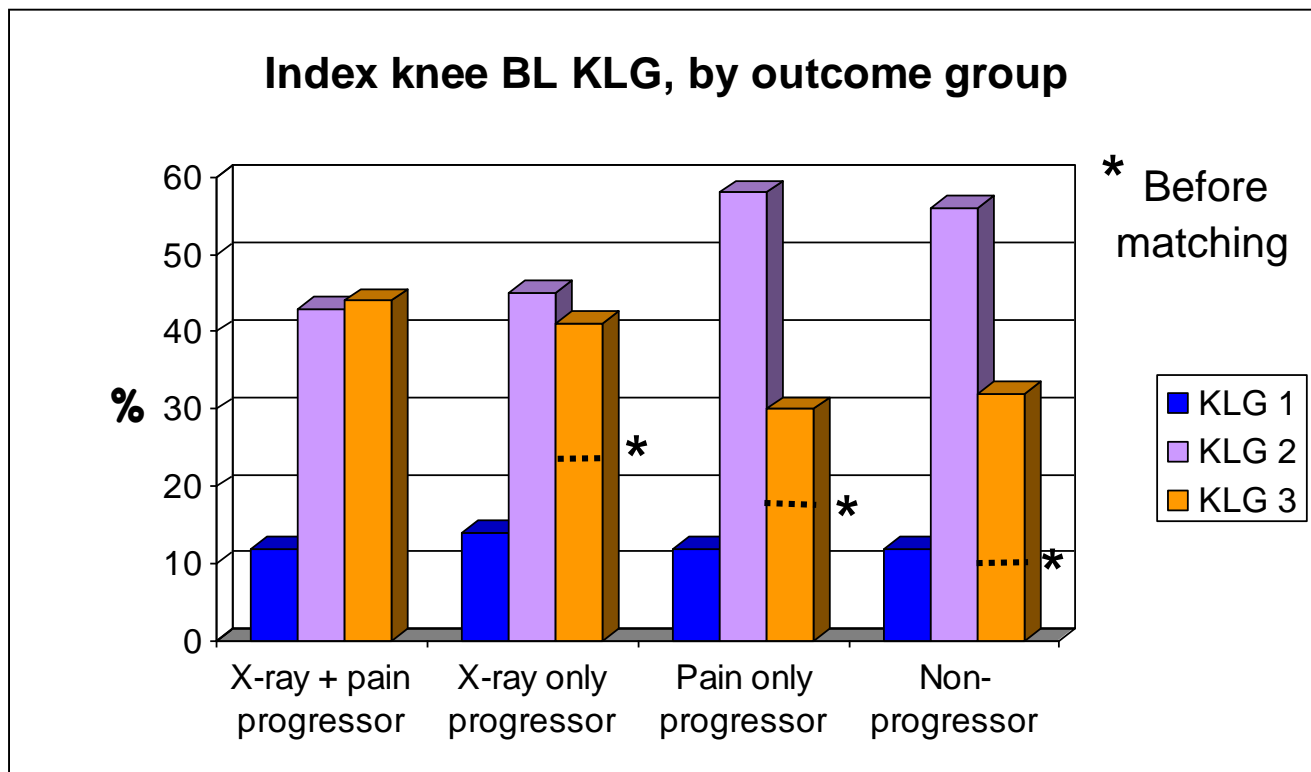
- Improved balance in baseline KLG
- Pain only progressors and non-progressors, fewer KLG 3 knees
- KLG a covariate in analyses

Baseline Subject Characteristics

	X-ray + pain progressor	X-ray only progressor	Pain only progressor	Non-progressor	P-value
Age (SD)	62.0 (8.8)	63.1 (8.3)	58.0 (8.7)	61.5 (9.1)	0.011
Male	43%	55%	35%	35%	0.003
Nonwhite	20%	12%	28%	22%	0.029
Pain meds for knees most days, past year	32%	21%	36%	28%	0.088
Glucosamine most days, past mo	33%	33%	29%	26%	0.290

Baseline Index Knee Characteristics

	X-ray + pain progressor	X-ray only progressor	Pain only progressor	Non-progressor	P-value
Hx of knee injury*	35%	40%	37%	33%	0.874
WOMAC knee pain (SD) (0-100)	20 (26)	33 (40)	19 (27)	26 (32)	0.002
Medial JSN gr 2 (vs 0-1)	44%	41%	28%	31%	0.009

* self-report of knee injury causing difficulty walking for >= 2 days

Limitations

- Partial overlap of ΔBiomarker and progression outcome assessment periods combines predictive and concurrent validity
  - Analyze <u>early</u> (BL-24mo) vs. <u>late</u> (BL -36/48 mo) progressors
  - Analyze ΔBiomarkers from BL to 12 mo as predictors

- Other definitions of pain progression may give different results

Strengths

- Clinically relevant outcomes (structure + pain) and assessment intervals
  - ΔBiomarkers over 24 mo and progression outcomes over 48 mo

- Large sample size

- Can compare biomarker performance for progression outcomes defined in several ways

- Publicly available data
  - Link biomarker and outcome data of study subjects and knees to all other OAI variables

Pre-Specified Analyses

Primary analysis

Case: knees with <u>both</u> X-ray and pain progression (n=194) vs.

Control: knees that <u>did not have both</u> X-ray and pain progression (n=406)

Secondary analyses

Method 1
Comparison of 4-level outcome groups

X-ray + pain progressor (n=194)	X-ray only progressor (n=103)	Pain only progressor (n=103)	Non-progressors (n=200)

Method 2
All progressors (n=400) vs. non-progressors (n=200)

Method 3
X-ray progressor (n=297) vs. X-ray non-progressors (n=303)

Method 4
Pain progressor (n=297) vs. Pain non-progressors (n=303)

FNIH OA Biomarkers Consortium Project -
Data Access on the OAI Database

http://oai.epi-ucsf.org/datarelease/

Option 1:

■ Click on the Data & Documentation tab at the top of the page

■ Once on the Data & Documentation page, click on the Summary of Data, Biospecimens and Images Currently Available link

■ Scroll down to OA Biomarkers Consortium FNIH Project and click on that link

• This takes you to the dedicated FNIH page on OAI Online

○ *Requires you to re-enter logon credentials and then redirects to data pages*

Option 2:

■ Alternatively, you can just bookmark/click on this link:

• https://www.oai.ucsf.edu/datarelease/FNIH.asp

○ This takes you to the OAIOnline logon page then immediately redirected to the FNIH page.

Imaging Biomarkers

David J. Hunter

Jamie E. Collins

Virginia Byers Kraus

Image Analysis

	Imaging Biomarker	Analytic Group	Parameter(s) Measured
Radiography			
	Minimum joint space width (JSW) & joint space area (JSA) and bone trabecular integrity (BTI) by fractal signature analysis (FSA)	Duke Image Analysis Lab (DIAL)	Medial and lateral & minimum JSW and JSA; medial & lateral BTI/FSA
MRI			
	Quantitative cartilage morphometry	Chondrometrics	Cartilage volume, thickness, denuded surface area
	Quantitative bone morphometry	Qmetrics	Bone area, bone curvature, bone/cartilage interface signal contrast
	Quantitative bone morphometry	Imorphics	Area of bone covered by cartilage (tAB) & volume of osteophytes
	Semi-quantitative whole joint scoring	Boston Image Core Lab (BICL)	Assessment of the joint organ morphology using the MRI OA Knee Score (MOAKS) system
	Quantitative cartilage and meniscus morphometry	Biomediq	Cartilage and meniscus volume

Analytic Overview

- **Descriptive Statistics**
 - n (%) for categorical variables, mean (SD) for continuous variables

- **Logistic Regression for primary comparison of cases (x-ray + pain progressors) vs. controls (do not have x-ray + pain progression)**
  - Multivariable models adjusted for sex, race, and baseline JSW, KL, age, WOMAC Pain
  - For continuous variables, ORs presented as the odds of being a case for each 1 SD increase in biomarker

- **Multinomial Logistic Regression for secondary comparison of 4-level outcome status, with non-progressors (subjects not progressing in x-ray or pain) used as reference group**

Quantitative Cartilage Morphometry

- Sagittal 3D DESSwe images (3T) @ baseline, 12 and 24 months

- analyzed by 12 readers (Chondrometrics GmbH)
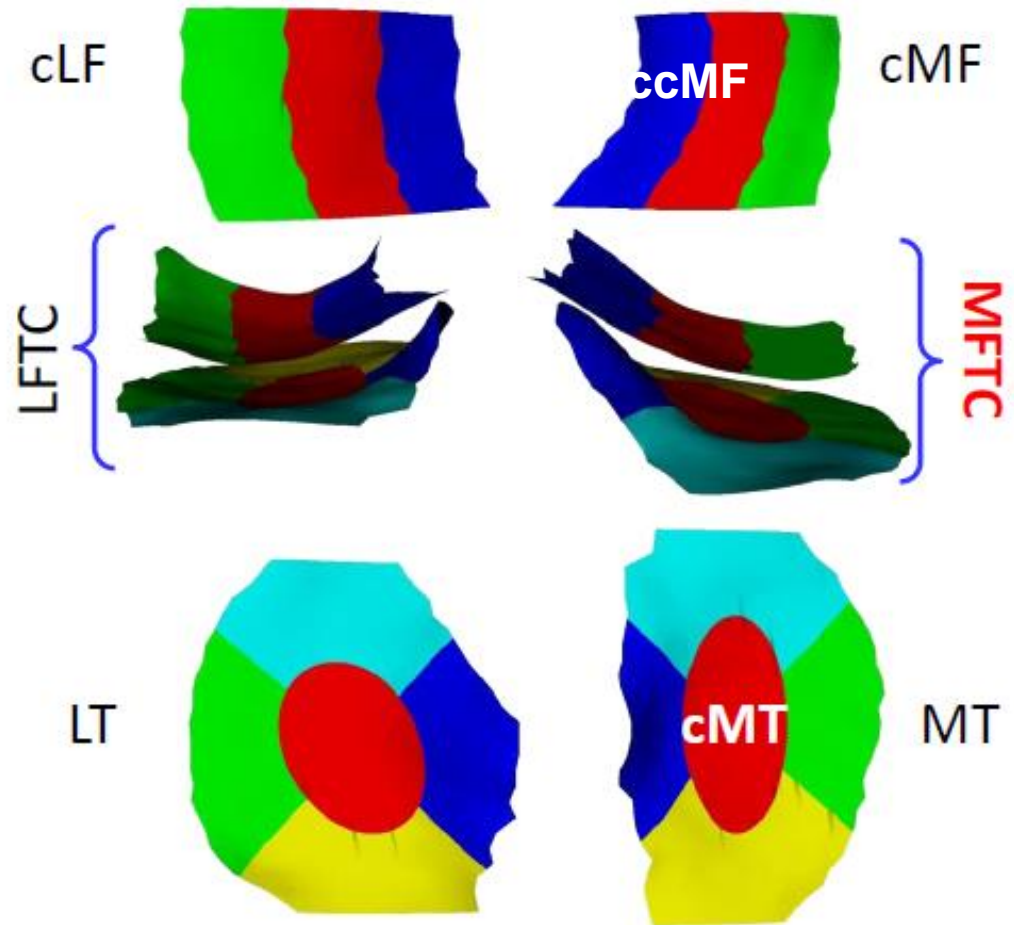
Cartilage Thickness Analysis: Predictors

- **24 month change in mean cartilage thickness**
  - Central medial femorotibial compartment (cMFTC)
  - Central medial tibia (cMT)
  - Central medial weight-bearing femur (ccMF)
  - Total medial femorotibial compartment (MFTC)

- **Location-independent measures**
  - Ordered Values
    - Cartilage change in each of the 16 subregions is computed, and then the regions are sorted by the amount of change
      - OV1: the smallest value/most negative change (the subregion with the greatest rate of cartilage thinning)
      - OV16: the largest value (the subregion with the least thinning or greatest thickening)
  - Cartilage thinning score: sum of all negative cartilage thickness changes
  - Cartilage thickening score: sum of all positive cartilage thickness changes

Cartilage Thickness: Primary Analysis Results

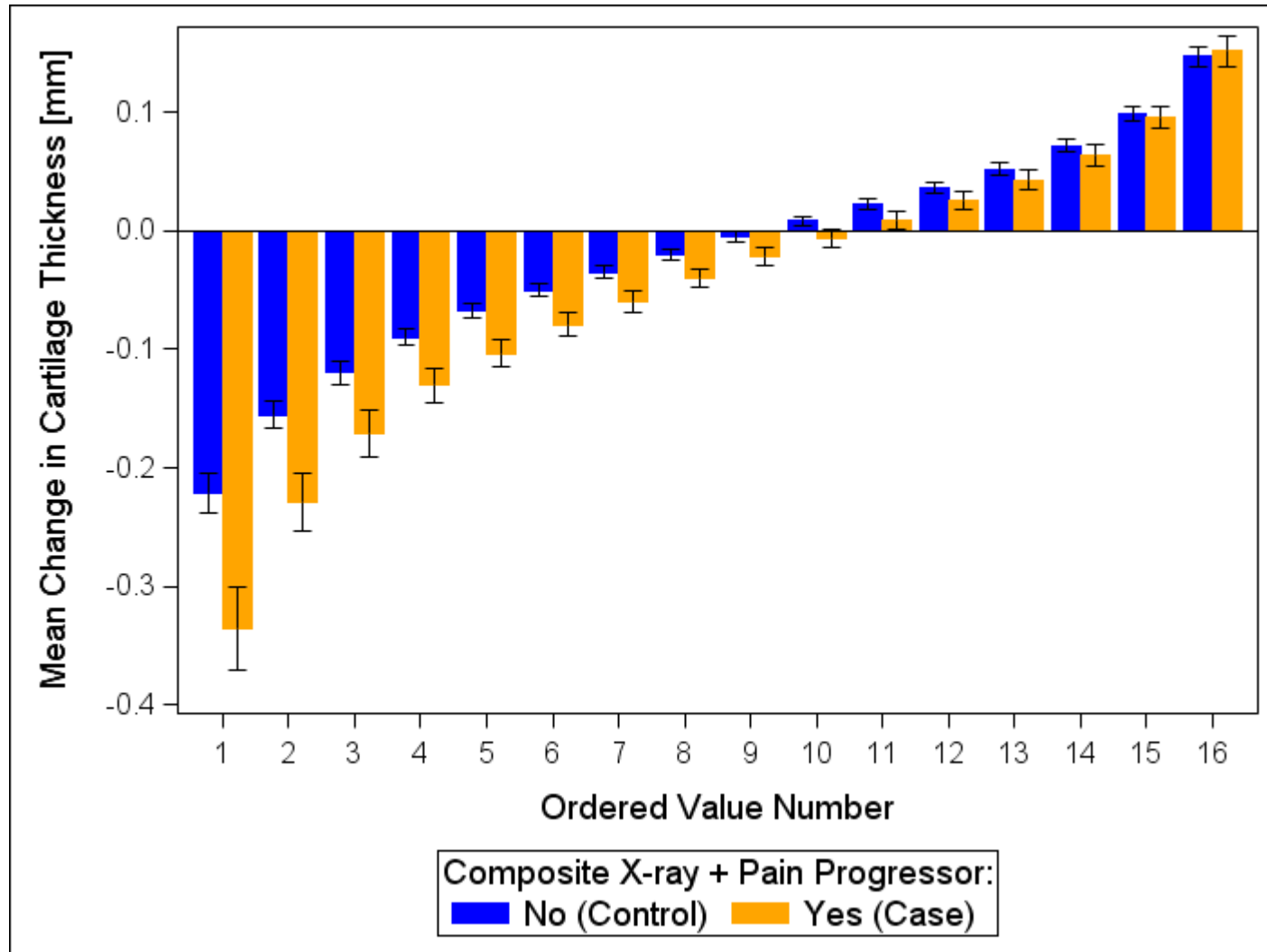## Change in Cartilage Thickness [mm] over 24 Months by Case-Control Status

Predictor	Case (mean (sd))	Control (mean (sd))	OR*	p-value*
Central medial femorotibial compartment (cMFTC)	-0.32 (0.40)	-0.12 (0.28)	1.9 (1.6, 2.3)	<.0001
Central medial tibia (cMT)	-0.12 (0.19)	-0.05 (0.13)	1.6 (1.3, 1.9)	<.0001
Central medial weight-bearing femur (ccMF)	-0.21 (0.28)	-0.08 (0.20)	1.8 (1.5, 2.2)	<.0001
Total medial femorotibial compartment (MFTC)	-0.18 (0.24)	-0.06 (0.18)	1.9 (1.6, 2.4)	<.0001
Cartilage thinning score	-1.26 (0.93)	-0.84 (0.65)	1.3 (1.1, 1.5)	0.0085
Cartilage thickening score	0.48 (0.37)	0.51 (0.38)	1.0 (0.8, 1.2)	0.9765

*Adjusted for BL JSW, BL WOMAC Pain, BL age, BL BMI, BL KLG, BL pain medication use, sex, and race

Odds Ratio = Odds of being a composite x-ray and pain progressor vs. not having both x-ray and pain progression for each 1 SD increase in biomarker
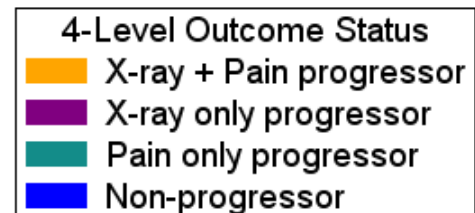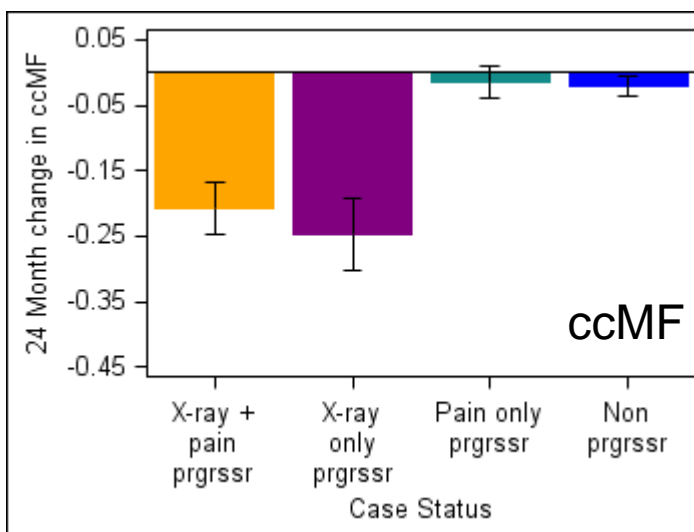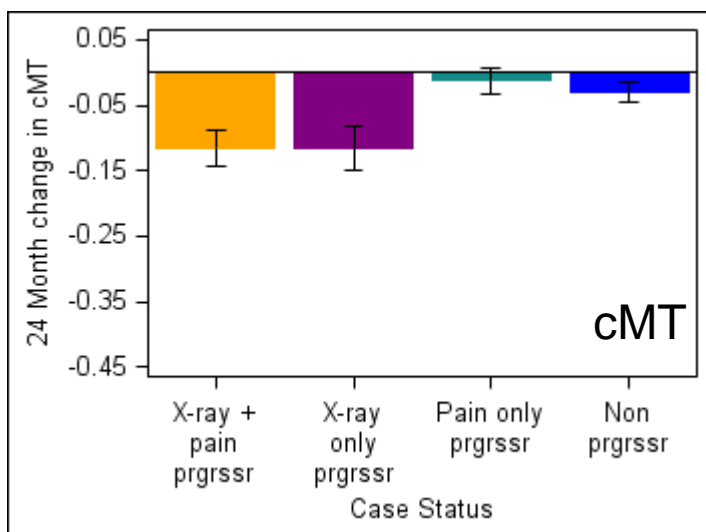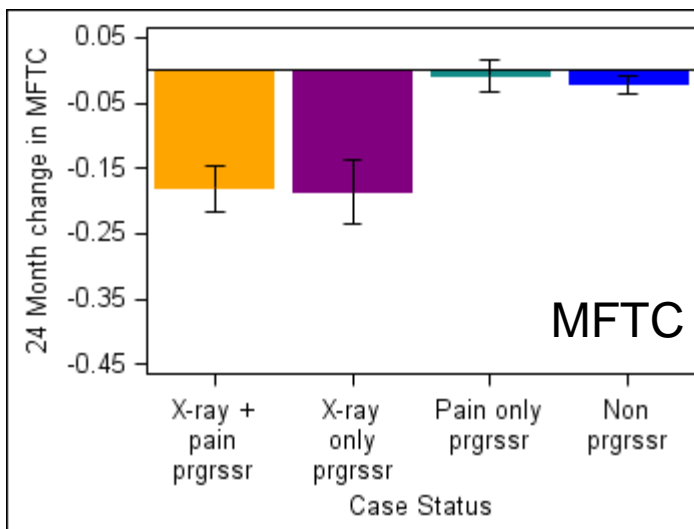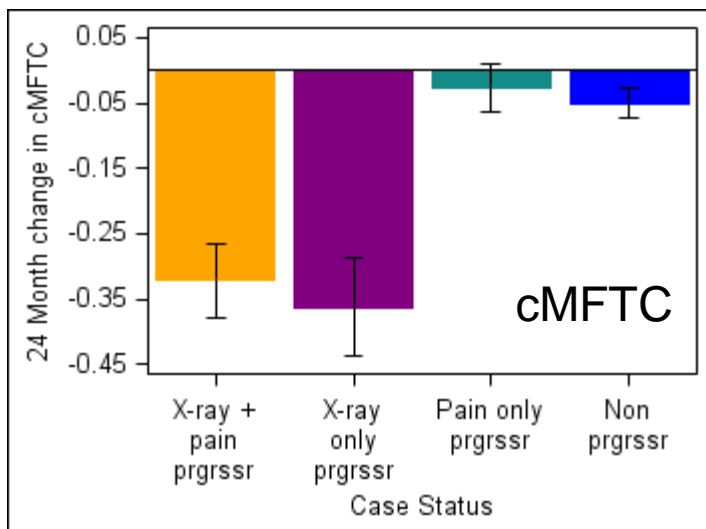
Cartilage Thickness: Primary Analysis Results

**24 Month Ordered Values for Change in Cartilage Thickness by Case-Control Status with 95% Confidence Intervals**

Cartilage Thickness: Secondary Analysis Results

Change in Cartilage Thickness [mm] over 24 Months by Case Status (95% CI)

Quantitative Subchondral Bone Plate Morphometry

- Automated segmentations[1] were reconstructed as 3D surfaces

- Measurements were taken at thousands of individual points across the surface

- Statistical Descriptors (Mean, Std. Dev., Low and High tails) were provided for Medial / Lateral Central Femur and Tibia
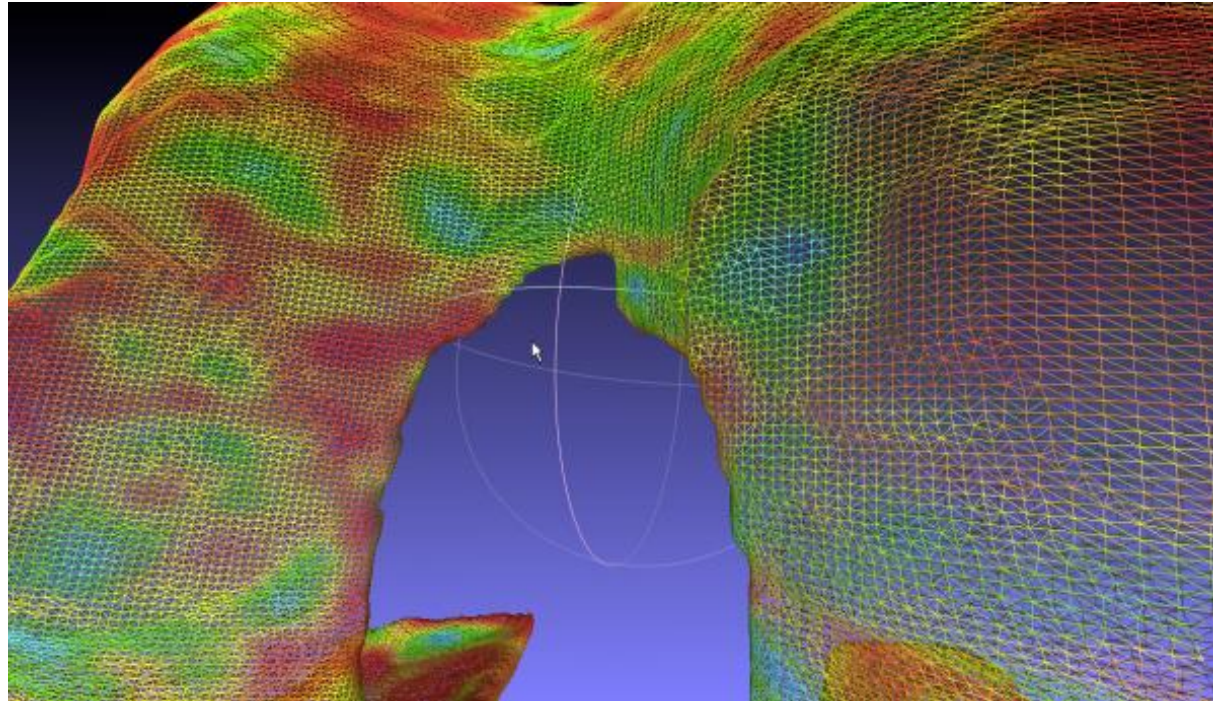
1: Tamez-Peña JG, Farber J, González PC, Schreyer E, Schneider E, Totterman S. Unsupervised segmentation and quantification of anatomical knee features: data from the Osteoarthritis Initiative. IEEE Trans Biomed Eng. 2012 Apr;59(4):1177-86. doi: 10.1109/TBME.2012.2186612. Epub 2012 Feb 3. PubMed PMID: 22318477.

Subchondral Bone Plate Analysis: Predictors

■ Subchondral Bone Plate (SBP) Area

- Central Medial Femur
- Central Lateral Femur
- Medial Tibia
- Lateral Tibia

■ Subchondral Bone Plate (SBP) Mean Curvature

- Central Medial Femur
- Central Lateral Femur
- Medial Tibia
- Lateral Tibia

SBP Area - Primary Analysis Results

Baseline SBP Area by Case-Control Status

Predictor	Case (mean (sd))	Control (mean (sd))	OR*	p-value*
Central MedFem Area	889.3 (156.4)	891.1 (153.4)	0.83 (0.6, 1.1)	0.1969
Central LatFem Area	808.4 (136.9)	802.0 (139.9)	1.0 (0.7, 1.3)	0.9070
MedTib Area	910.8 (178.0)	911.6 (178.3)	0.82 (0.6, 1.1)	0.1625
LatTib Area	809.6 (158.0)	800.1 (161.8)	1.0 (0.8, 1.4)	0.8528

*Adjusted for BL JSW, BL Pain, BL age, BL BMI, BL KLG, BL pain med use, sex, and race

Odds Ratio = Odds of being a composite x-ray and pain progressor vs. not having both x-ray and pain progression for each 1 SD increase in biomarker

SBP Curvature - Primary Analysis Results

Baseline SBP Curvature by Case-Control Status

Predictor	Case (mean (sd))	Control (mean (sd))	OR*	p-value*
Central MedFem Mean Curvature	0.0274 (0.0064)	0.0290 (0.0059)	0.77 (0.6, 0.9)	0.0101
Central LatFem Mean Curvature	0.0255 (0.0046)	0.0264 (0.0046)	0.81 (0.7, 0.99)	0.0380
MedTib Mean Curvature	-0.0277 (0.0067)	-0.0284 (0.0062)	1.1 (0.9, 1.4)	0.2779
LatTib Mean Curvature	-0.0118 (0.0064)	-0.0117 (0.0064)	1.0 (0.8, 1.2)	0.7391

*Adjusted for BL JSW, BL Pain, BL age, BL BMI, BL KLG, BL pain med use, sex, and race

Odds Ratio = Odds of being a composite x-ray and pain progressor vs. not having both x-ray and pain progression for each 1 SD increase in biomarker

SBP Curvature - Primary Analysis Results

Baseline SBP Curvature by Case-Control Status

Predictor	Case (mean (sd))	Control (mean (sd))	OR*	p-value*
Central MedFem Mean Curvature	0.0274 (0.0064)	0.0290 (0.0059)	0.77 (0.6, 0.9)	0.0101
Central LatFem Mean Curvature	0.0255 (0.0046)	0.0264 (0.0046)	0.81 (0.7, 0.99)	0.0380
MedTib Mean Curvature	-0.0277 (0.0067)	-0.0284 (0.0062)	1.1 (0.9, 1.4)	0.2779
LatTib Mean Curvature	-0.0118 (0.0064)	-0.0117 (0.0064)	1.0 (0.8, 1.2)	0.7391

*Adjusted for BL JSW, BL Pain, BL age, BL BMI, BL KLG, BL pain med use, sex, and race

Odds Ratio = Odds of being a composite x-ray and pain progressor vs. not having both x-ray and pain progression for each 1 SD increase in biomarker

SBP Area - Primary Analysis Results

24 Month Change in SBP Area by Case-Control Status

Predictor	Case (mean (sd))	Control (mean (sd))	OR[*]	p-value[*]
Central MedFem Area	-18.4 (58.0)	-1.9 (35.6)	0.68 (0.6, 0.8)	0.0001
Central LatFem Area	-5.6 (33.4)	-5.9 (29.5)	0.98 (0.8, 1.2)	0.8667
MedTib Area	-5.4 (43.2)	0.9 (35.4)	0.85 (0.7, 1.02)	0.0779
LatTib Area	-3.6 (36.7)	-5.3 (35.3)	1.03 (0.9, 1.2)	0.7698

*Adjusted for BL JSW, BL Pain, BL age, BL BMI, BL KLG, BL pain med use, sex, and race

Odds Ratio = Odds of being a composite x-ray and pain progressor vs. not having both x-ray and pain progression for each 1 SD increase in biomarker

SBP Area - Primary Analysis Results

24 Month Change in SBP Area by Case-Control Status

| Predictor	Case (mean (sd))	Control (mean (sd))	OR*	p-value*
Central MedFem Area	-18.4 (58.0)	-1.9 (35.6)	0.68 (0.6, 0.8)	0.0001
Central LatFem Area	-5.6 (33.4)	-5.9 (29.5)	0.98 (0.8, 1.2)	0.8667
MedTib Area	-5.4 (43.2)	0.9 (35.4)	0.85 (0.7, 1.02)	0.0779
LatTib Area	-3.6 (36.7)	-5.3 (35.3)	1.03 (0.9, 1.2)	0.7698

*Adjusted for BL JSW, BL Pain, BL age, BL BMI, BL KLG, BL pain med use, sex, and race

Odds Ratio = Odds of being a composite x-ray and pain progressor vs. not having both x-ray and pain progression for each 1 SD increase in biomarker

SBP Curvature - Primary Analysis Results

24 Month Change in SBP Curvature by Case-Control Status

Predictor	Case (mean (sd))	Control (mean (sd))	OR*	p-value*
Central MedFem Mean Curvature	-0.0013 (0.0037)	-0.0007 (0.0028)	0.85 (0.7, 1.02)	0.0830
Central LatFem Mean Curvature	-0.0008 (0.0022)	-0.0005 (0.0019)	0.86 (0.7, 1.03)	0.1063
MedTib Mean Curvature	-0.0008 (0.0039)	-0.0002 (0.0032)	0.83 (0.7, 1.00)	0.0456
LatTib Mean Curvature	-0.0002 (0.0029)	-0.0003 (0.0028)	1.01 (0.8, 1.2)	0.9073

*Adjusted for BL JSW, BL Pain, BL age, BL BMI, BL KLG, BL pain med use, sex, and race

Odds Ratio = Odds of being a composite x-ray and pain progressor vs. not having both x-ray and pain progression for each 1 SD increase in biomarker
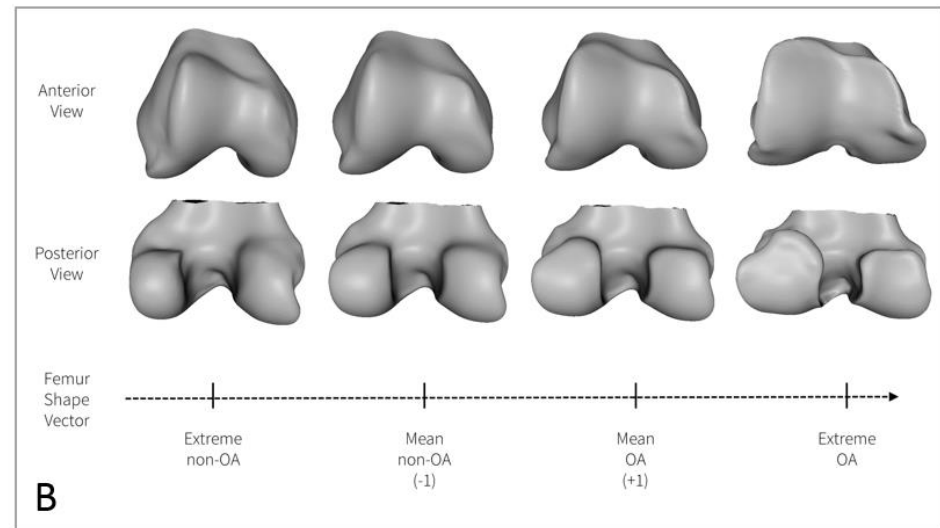
Quantitative Bone Morphometry:
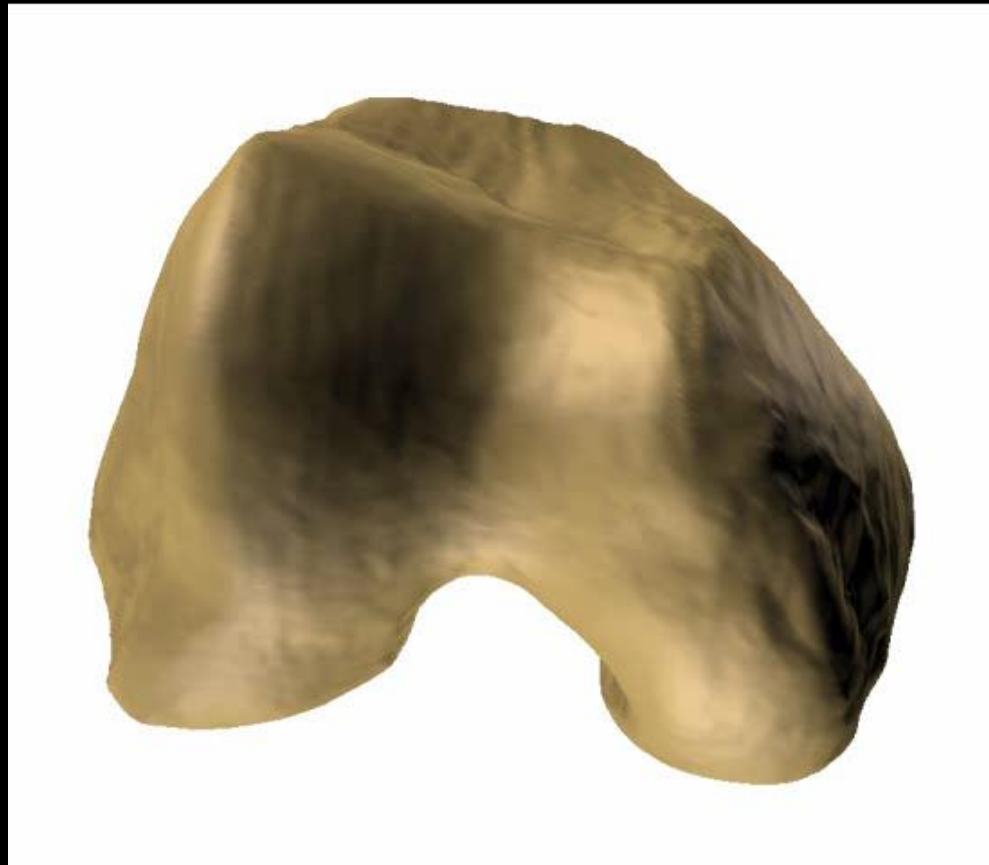## SBP - Total Bone Area (tAB) and 3D Shape

- Change in total bone area (tAB) on the medial and lateral femur, tibia and patella was measured directly from the automated segmentations.

- Overall 3D shape for the femur, tibia and patella, was obtained by projecting the shape from each segmented bone surface onto an OA vector for each bone.
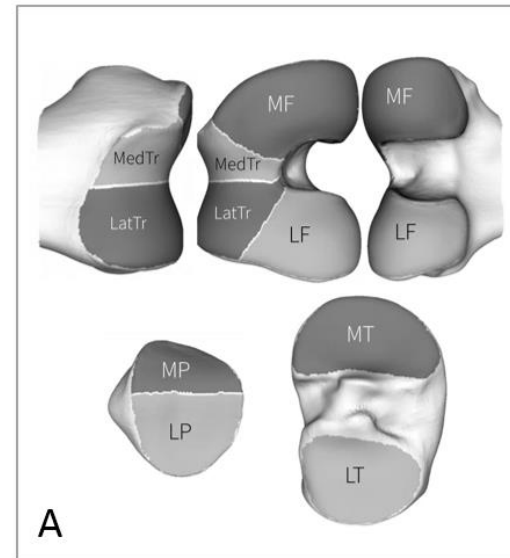
Video Showing the 2 Ends of Vector

SBP - tAB and 3D Shape Analysis: Predictors

■ 24 month change in area of subchondral bone (tAB)

Medial	Femur
	Tibia
	Patella
Lateral	Femur
	Tibia
	Patella
	Notch
Medial	Trochlea
Lateral	Trochlea

■ 24 month change in position on 3D shape vectors
- Femur
- Tibia
- Patella

Change in area of bone (tAB) [mm^2] over 24 Months by Case-Control Status

Predictor	Case (mean (sd))	Control (mean (sd))	OR*	p-value*
Medial Femur (tAB)	37.48 (54.82)	16.13 (41.44)	1.7 (1.4, 2.0)	<.0001
Medial Tibia (tAB)	16.99 (22.11)	10.22 (19.14)	1.4 (1.2, 1.7)	<.0001
Medial Patella (tAB)	7.25 (29.74)	3.10 (16.10)	1.3 (1.1, 1.7)	0.0160
Lateral Femur (tAB)	7.98 (47.43)	-0.45 (42.84)	1.3 (1.0, 1.5)	0.0222
Lateral Tibia (tAB)	11.01 (17.23)	5.53 (14.40)	1.5 (1.2, 1.8)	<.0001
Lateral Patella (tAB)	9.36 (37.41)	3.76 (21.21)	1.3 (1.1, 1.7)	0.0129

*Adjusted for BL JSW, BL WOMAC Pain, BL age, BL BMI, BL KLG, BL pain medication use, sex, and race

Odds Ratio = Odds of being a composite x-ray and pain progressor vs. not having both x-ray and pain progression for each 1 SD increase in biomarker

Change in area of bone (tAB) [mm^2] and Bone Shape over 24 Months by Case-Control Status

Predictor	Case (mean (sd))	Control (mean (sd))	OR*	p-value*
Femoral Notch (tAB)	13.09 (26.47)	6.22 (22.98)	1.3 (1.1, 1.6)	0.0045
Lateral PF Femur (tAB)	7.09 (21.04)	0.57 (18.75)	1.4 (1.2, 1.8)	0.0002
Medial PF Femur (tAB)	12.34 (14.55)	5.77 (11.24)	1.7 (1.4, 2.1)	<.0001
Femoral Vector of 3D Shape	0.30 (0.35)	0.16 (0.27)	1.7 (1.4, 2.0)	<.0001
Tibial Vector of 3D Shape	0.35 (0.45)	0.22 (0.43)	1.4 (1.2, 1.7)	0.0003
Patella Vector of 3D Shape	0.29 (0.68)	0.17 (0.68)	1.2 (1.0, 1.5)	0.0352

*Adjusted for BL JSW, BL WOMAC Pain, BL age, BL BMI, BL KLG, BL pain medication use, sex, and race

Odds Ratio = Odds of being a composite x-ray and pain progressor vs. not having both x-ray and pain progression for each 1 SD increase in biomarker
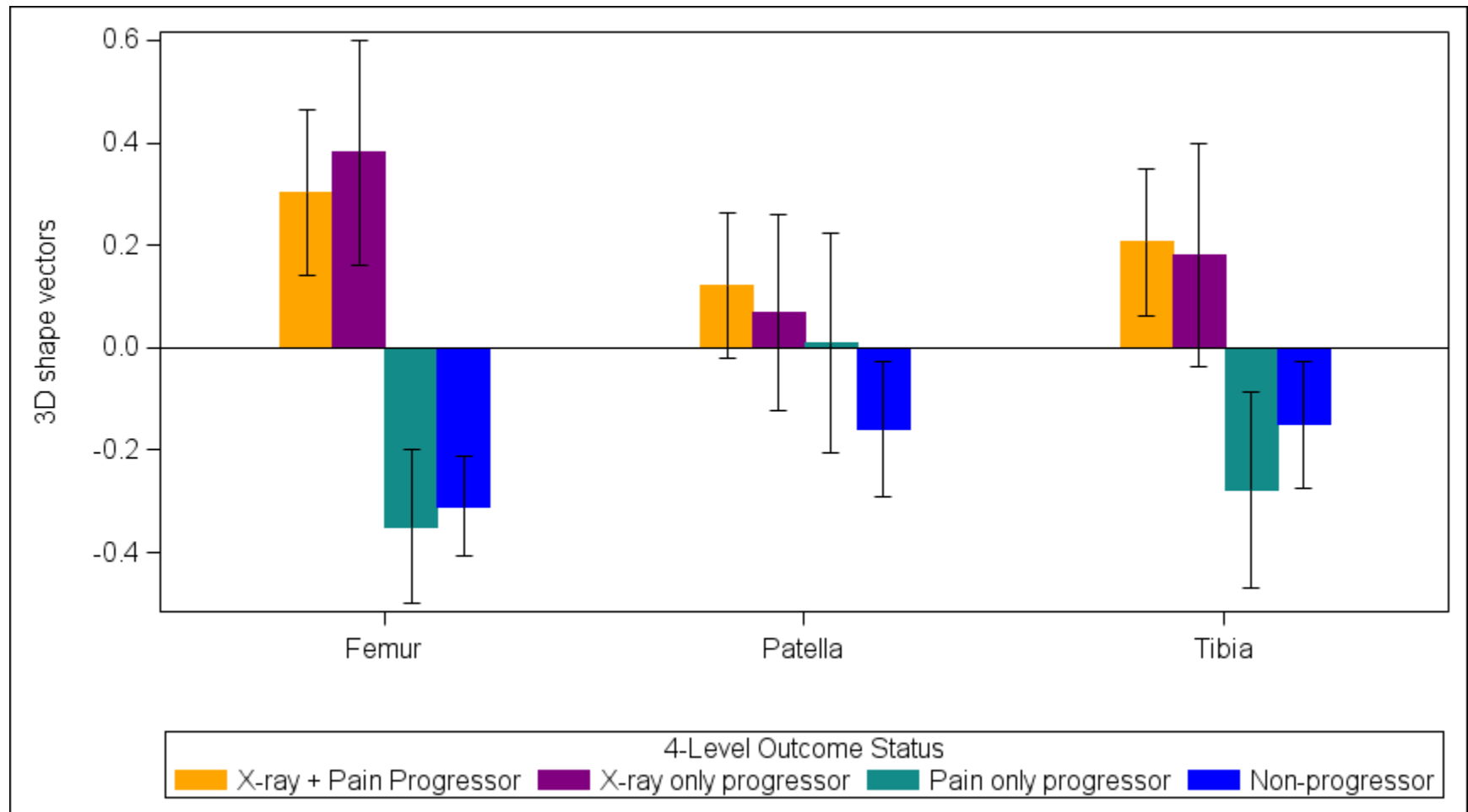
Change in area of the bone (tAB) in the medial compartments over 24 Months by 4-Level Case-Control Status (normalized, with 95% CIs)
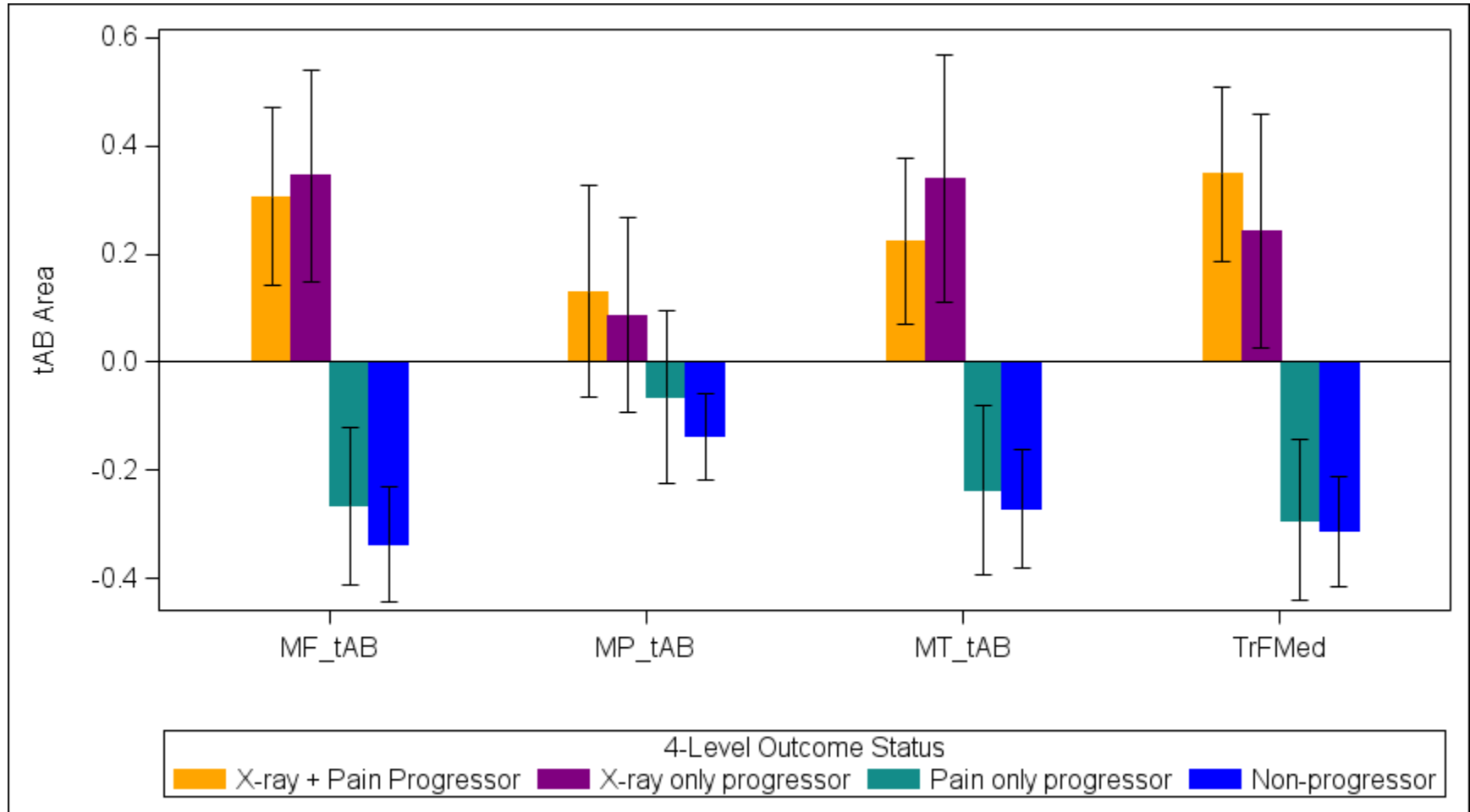
SBP 3D Shape: Secondary Analysis Results

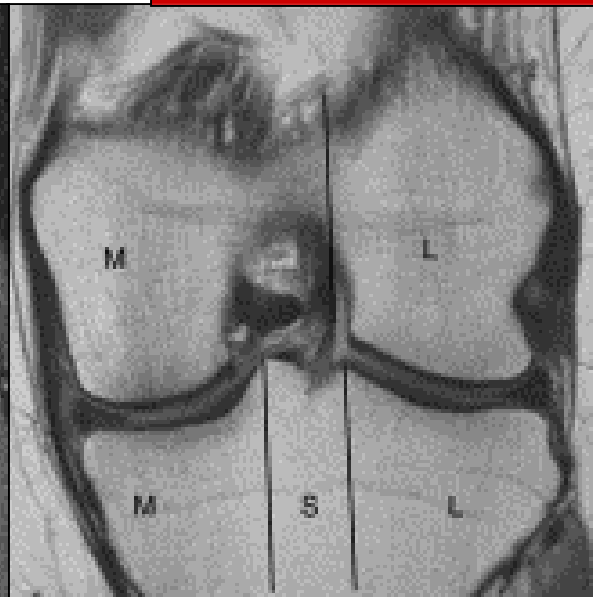**Change in 3D Shape Vector over 24 Months by 4-Level Case-Control Status (normalized, with 95% CIs)**
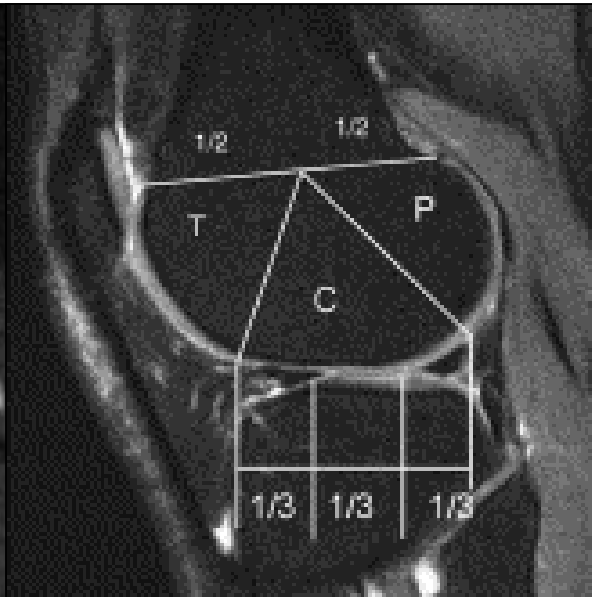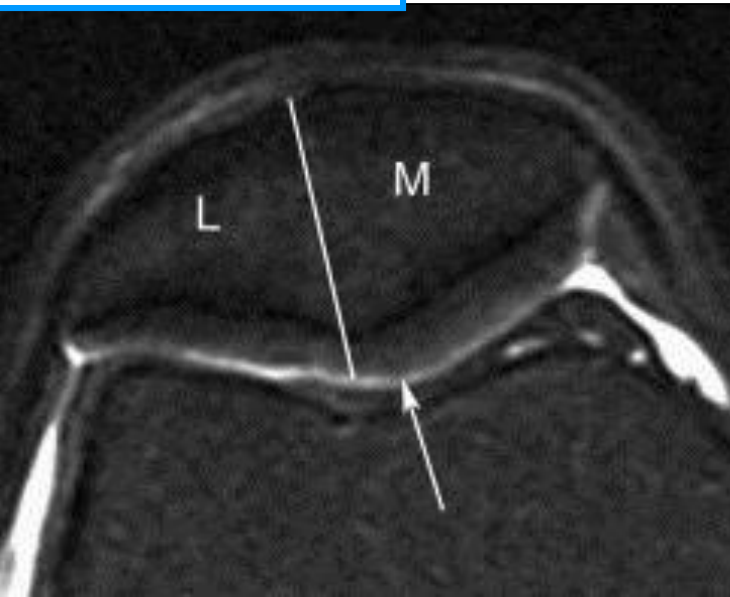
Semi-Quantitative Whole Joint Scoring

- **MOAKS = MRI Osteoarthritis Knee Score**
  Osteoarthritis Cartilage 2011;19:990-1002
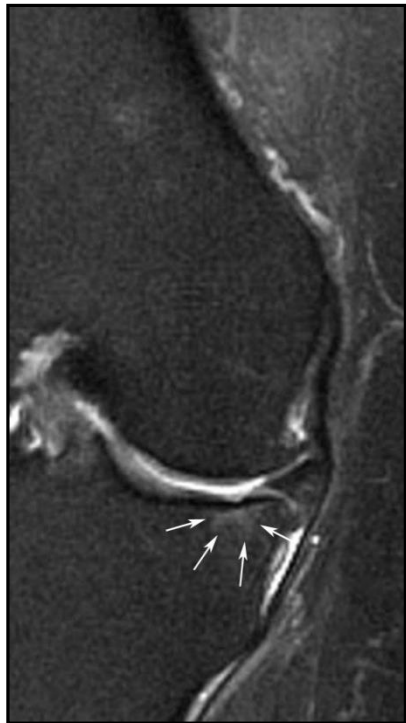
Semi-Quantitative Scoring - MOAKS

■ Assessment of articular cartilage directly

■ Assessment of other important structures

- Meniscus
- Osteophytes
- Attrition
- Subchondral bone marrow lesions and cysts
- Ligaments
- Synovium
- Effusion
- periarticular structures

Adapted from: Guermazi et al. Nat Rev Rheumatol 2013;9:236-51

BML Scoring: MOAKS Size

Grade 1 BML   Grade 2 BML   Grade 3 BML   BML consisting of non-cystic/ill-defined portion and cystic part

MOAKS Analysis: Predictors

- **Six Domains**
  - BMLs
  - Osteophytes
  - Meniscus
  - Cartilage
  - Synovitis
  - Effusion

MOAKS Analysis: Predictors

Domain	Predictor (24 Month change)
BML	Change in number of subregions affected by any BML
	Max change in BML score across all subregions
Osteophyte	Change in number of subregions affected by any Osteophyte
	Max change in Osteophyte score across all subregions in knee
Meniscus	Number of regions with worsening in meniscal morphology
	Worsening in meniscal extrusion
Cartilage	Number of areas with worsening in thickness
	Number of areas with worsening in surface area (include within-grade change)
	Number of areas with worsening in surface area (excluding within-grade change)
Synovitis	Change in Inter-Condylar Synovitis
Effusion	Change in Whole Knee Effusion

BML - Primary Analysis Results

Variable	Category	Case	Control	OR (95% CI)	P-value
Change in Number of subregions affected by any BML	Improvement	26 (13.4%)	55 (13.6%)	1.1 (0.6, 1.8)	0.318
	No Change	95 (49.0%)	214 (52.8%)	REF	
	Worsen in 1 subregion	49 (25.3%)	105 (25.9%)	1.1 (0.7, 1.6)	
	Worsen in 2+ subregions	24 (12.4%)	31 (7.7%)	1.7 (1.0, 3.1)	
Max change in BML score	No Change	53 (27.3%)	138 (34.1%)	REF	0.003
	Within grade worsening	12 (6.2%)	24 (5.9%)	1.3 (0.6, 2.8)	
	Worsening by 1 grade	81 (41.8%)	192 (47.4%)	1.1 (0.7, 1.7)	
	Worsening by 2+ grades	48 (24.7%)	51 (12.6%)	2.5 (1.5, 4.1)	

Osteophyte and Meniscus - Primary Analysis Results

Variable	Category	Case	Control	OR (95% CI)	P-value
Increase in number of subregions affected by any Osteophyte	No	173 (89.2%)	371 (91.4%)	REF	0.386
	Yes	21 (10.8%)	35 (8.6%)	1.3 (0.7, 2.3)	
Max change in Osteophyte score >=1 across all subregions in knee	No	151 (77.8%)	347 (85.5%)	REF	0.021
	Yes	43 (22.2%)	59 (14.5%)	1.7 (1.1, 2.6)	
Meniscal Morphology: 24 Month any regions with worsening	No	140 (72.2%)	365 (90.1%)	REF	<0.001
	Yes	54 (27.8%)	40 (9.9%)	3.5 (2.2, 5.5)	
Meniscal Extrusion Medial - 24 Month worsening	No	143 (74.1%)	369 (91.3%)	REF	<0.001
	Yes	50 (25.9%)	35 (8.7%)	3.7 (2.3, 5.9)	

Cartilage - Primary Analysis Results

Variable	Category	Case	Control	OR (95% CI)	P-value
Cartilage Morphology - worsening in thickness	No Change	82 (42.3%)	266 (65.5%)	REF	<0.001
	Worsen in 1 subreg	49 (25.3%)	83 (20.4%)	1.9 (1.2, 2.9)	
	Worsen in 2 subreg	38 (19.6%)	39 (9.6%)	3.2 (1.9, 5.3)	
	Worsen in 3+ subreg	25 (12.9%)	18 (4.4%)	4.5 (2.3, 8.7)	
Cartilage Morphology - worsening in surface area (incl within-grade chg)	No Change	53 (27.3%)	193 (47.5%)	REF	<0.001
	Worsen in 1 subreg	54 (27.8%)	122 (30.0%)	1.6 (1.0, 2.5)	
	Worsen in 2 subreg	39 (20.1%)	52 (12.8%)	2.7 (1.6, 4.6)	
	Worsen in 3+ subreg	48 (24.7%)	39 (9.6%)	4.5 (2.7, 7.5)	
Cartilage Morphology - worsening in surface area (excl within-grade chg)	No Change	105 (54.1%)	277 (68.2%)	REF	<0.001
	Worsen in 1 subreg	41 (21.1%)	87 (21.4%)	1.2 (0.8, 1.9)	
	Worsen in 2 subreg	25 (12.9%)	31 (7.6%)	2.1 (1.2, 3.8)	
	Worsen in 3+ subreg	23 (11.9%)	11 (2.7%)	5.5 (2.6, 11.7)	

Synovitis and Effusion - Primary Analysis Results

Variable	Category	Case	Control	OR (95% CI)	P-value
Change MOAKS Inter-Condylar Synovitis	Improvement	3 (1.5%)	7 (1.7%)	1.0 (0.3, 4.0)	0.002
	No Change	158 (81.4%)	374 (92.1%)	REF	
	Worsen	33 (17.0%)	25 (6.2%)	3.1 (1.8, 5.4)	
Change in MOAKS Whole Knee Effusion	Improvement	17 (8.8%)	62 (15.3%)	0.8 (0.4, 1.4)	<0.001
	No Change	98 (50.5%)	269 (66.3%)	REF	
	Worsen	79 (40.7%)	75 (18.5%)	2.9 (2.0, 4.3)	

Preliminary Results of Multivariable Models
Combining Domains (1 out of 3) - Cartilage

	Model 1 Cartilage
C-statistic	0.686
Cartilage - areas with worsening in thickness	P=0.0004
None	REF
1 subregion	1.7 (1.1, 2.6)
2 subregions	2.5 (1.4, 4.2)
3+ subregions	3.2 (1.6, 6.5)
Cartilage - areas with worsening in surface area (incl within-grade change)	P=0.0003
None	REF
1 subregion	1.3 (0.8, 2.1)
2 subregions	1.9 (1.1, 3.3)
3+ subregions	3.3 (1.9, 5.6)

Preliminary Results of Multivariable Models
Combining Domains (2 out of 3) – Cartilage + Meniscus

|	Model 1 Cartilage	Model 2 Model 1 + Meniscus
C-statistic	0.686	0.711
Cartilage - areas with worsening in thickness	P=0.0004	P=0.0028
None	REF	REF
1 subregion	1.7 (1.1, 2.6)	1.6 (1.0, 2.6)
2 subregions	2.5 (1.4, 4.2)	2.2 (1.3, 3.9)
3+ subregions	3.2 (1.6, 6.5)	2.8 (1.4, 5.8)
Cartilage - areas with worsening in surface area (incl within-grade change)	P=0.0003	P=0.0291
None	REF	REF
1 subregion	1.3 (0.8, 2.1)	1.2 (0.8, 2.0)
2 subregions	1.9 (1.1, 3.3)	1.6 (0.9, 2.9)
3+ subregions	3.3 (1.9, 5.6)	2.3 (1.3, 4.2)
Meniscus: Meniscal Morphology: Any regions with worsening (Yes vs. No)		1.8 (1.0, 3.0) P=0.0420
Meniscus: Meniscal Extrusion Medial worsening (Yes vs. No)		1.9 (1.1, 3.3) P=0.0304

Preliminary Results of Multivariable Models
## Combining Domains (2 out of 3) – Cartilage + Meniscus + Effusion

	Model 1	Model 2	Model 3
	Cartilage	Model 1 + Meniscus	Model 2 + Effusion
C-statistic	0.686	0.711	0.725
Cartilage - areas with worsening in thickness	P=0.0004	P=0.0028	P=0.0087
None	REF	REF	REF
1 subregion	1.7 (1.1, 2.6)	1.6 (1.0, 2.6)	1.6 (1.0, 2.5)
2 subregions	2.5 (1.4, 4.2)	2.2 (1.3, 3.9)	2.0 (1.1, 3.5)
3+ subregions	3.2 (1.6, 6.5)	2.8 (1.4, 5.8)	2.8 (1.3, 5.7)
Cartilage - areas with worsening in surface area (incl within-grade change)	P=0.0003	P=0.0291	P=0.0804
None	REF	REF	REF
1 subregion	1.3 (0.8, 2.1)	1.2 (0.8, 2.0)	1.2 (0.8, 1.9)
2 subregions	1.9 (1.1, 3.3)	1.6 (0.9, 2.9)	1.5 (0.8, 2.7)
3+ subregions	3.3 (1.9, 5.6)	2.3 (1.3, 4.2)	2.1 (1.2, 3.9)
Meniscus: Meniscal Morphology: Any regions with worsening (Yes vs. No)		1.8 (1.0, 3.0) P=0.0420	1.8 (1.0, 3.0) P=0.0416
Meniscus: Meniscal Extrusion Medial worsening (Yes vs. No)		1.9 (1.1, 3.3) P=0.0304	1.9 (1.1, 3.3) P=0.1249
Effusion : Change in Effusion Category			P=0.0010
Improvement			REF
No change			1.5 (0.8, 2.7)
worsening			2.9 (1.5, 5.7)

Meniscus - Secondary Analysis Results

Change in cartilage and meniscus over 24 Months by 4-Level Case-Control Status
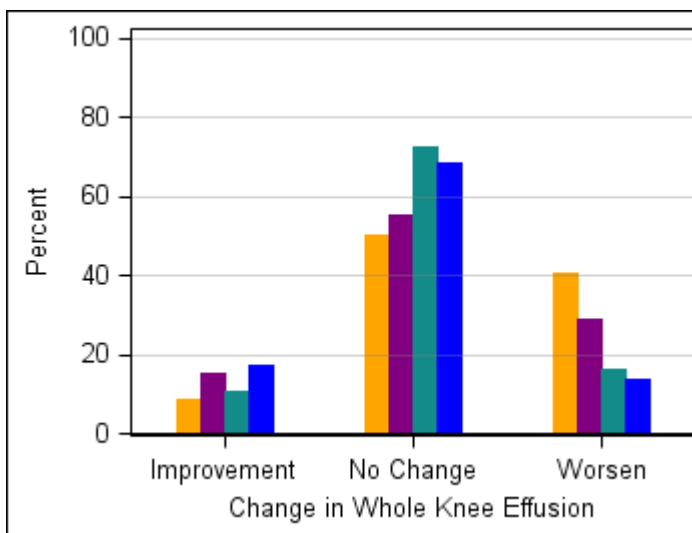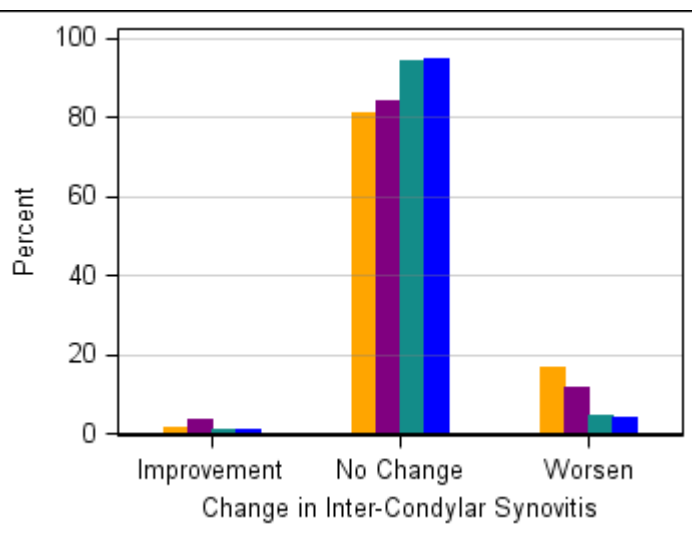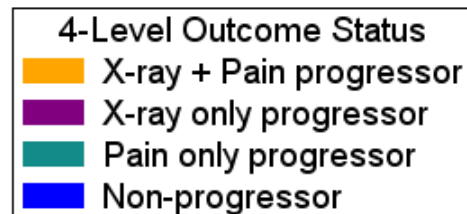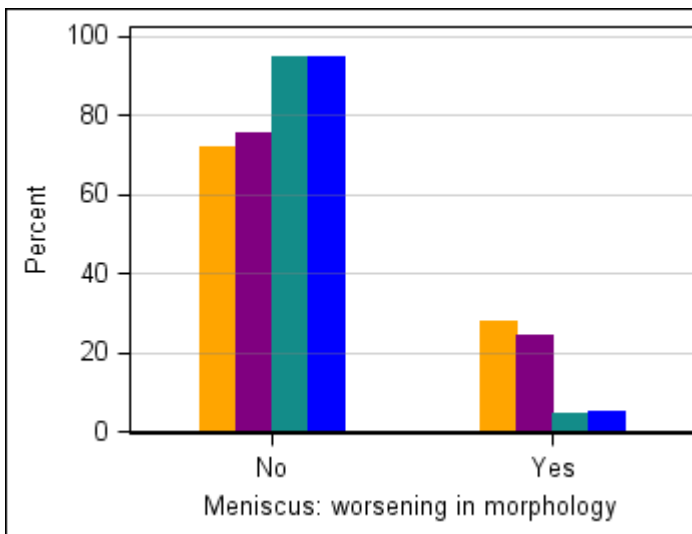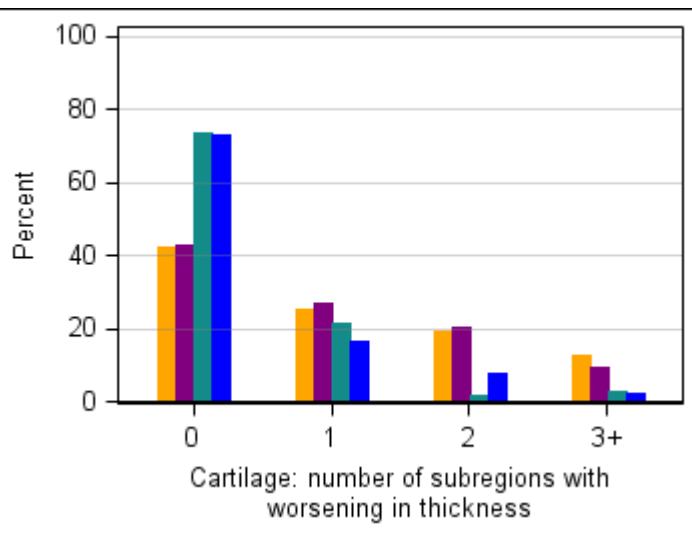
MR Imaging Biomarkers Discussion

- Associations of biomarkers with X-ray+ pain progressors and with X-ray only progressors are similar

- Associations of biomarkers with pain only progressors generally not significant

- The imaging technologies differ in the extent to which they are able to distinguish cases and controls

- **Next steps:** multivariable models comparing the different technologies

Bone Trabecular Integrity (BTI)

- BTI: measure of trabecular structure or 'texture'

- Measured in subchondral region of tibia

- Horizontal and vertical components
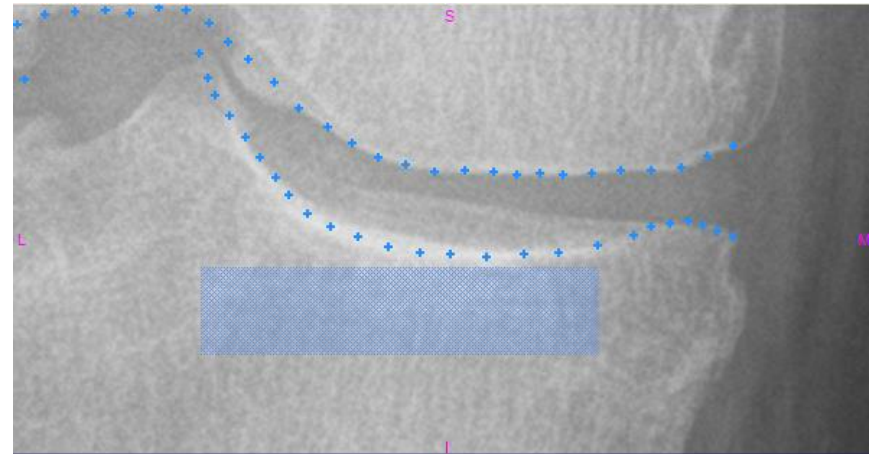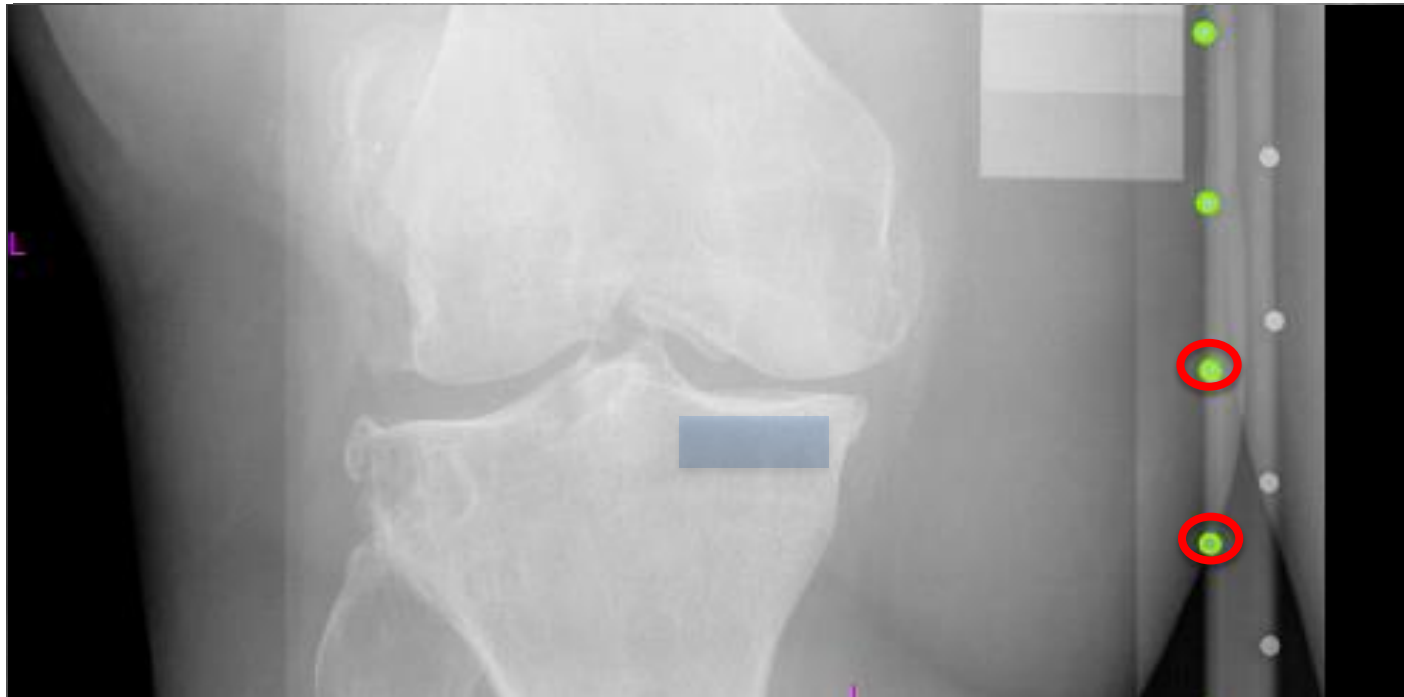
Bone Trabecular Integrity (BTI)

- Technique has a long publication history
  - Starting with J Lynch et al, Med Phys (1991)

- Characteristics of subchondral bone trabeculae are analyzed from knee radiographs (x-rays)

- Technique has been shown to be robust to:
  - Variations in pixel size
  - X-ray exposure
  - Patient positioning
  - Digitisation parameters

Bone Trabecular Integrity (BTI)

■ BTI has demonstrated a strong association with the progression of OA based on radiographic and MRI outcomes:

- JC Buckland-Wright et al, Rheumatology (2007)
- EA Messent, C Buckland-Wright et al, OAC (2006)
- VB Kraus, et al, Arthritis Rheum (2009)
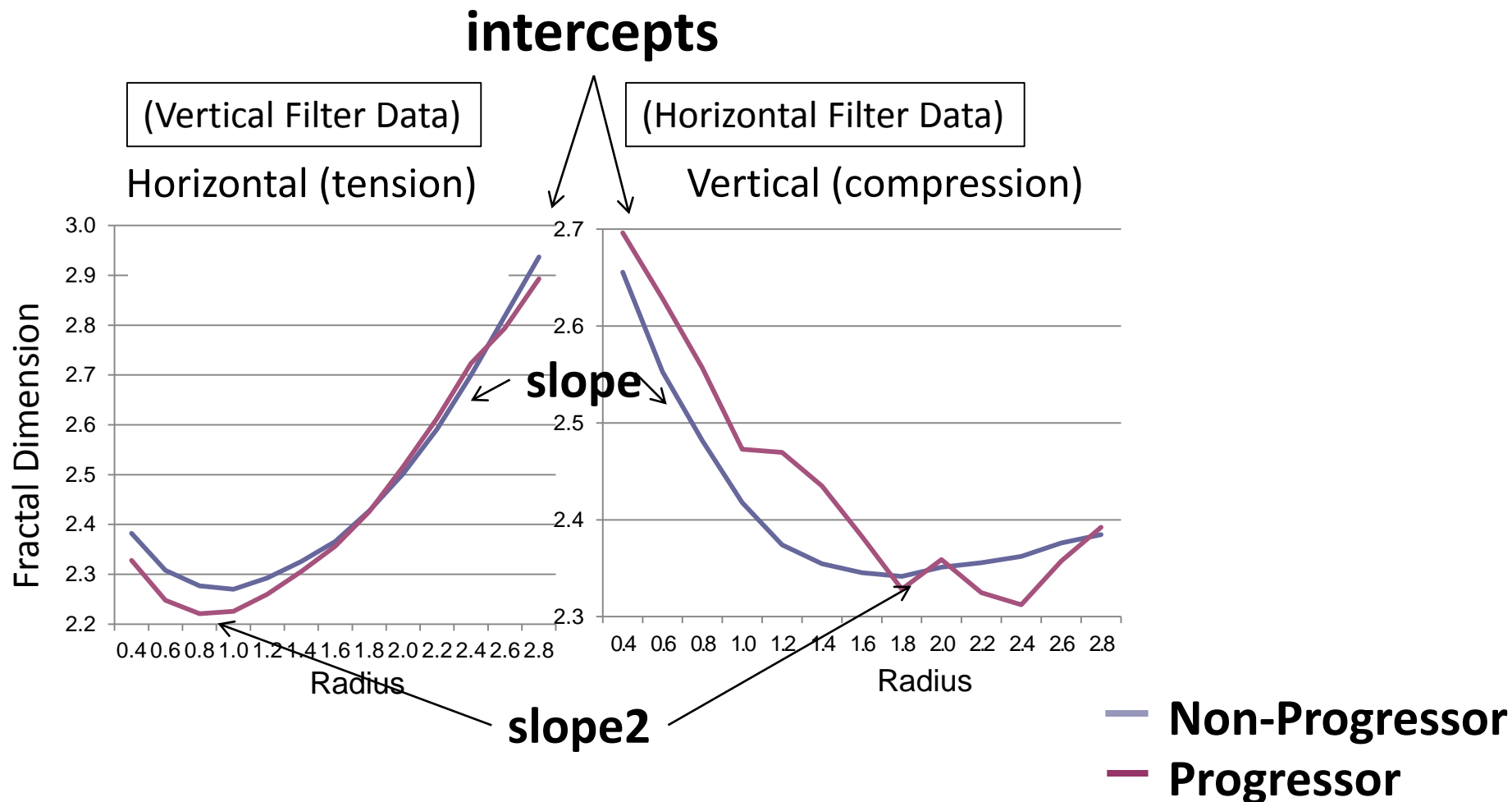- VB Kraus, et al, Arthritis Rheum (2013)

BTI: Image Analysis Implementation

- KneeAnalyzer™ markup of 6 initialisation points on x-ray
- Tibial subchondral region of interest automatically placed
- Fractal Signature Analysis (FSA) of medial compartment region
- Calibration using the Synaflexer™ beads

■ Extraction of 6 BTI parameters from FSA curves

Baseline Bone Trabecular Integrity by Case-Control Status

Area	Case (mean (sd))	Control (mean (sd))	OR[*] (z score)	p-value[*]
Intercept (Horizontal)	2.77 (0.20)	2.74 (0.21)	1.45 (0.9 ,2.4)	0.1337
Slope (Horizontal)	-0.18 (0.05)	-0.19 (0.05)	1.02 (0.8 ,1.3)	0.8796
Quadratic Term (Horizontal)	0.10 (0.07)	0.10 (0.06)	1.14 (0.9 ,1.5)	0.3863
Intercept (Vertical)	2.61 (0.17)	2.59 (0.16)	0.74 (0.5 ,1.1)	0.1625
Slope (Vertical)	0.03 (0.09)	0.04 (0.10)	0.99 (0.7 ,1.3)	0.9214
Quadratic Term (Vertical)§	0.25 (0.09)	0.26 (0.09)	0.72 (0.5 ,1.0)	0.0274

[*]Adjusted for BL JSW, BL WOMAC Pain, BL age, BL BMI, BL KLG, BL pain medication use, sex, and race

§Not significantly associated with any covariate

Odds Ratio = Odds of being a composite JSL and pain progressor vs. not having both JSL and pain progression for each 1 SD increase in biomarker

BTI Analysis: Results

Single baseline BTI parameter predicts any progression, joint space loss and pain progression over 48 months

Label	Method 2: all progressors vs non-progressors		Method 3: Joint space loss (JSL) progressors vs JSL non-progressors		Method 4: pain progressors vs non-progressors	
Intercept (Horizontal)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)
Quadratic Term (Vertical)	0.0108	0.79 (0.66, 0.95)	0.0328	0.83 (0.70, 0.98)	0.0202	0.81 (0.69, 0.97)

Baseline Bone Trabecular Integrity by Case-Control Status

Area	Case (mean (sd))	Control (mean (sd))	OR* (z score)	p-value*
Intercept (Horizontal)	2.77 (0.20)	2.74 (0.21)	1.45 (0.9 ,2.4)	0.1337
Slope (Horizontal)	-0.18 (0.05)	-0.19 (0.05)	1.02 (0.8 ,1.3)	0.8796
Quadratic Term (Horizontal)	0.10 (0.07)	0.10 (0.06)	1.14 (0.9 ,1.5)	0.3863
Intercept (Vertical)	2.61 (0.17)	2.59 (0.16)	0.74 (0.5 ,1.1)	0.1625
Slope (Vertical)	0.03 (0.09)	0.04 (0.10)	0.99 (0.7 ,1.3)	0.9214
Quadratic Term (Vertical)§	0.25 (0.09)	0.26 (0.09)	0.72 (0.5 ,1.0)	0.0274

*Adjusted for BL JSW, BL WOMAC Pain, BL age, BL BMI, BL KLG, BL pain medication use, sex, and race

§Not significantly associated with any covariate

Odds Ratio = Odds of being a composite JSL and pain progressor vs. not having both JSL and pain progression for each 1 SD increase in biomarker

Baseline composite BTI score predicts case status at 48 months

Model	Unadjusted			Adjusted		
	OR* (95% CI)	p-value	C statistic	OR* (95% CI)	p-value	C statistic
Composite BTI Z-Score	1.21 (1.02, 1.45)	0.0308	0.552	1.24 (1.03, 1.49)	0.0213	0.631
Covariates Only						0.608

sum of all markers z-scores (vertical parameters reverse coded)

*Odds of being a composite x-ray + pain progressor for each 1 SD increase in composite score.

Covariates: BL JSW, BL WOMAC Pain, BL age, BL BMI, BL KLG, BL pain medication use, sex, and race

BTI Analysis: Future

- Analyze Joint Space Area

- Analyze Anatomic Axis Angle to assess malalignment as a predictor

- Evaluate longitudinal BTI over 12 and 24 months

- BTI 6 parameter extraction now automated by Duke software that interfaces with KneeAnalyzer

- X-ray analysis and parameter extraction will require ~$\leq$30 seconds per image

- Technique being developed as a point of care tool by Optasia Medical in Collaboration with Parexel

FSA/BTI Acknowledgements

- **H Cecil Charles, PhD**
  - DIAL Director, Co-Director - Center for Advanced Magnetic Resonance Development, Associate Professor of Radiology

- **Carl F Pieper, DPH**
  - Associate Professor of Biostatistics and Bioinformatics

- **Lawrence Whitley**
  - Data Analyst, Department of Medicine

Duke University School of Medicine

Serum and Urine Soluble Biomarkers

Virginia Byers Kraus

Elena Losina

Soluble BM: Panel Analyzed

Biomarker	Process (preliminary)	BIPEDS Classifications	Surrogacy Based on Human Clinical Trials (preliminary)	ELISA assay type
urinary CTX-II	type II collagen degradation	Knee: BPED Hip: BPD	characterization: changed significantly in 3 pharmacologic trials that met primary clinical endpoints (Christgau 2004, Gineyts 2004, Manicourt 2006)	competitive-inhibition
serum COMP	cartilage degeneration	Knee: BPD Hip: BPD	exploration: not used to date in pharmacologic trial	competitive-inhibition & sandwich
serum HA	osteophyte burden, synovitis	Knee: BPED Hip: P	demonstration: changed significantly in one pharmacologic trial that met primary clinical endpoints (Manicourt 2006)	sandwich protein binding assay
serum and urine C1,2C	Types I and II collagen degradation	Knee: D(u) Hip: none	exploration: nonsignificant change in one pharmacologic trial that met primary clinical endpoint (Mazzuca 2006)	competitive-inhibition
serum and urine C2C	type II collagen degradation	Knee: E(s), D(u) Hip: B(s)	demonstration: nonsignificant change in one pharmacologic trial that met primary clinical endpoint (Mazzuca 2006)	competitive-inhibition
serum and urine Coll2-1NO2	type II collagen degradation	Knee: D(s),B(u),P(u) Hip: D(s)	exploration: not used to date in pharmacologic trial	competitive-inhibition
serum CPII	type II collagen synthesis	Knee: D(s) Hip: B(s)	exploration: nonsignificant change in one pharmacologic trial that met primary clinical endpoint (Mazzuca 2006)	competitive-inhibition
Serum PIIANP	Type II collagen synthesis	Knee: BPD Hip: none	exploration: not used to date in pharmacologic trial	competitive-inhibition
urine/serum NTX-1	bone resorption	Knee: P(u),E(u) Hip: P(s)	demonstration: changed significantly in one pharmacologic trial that met primary clinical (WOMAC) endpoint (Spector 2005)	competitive-inhibition
Urine CTXI alpha and beta/serum CTX-1	bone resorption	Knee: B(u), D(s/u), P(u) Hip: none	exploration: not used to date in pharmacologic trial	competitive-inhibition
serum CS846	cartilage aggrecan synthesis/turnover	Knee: P Hip: none	exploration: nonsignificant change in one pharmacologic trial that met primary clinical endpoint (Mazzuca 2006) but changed associated with concurrent JSN	competitive-inhibition
serum MMP-3	protease involved with joint tissue degradation	Knee: E Hip: none	characterization: changed significantly in two pharmacologic trials that met primary clinical endpoints (Lohmander 2005, Manicourt 2006)	sandwich for total MMP-3 assay

Soluble BM: Assays

- 12 biomarkers (18 total by format) were chosen by consensus of an expert working group (Kraus VB, et al, OAC 2011)

- Selection required the biomarker be available "off the shelf" as a commercially available kit

- LabCorp Clinical Trials (San Leandro, CA)--CLIA and CAP certified division within LabCorp measured all biomarkers except urine Col2-1 NO2

- Artialis (Liege, Belgium)--Good Laboratory Practice (GLP) certified facility, measured urine Col2-1 NO2

- Duplicate analyses of baseline, 12m and 24 m samples

- Same lot of kits used for all analyses of each biomarker

Soluble BM: Samples

- N=1785 samples were available for analysis from 600 subjects (15 of the 12 month samples missing)

- Nearly all (92-98%) serum and urine were >8 hrs fasting samples

- All samples encoded by UCSF

- Unthawed stock serum sample provided to LabCorp

- An unthawed stock urine sample was aliquoted by LabCorp and an aliquot provided to Artialis (Liege, Belgium) for Col2-1 NO2

- Freeze thaws were minimized and assays optimally sequenced per available information of biomarker stability

- Clustering of samples by individual was performed to minimize technical variability of longitudinal analyses (running all samples for a particular individual on the same plate)

Soluble BM: Imputation Strategy

- For results above the highest standard, the sample was diluted more and reanalyzed

- For results below the lowest standard
  - The kit manufacturer was consulted and when deemed appropriate, the sample was diluted less and reanalyzed

  - For samples still yielding values below the lowest standard, results were imputed by interpolation from the standard curve extended from the lowest standard to zero.

  - This method was deemed superior to random imputation particularly as some of the biomarkers had linear standard curves in this low range (HA and CS-846)

Soluble BM: Coefficients of Variation
(from smallest to largest)

Biomarker (units)	CV
serum CTX-1 (ng/ml)	5%
serum COMP (ng/ml)	5%
serum HA (ng/ml)	7%
serum NTX-1 (nm BCE)	7%
serum MMP-3 (ng/ml)	10%
serum C2C (ng/ml)	12%
serum CPII (ng/ml)	12%
serum PIIANP (ng/ml)	12%
serum Coll2-1 NO2 (nM)	14%
serum CS846 (ng/ml)	17%
serum C1,2C (µg/ml)	23%

Biomarker (units)	CV
urine NTX-1 (nM BCE/mmol Cr)	3%
urine Creatinine (mmol/L)	3%
urine CTX-1α (µg/mmol Cr)	4%
urine CTX-II (ng/mmol Cr)	5%
urine C2C HUSA (ng/mmol Cr)	6%
urine CTX-1β (µg/mmol Cr)	8%
Urine Col-2-1NO2 (nM/mmol Cr)	9%
urine C1,2C (ng/mmol Cr)	22%

Soluble BM: Covariates

- Several of the baseline biomarker concentrations were associated with one or more demographic or baseline characteristics including sex, age, BMI, race, baseline joint space width, baseline WOMAC pain and baseline use of pain medications

- These covariates were therefore used for the final analyses but did not alter any of the results

Soluble BM: Covariates -- Serum

| Biomarker (z scored)	Sex*	Pain Meds*	Race*	BL JSW[+]	BL WOMAC Pain[+]	BL Age[+]	BL BMI[+]
serum C12C	**-0.2381 (<0.0001)**	0.0325 (0.4219)	**0.3425 (0.0404)**	0.0455 (0.2659)	-0.0347 (0.3960)	0.0552 (0.1773)	-0.0443 (0.2799)
serum C2C	-0.2034 (0.3710)	-0.1013 (0.0613)	0.4871 (0.0263)	-0.0203 (0.6250)	0.0306 (0.4613)	0.0359 (0.3872)	0.0655 (0.1147)
serum COLL2-1 NO2	-0.4487 (0.1292)	-0.1273 (0.1427)	**0.5834 (<0.0001)**	-0.0161 (0.6986)	0.0522 (0.2084)	0.0098 (0.8127)	-0.0664 (0.1099)
serum CPII	-0.2794 (0.5065)	**-0.0741 (<0.0001)**	**0.7965 (<0.0001)**	0.0107 (0.7962)	0.0183 (0.6591)	0.0269 (0.5173)	-0.0484 (0.2439)
serum CS846	**-0.1115 (0.0100)**	-0.0212 (0.6736)	0.0068 (0.5599)	-0.0051 (0.9022)	0.0186 (0.6550)	-0.0349 (0.4007)	0.0155 (0.7084)
serum CTXI	**-0.1479 (<0.0001)**	0.1160 (0.0541)	**-0.1441 (0.0310)**	0.0267 (0.5145)	-0.0427 (0.2970)	-0.0152 (0.7103)	**-0.1228 (0.0026)**
serum COMP	0.1469 (0.0927)	0.0558 (0.7808)	0.0809 (0.0863)	0.0197 (0.6352)	-0.0636 (0.1256)	**0.2454 (<0.0001)**	-0.0629 (0.1301)
serum HA	0.0159 (0.5384)	-0.0775 (0.1558)	**0.3355 (<0.0001)**	**-0.0993 (0.0165)**	-0.0054 (0.8965)	**0.3808 (<0.0001)**	0.0483 (0.2447)
serum MMP-3	**1.0604 (<0.0001)**	-0.0052 (0.4516)	-0.1361 (0.0698)	0.0388 (0.3497)	-0.0774 (0.0619)	**0.0949 (0.0220)**	**-0.1138 (0.0060)**
serum NTXI	**-0.1654 (0.0001)**	0.1755 (0.8993)	0.0210 (0.0898)	0.0032 (0.9378)	-0.0024 (0.9543)	0.0402 (0.3327)	-0.0231 (0.5791)
serum PIIANP	-0.0377 (0.3946)	-0.0556 (0.5065)	0.3650 (0.5638)	-0.0100 (0.8066)	**0.0819 (0.0451)**	-0.0006 (0.9891)	**0.1517 (0.0002)**

Soluble BM: Covariates -- Serum

| Biomarker (z scored)	Sex*	Pain Meds*	Race*	BL JSW[+]	BL WOMAC Pain[+]	BL Age[+]	BL BMI[+]
urine Col2-1 NO2	**-0.2646 (0.0142)**	**-0.2250 (<0.0001)**	**-0.2401 (<0.0001)**	0.0211 (0.6114)	-0.0013 (0.9746)	0.0216 (0.6019)	-0.0103 (0.8041)
urine C12C	**0.2635 (0.0142)**	0.0324 (0.5869)	-0.0735 (0.5688)	0.0633 (0.1268)	-0.0000 (0.9998)	**-0.1040 (0.0120)**	0.0745 (0.0724)
urine C2C	**-0.1311 (0.0334)**	0.0191 (0.8064)	**0.0247 (0.0131)**	**-0.1006 (0.0151)**	-0.0308 (0.4585)	**0.2741 (<0.0001)**	-0.0299 (0.4719)
urine CTXII	**-0.2953 (<0.0001)**	**0.0630 (0.0364)**	0.2086 (0.0381)	-0.0537 (0.1954)	**0.1057 (0.0106)**	**0.1486 (0.0003)**	0.0791 (0.0564)
urine NTXI	**-0.4228 (<0.0001)**	**0.1796 (0.0278)**	-0.1890 (0.0564)	-0.0258 (0.5348)	-0.0473 (0.2539)	0.0330 (0.4260)	**-0.1075 (0.0094)**
urine CTXIα	**-0.2906 (<0.0001)**	**0.1931 (0.0500)**	-0.0233 (0.6575)	0.0005 (0.9900)	-0.0565 (0.1730)	0.0039 (0.9255)	**-0.0868 (0.0363)**
urine CTXIβ	**-0.2977 (<0.0001)**	0.1965 (0.3447)	0.0398 (0.4598)	0.0273 (0.5111)	-0.0203 (0.6251)	-0.0594 (0.1523)	-0.0782 (0.0594)

- The dynamic nature of OA-related biochemical markers is known: Sharif M, et al, Arthritis Rheum (2004); Sharif M, et al, Rheumatology (Oxford) (2007).

24 progressors and 60 non-progressors.

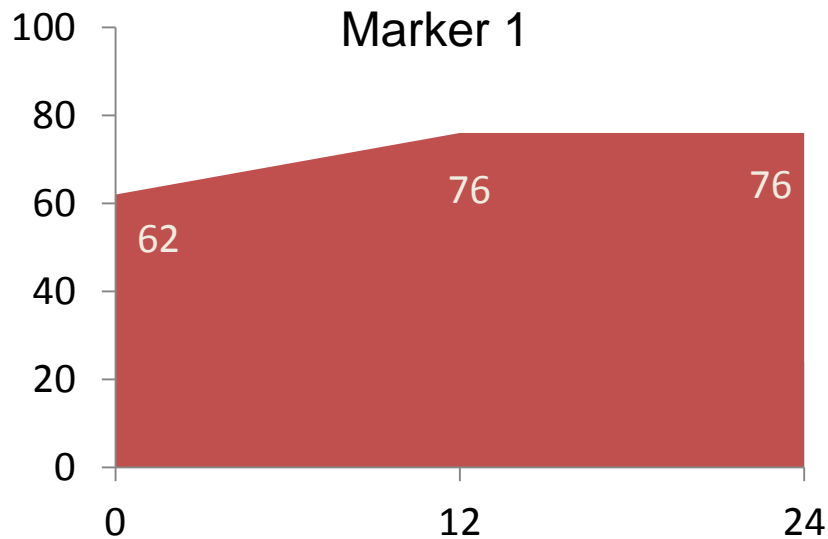Change Scores ~=100 both groups
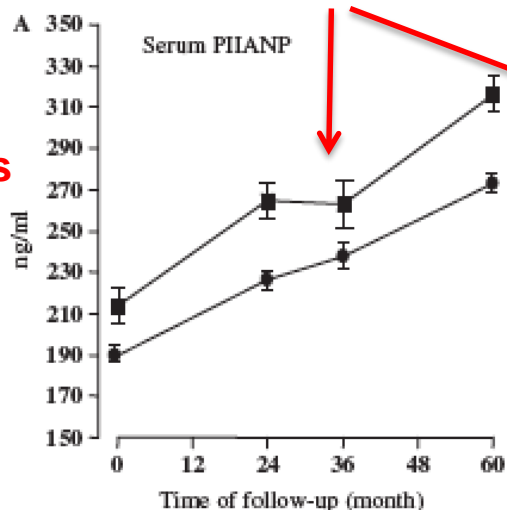
Change Scores ~=0 both groups
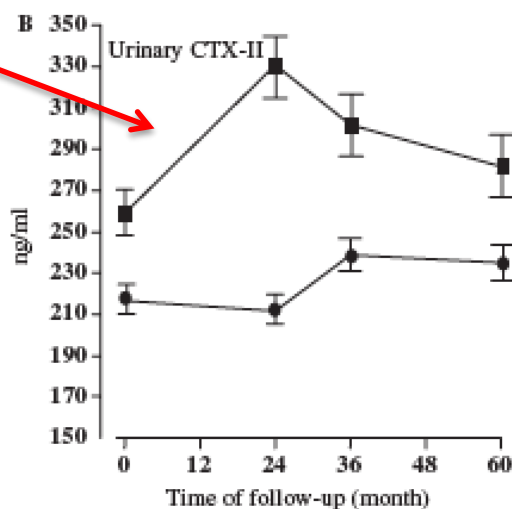
Sharif, et al. (2007) Rheumatology (Oxford) 46(6): 938-943.

- <u>Time-Integrated Concentrations</u> can overcome these issues and can be used to evaluate the longitudinal dynamic change in the biomarkers

Defining Time-Integrated Concentrations

Marker 1

Marker 1:

Change : 76-62=14

TIC: (62+76)/2+(76+76)/2=145

Marker 2:

Change : 36-22=14

TIC: (22+60)/2+(60+36)/2=89
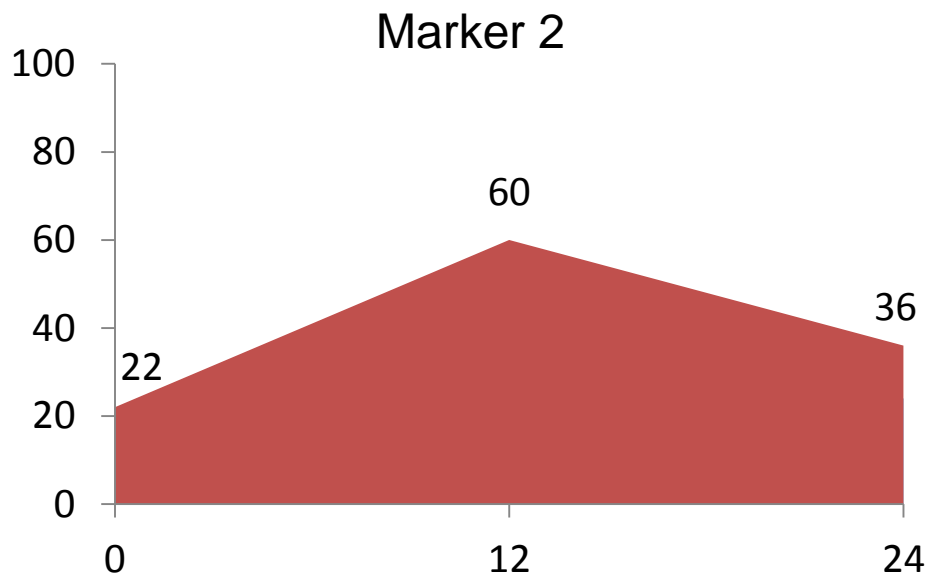
Marker 2

Soluble BM: Statistical Analytic Strategy

- Algorithm
  - Calculate Time-Integrated-Concentration (TIC) for each marker for each subject
    - Use interpolated research value
  - Determine mean and SD of the TIC for each biomarker
  - Convert to Z- score by subtracting the mean and dividing by SD
  - Z-scores standardization helps to compare across multiple biochemical markers

- Analyzed several standardized (z-score converted) measures: baseline, 12 change, 24-month TIC for each biomarkers
  - Univariate analysis for each biomarker
  - combinatorial approach using 24-month TIC for multiple biomarkers to optimize prediction

Soluble BM: Single Biomarker Associations
(adjusted for baseline covariates — Serum 1 of 2)

Biomarker	Baseline Concentration (z-score)			12 Month TIC (Z-score)			24 Month TIC (z-score)		
	Mean (SD) Median		OR, 95% CI, P value	Mean (SD) Median		OR, 95% CI, P value	Mean (SD) Median		OR, 95% CI, P value
	Comparators	Cases		Comparators	Cases		Comparators	Cases	
C12C	-0.03 (0.98) -0.09	0.06 (1.04) -0.02	1.08 (0.91, 1.29) 0.3868	-0.01 (1.00) -0.04	0.02 (1.00) -0.04	1.02 (0.85, 1.22) 0.8637	0.01 (1.01) -0.15	-0.01 (0.99) -0.06	0.97 (0.81, 1.15) 0.7031
C2C	-0.02 (0.94) -0.16	0.04 (1.11) -0.12	1.05 (0.88, 1.25) 0.5778	-0.00 (0.98) -0.10	0.01 (1.05) -0.09	0.98 (0.82, 1.18) 0.8536	0.04 (0.95) 0.04	-0.07 (1.10) -0.03	0.88 (0.73, 1.05) 0.1548
COLL2-1 NO2	0.00 (1.03) -0.18	-0.01 (0.94) -0.15	1.00 (0.84, 1.21) 0.9598	0.00 (1.00) -0.22	-0.01 (1.00) -0.18	1.00 (0.82, 1.21) 0.9930	0.02 (1.01) -0.20	-0.04 (0.98) -0.15	0.96 (0.79, 1.16) 0.6753
CPII	0.01 (1.03) -0.12	-0.01 (0.95) -0.18	0.96 (0.80, 1.16) 0.6848	0.02 (1.00) -0.09	-0.04 (1.00) -0.18	0.92 (0.75, 1.12) 0.3963	0.05 (1.00) -0.07	-0.10 (1.01) -0.17	0.84 (0.69, 1.02) 0.0814
CS846	-0.01 (0.96) -0.20	0.02 (1.09) -0.23	1.05 (0.88, 1.24) 0.6071	-0.01 (0.95) -0.20	0.02 (1.11) -0.28	1.04 (0.87, 1.25) 0.6637	0.00 (0.95) -0.18	-0.00 (1.10) -0.30	1.00 (0.84, 1.20) 0.9749

Soluble BM: Single Biomarker Associations
(adjusted for baseline covariates — Serum 2 of 2)

Biomarker	Baseline Concentration			12 Month TIC			24 Month TIC		
	Mean (SD) Median z score		OR, 95% CI, P value	Mean (SD) Median z score		OR, 95% CI, P value	Mean (SD) Median z score		OR, 95% CI, P value
	Comparators	Cases		Comparators	Cases		Comparators	Cases	
CTXI	-0.05 (1.01) -0.28	0.10 (0.96) -0.04	1.18 (0.99, 1.40) 0.0642	-0.07 (0.98) -0.24	0.16 (1.04) 0.02	**1.28 (1.07, 1.53) 0.0066**	-0.06 (0.96) -0.23	0.12 (1.07) 0.01	**1.21 (1.02, 1.44) 0.0277**
COMP	0.02 (1.02) -0.12	-0.05 (0.96) -0.24	0.89 (0.74, 1.08) 0.2356	0.02 (1.01) -0.11	-0.03 (0.98) -0.24	0.91 (0.75, 1.10) 0.3446	0.03 (1.00) -0.05	-0.07 (1.01) -0.21	0.86 (0.71, 1.04) 0.1147
HA	-0.04 (1.02) -0.34	0.08 (0.96) -0.22	1.09 (0.90, 1.31) 0.3818	-0.06 (0.99) -0.35	0.12 (1.02) -0.19	1.18 (0.97, 1.43) 0.0970	-0.05 (0.96) -0.36	0.11 (1.06) -0.16	1.16 (0.96, 1.41) 0.1159
MMP-3	-0.02 (1.00) -0.22	0.04 (1.01) -0.22	1.00 (0.81, 1.22) 0.9805	-0.02 (1.01) -0.23	0.05 (0.99) -0.14	1.02 (0.82, 1.28) 0.8342	-0.02 (1.00) -0.22	0.05 (0.99) -0.13	1.00 (0.81, 1.24) 0.9876
NTXI	-0.05 (0.99) -0.20	0.11 (1.02) 0.01	1.19 (1.00, 1.42) 0.0514	-0.08 (0.97) -0.19	0.18 (1.05) 0.02	**1.29 (1.08, 1.55) 0.0056**	-0.05 (0.94) -0.14	0.10 (1.11) -0.01	1.16 (0.97, 1.38) 0.0951
PIIANP	0.04 (0.99) -0.03	-0.09 (1.03) -0.16	0.88 (0.74, 1.05) 0.1577	0.06 (0.98) 0.09	-0.13 (1.04) -0.18	**0.83 (0.69, 0.99) 0.0431**	0.07 (0.95) 0.13	-0.16 (1.09) -0.20	**0.79 (0.66, 0.94) 0.0076**

Soluble BM: Single Biomarker Associations
(adjusted for baseline covariates — Urine, Creatinine adjusted)

Biomarker	Baseline Concentration			12 Month TIC			24 Month TIC		
	Mean (SD) Median z score		OR, 95% CI, P value	Mean (SD) Median z score		OR, 95% CI, P value	Mean (SD) Median z score		OR, 95% CI, P value
	Comparators	Cases		Comparators	Cases		Comparators	Cases	
Coll2-1 NO2	-0.02 (1.01) -0.27	0.04 (0.98) -0.19	1.08 (0.91, 1.28) 0.3677	-0.03 (1.01) -0.28	0.07 (0.99) -0.11	1.14 (0.95, 1.36) 0.1542	-0.01 (0.99) -0.23	0.03 (1.02) -0.20	1.07 (0.90, 1.27) 0.4447
C12C	0.04 (1.02) -0.12	-0.08 (0.96) -0.32	0.89 (0.74, 1.07) 0.2245	0.02 (1.02) -0.11	-0.04 (0.95) -0.09	0.96 (0.79, 1.15) 0.6335	0.02 (1.03) -0.13	-0.03 (0.94) -0.13	0.95 (0.79, 1.14) 0.5848
C2C HUSA	-0.05 (0.93) -0.20	0.11 (1.12) -0.06	1.15 (0.96, 1.38) 0.1222	-0.06 (0.97) -0.25	0.12 (1.05) -0.04	1.18 (0.98, 1.42) 0.0874	-0.06 (0.98) -0.26	0.12 (1.04) -0.07	1.17 (0.98, 1.41) 0.0885
CTXII	-0.07 (0.98) -0.35	0.15 (1.04) -0.08	**1.29 (1.07, 1.54) 0.0063**	-0.08 (1.01) -0.30	0.18 (0.96) 0.05	**1.34 (1.11, 1.62) 0.0020**	-0.07 (1.01) -0.28	0.15 (0.97) 0.08	**1.29 (1.08, 1.55) 0.0062**
NTXI	-0.06 (1.00) -0.24	0.12 (1.00) -0.05	**1.22 (1.02, 1.45) 0.0301**	-0.07 (0.98) -0.17	0.15 (1.03) -0.10	**1.28 (1.07, 1.55) 0.0083**	-0.06 (0.97) -0.18	0.11 (1.04) -0.16	**1.23 (1.03, 1.47) 0.0259**
CTX-1α	-0.06 (0.98) -0.26	0.12 (1.03) -0.08	**1.21 (1.02, 1.45) 0.0294**	-0.08 (0.97) -0.28	0.17 (1.04) -0.01	**1.29 (1.07, 1.55) 0.0063**	-0.07 (0.97) -0.28	0.15 (1.05) -0.01	**1.27 (1.06, 1.52) 0.0081**
CTX-1ß	-0.03 (1.01) -0.27	0.07 (0.98) -0.12	1.14 (0.95, 1.36) 0.1502	-0.06 (0.97) -0.27	0.13 (1.06) -0.10	**1.27 (1.05, 1.52) 0.0123**	-0.05 (0.95) -0.26	0.11 (1.09) -0.14	**1.22 (1.02, 1.46) 0.0299**

Selecting Markers for Combinatorial Approach Using Measures of Discrimination

- **Two stage approach**
  1. We selected markers for multivariable modeling (combinatorial approach) based on univariate p-values (p<0.10)

  2. For each marker selected we evaluated net reclassification (NRI)
     - NRI: measures the improvement in risk prediction between the old and new models by comparing number of advantageous reclassifications to the number of disadvantageous reclassifications (NRI>10%)

- **Additionally we took into consideration correlation among biomarkers to avoid colinearity problems in multivariable modeling**

Selecting Markers for Combinatorial Approach Using Measures of Discrimination

- **ROC analysis:**
  - Illustrates the performance of a set of variables as its discrimination threshold is varied
  - AUC: probability that a set of markers would rank a randomly chosen case higher than a randomly chosen control (c-statistics)

- **Cross-validation:**
  - Used 10-fold cross validation to assess the prediction error.
  - Split data randomly into 10 equally-sized subsets
    - Of the 10 subsets, 9 are used as training data to estimate the model, and model performance is tested on the 10th subset
    - Process is repeated 10 times, so that each observation is included exactly ones as part of the testing dataset

38% within 1/2 SD on either side of mean

68% within 1 SD on either side of mean

31% > 1 SD above mean
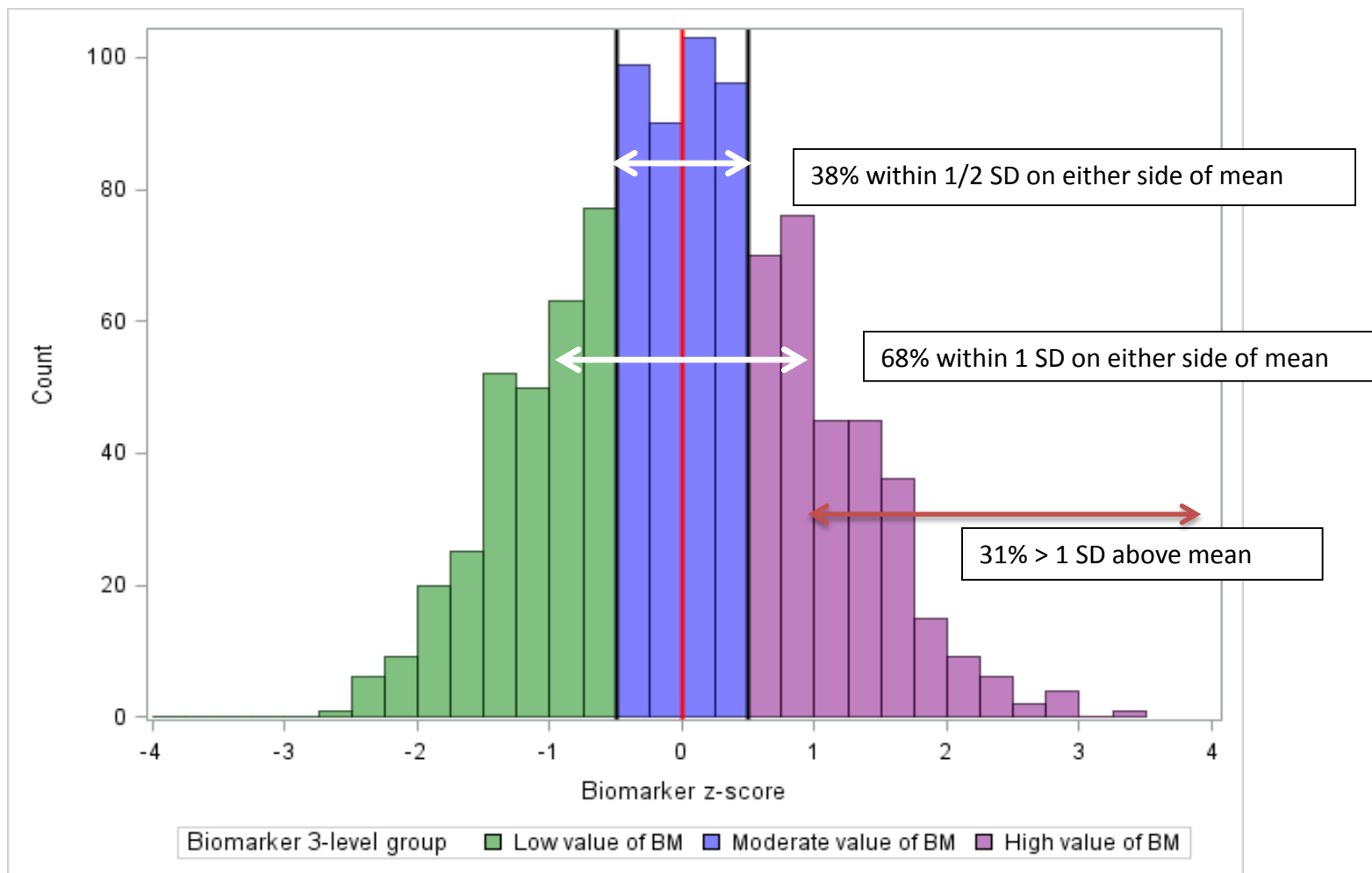
CTXI, serum
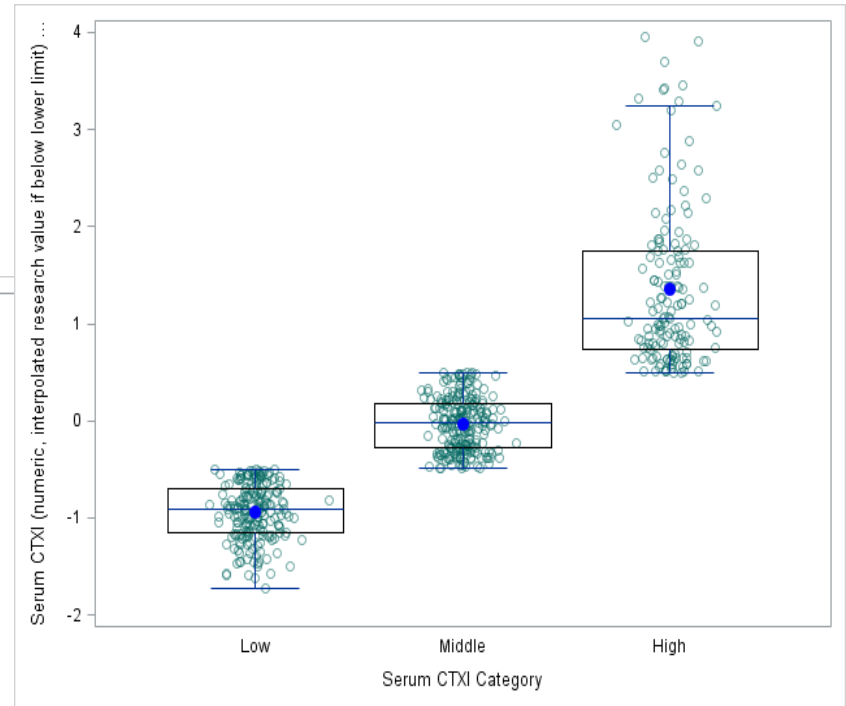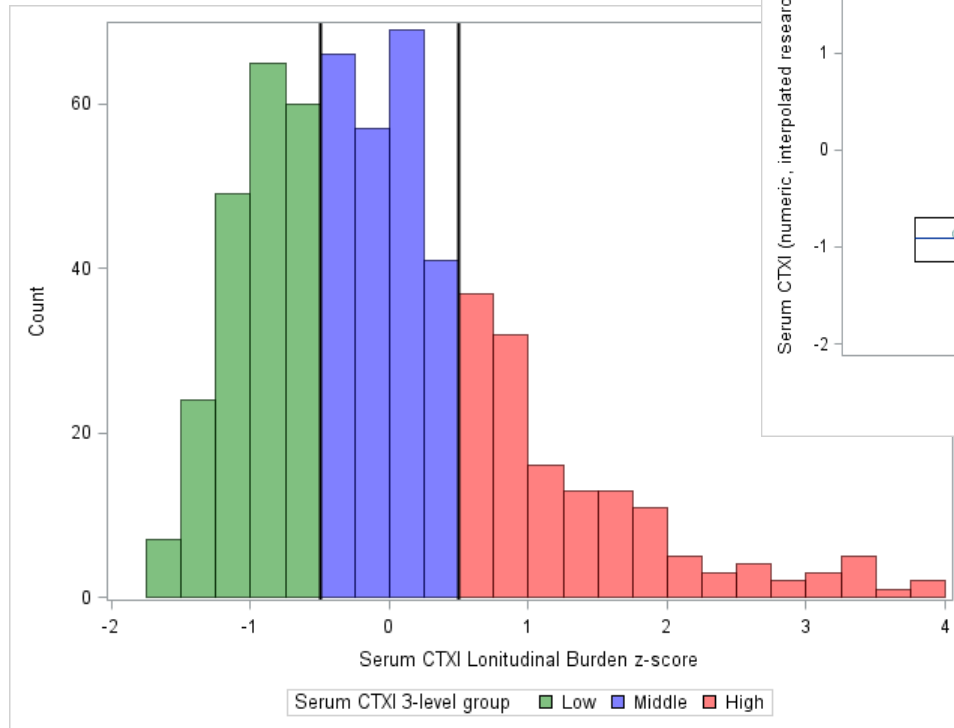
Soluble BM: Combinatorial Analytic Strategy

- For those biochemical markers selected for combinatorial approach we created 5-level categorical variables

- Categories were created based on z-score:
  - < - 1 SD below the mean
  - between 1 and 0.5 SDs below the mean
  - within 0.5 SDs on either side of the mean
  - between 0.5 and 1 SDs above the mean
  - > 1 SD above the mean

- Each category was assigned a score: -1, -0.5, 0, 0.5, 1

- Since lower levels of Serum PIIANP should be associated with being a case, the categories were reverse coded

Results: Selecting Markers for Combinatorial Analysis Using Measures of Discrimination

	OR	OR 95% CI	p-value	NRI	CI	NRI Cases - % correctly reclassified	Controls - % correctly reclassified
Serum CPII	0.84	0.69, 1.02	0.0814	0.0934	-.0777, .2646	23%	-13%
Serum CTXI	1.21	1.02, 1.44	0.0277	0.1924	0.0222, .3627	-3%	22%
Serum HA	1.16	0.96, 1.41	0.1159	0.1027	-.0718,0.2772	3%	7%
Serum NTXI	1.16	0.97, 1.38	0.0951	0.0890	-.0852,0.2632	10%	-1%
Serum PIIANP	0.79	0.66, 0.94	0.0076	0.2353	0.0646,0.4060	9%	15%
Urine CTX-1a (Ur_alpha)	1.27	1.06, 1.52	0.0081	0.1778	0.0039,0.3516	6%	12%
Urine CTX-1ß (Ur_beta)	1.22	1.02, 1.46	0.0299	0.0631	-.1110,0.2373	7%	-0%
Urine C2C	1.17	0.98, 1.41	0.0885	0.1152	-.0583,0.2888	12%	-0%
Urinary CTXII	1.29	1.08, 1.55	0.0062	0.2698	0.0970,0.4425	15%	12%
Urine NTXI	1.23	1.03, 1.47	0.0259	0.0326	-.1417,0.2069	5%	-2%

Pearson Correlations Among Selected Markers

Marker	Serum CTXI	Serum HA	Serum NTXI	Serum PIIANP	Urine C2C	Urine CTXII	Urine NTXI	Urine CTXI alpha	Urine CTXI beta
Serum CTXI		0.079	0.643	0.055	0.245	0.381	0.798	0.803	0.827
Serum HA			0.109	0.003	0.322	0.375	0.094	0.093	0.059
Serum NTXI				0.100	0.234	0.342	0.584	0.594	0.562
Serum PIIANP					0.120	0.072	0.053	0.042	0.058
Urine C2C						0.657	0.366	0.331	0.275
Urine CTXII							0.482	0.440	0.422
Urine NTXI								0.902	0.862
Urine CTXI alpha									0.850

Biochemical Markers: Mechanisms and Direction

Marker	Mechanism	If OA Progressing we expect...	
		High vs Low	TIC
Serum_CTXI	Bone Resorption (BR)	H	H
Serum_NTXI	Bone Resorption (BR)	H	H
Serum_HA	Inflammation (I)	H	H
Serum_PIIANP	Cartilage Synthesis (CS)	L	L
Urine CTXII	Cartilage Degradation (CD)	H	H
Urine C2C	Cartilage Degradation (CD)	H	H
Urine NTXI	Bone Resorption/Turnover (BR)	H	H
Urine CTX-1alpha	Bone Resorption/Turnover (BR) (new bone)	H	H
Urine CTX-1beta	Bone Resorption/Turnover (BR) (old bone)	H	H

Groupings for the Final Models: Predicting Case-Control Status Based on TIC

Marker	Groups
Serum CTXI Group 3-level	less than 0.5 SDs above the mean greater than 0.5 SD above the mean
Serum HA Group 2-level	Less than 0.5 SDs above the mean 0.5 SDs above the mean or greater
Serum NTXI Group 3-level	less than 1 SD above the mean greater than 1 SD above the mean
Serum PIIANP Group 2-level	Less than 0.5 SDs above the mean 0.5 SDs above the mean or greater
Urinary CTXII creatinine adj Group 2-level	at least 0.5 SDs below the mean 0.5 SDs below the mean or greater
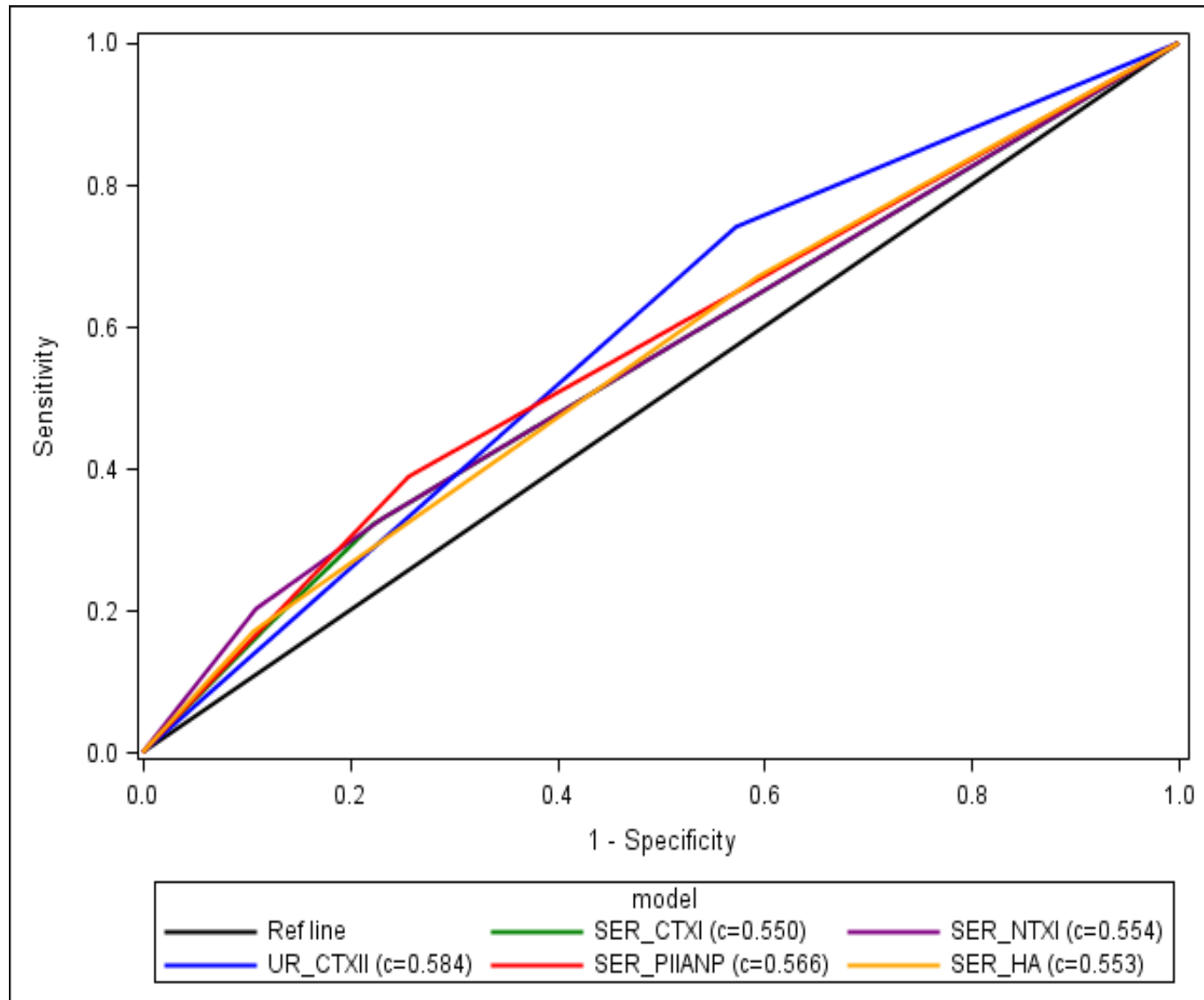
Additional Modeling Considerations

■ We evaluated the performance of various combinations of biomarkers:

- Including all 5 markers in a logistic model

- Including only markers that were statistically significant in the multivariable model (Urine CTXII, Serum PIIANP)

- Including all serum markers

- Combinations of the best performing markers in preliminary analysis (Urine CTXII, Serum PIIANP, Serum NTXI)

- For reference, we've also included the ROC curve for the best performing (by c-statistic) univariate marker Urine CTXII

ROC Analysis using Combination of Biomarkers
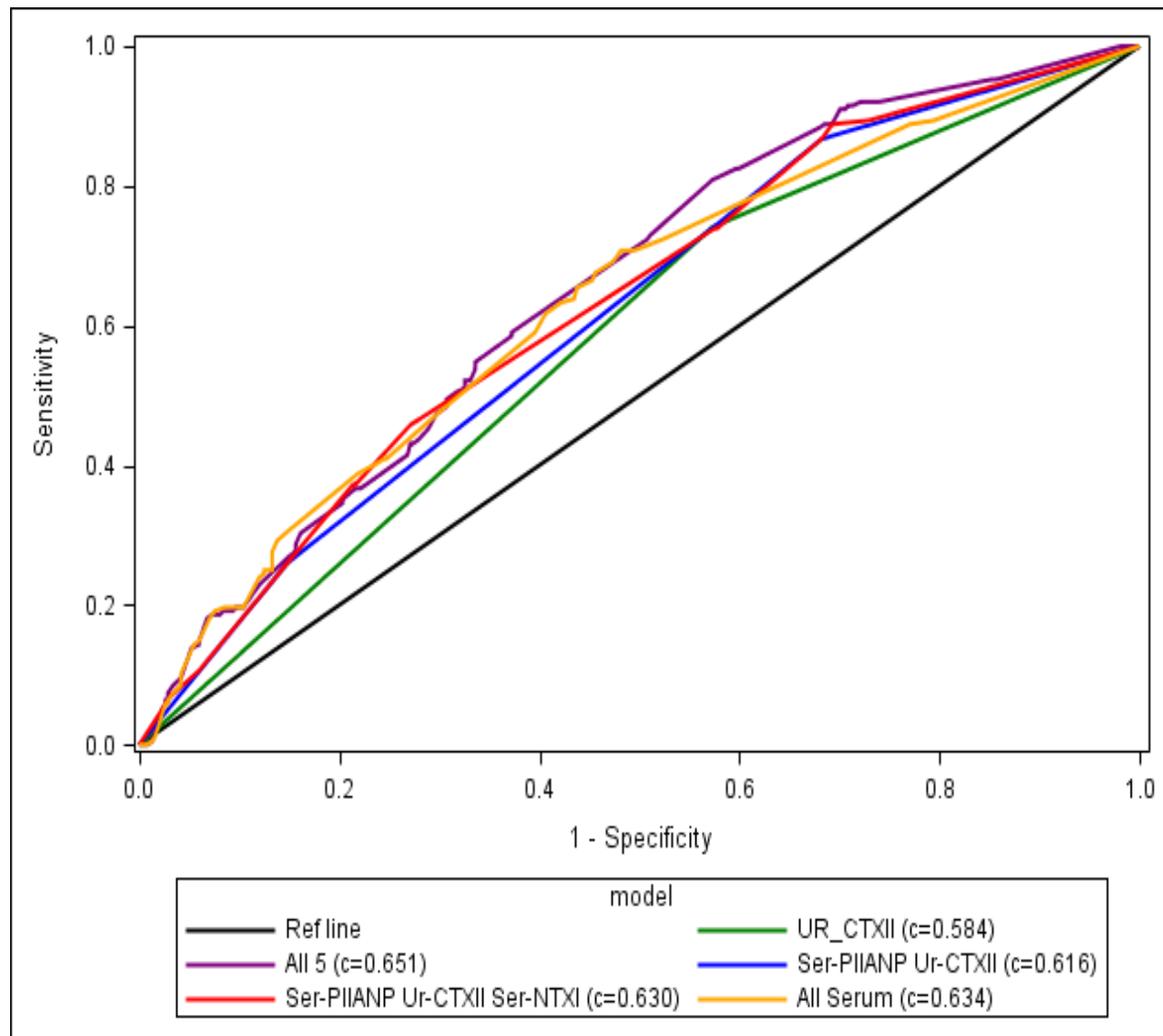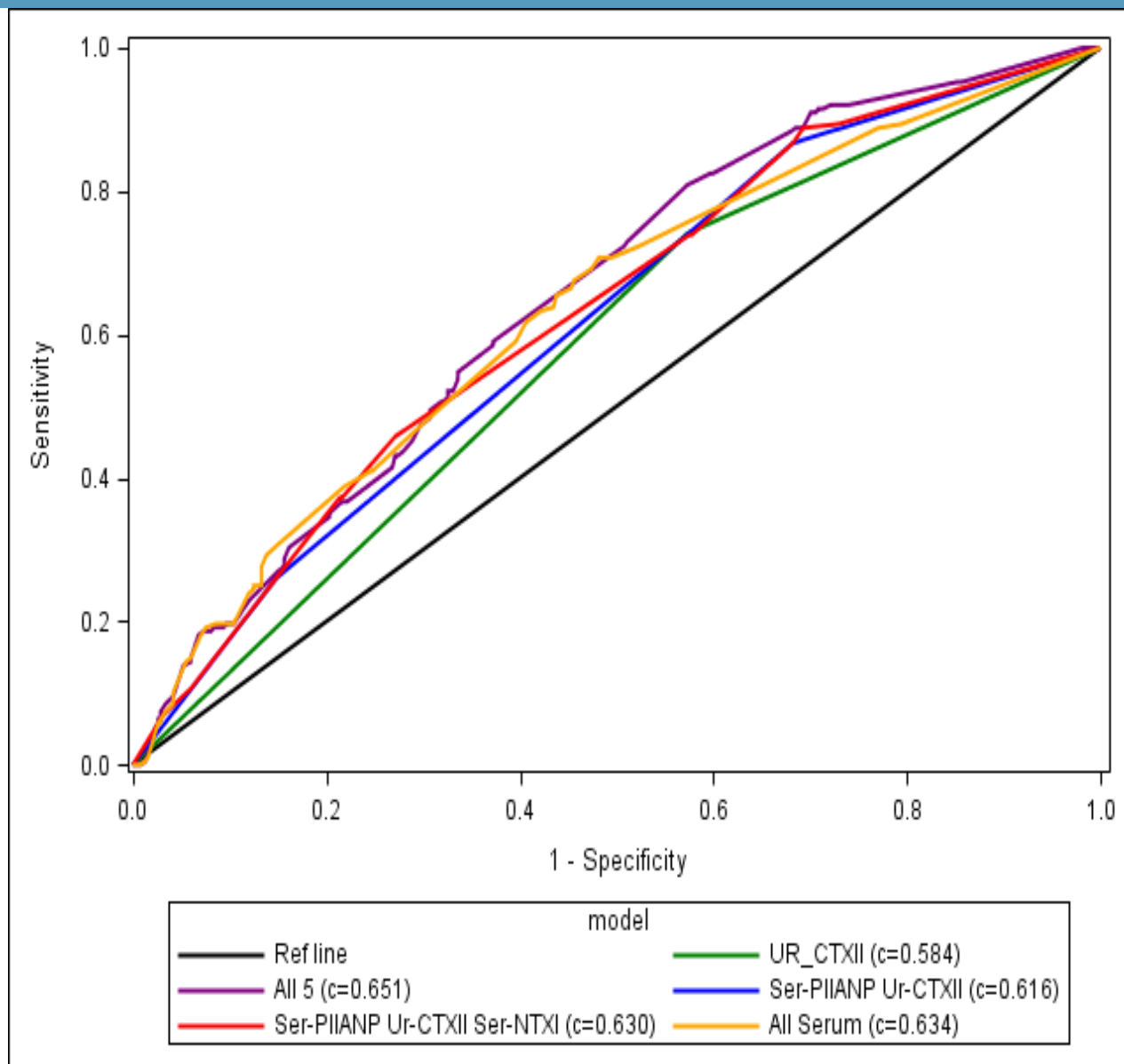
Soluble BM: Combinatorial Results: Adjusted for Covariates

Receiver Operator Characteristic (ROC) Curves for 24 month TICs
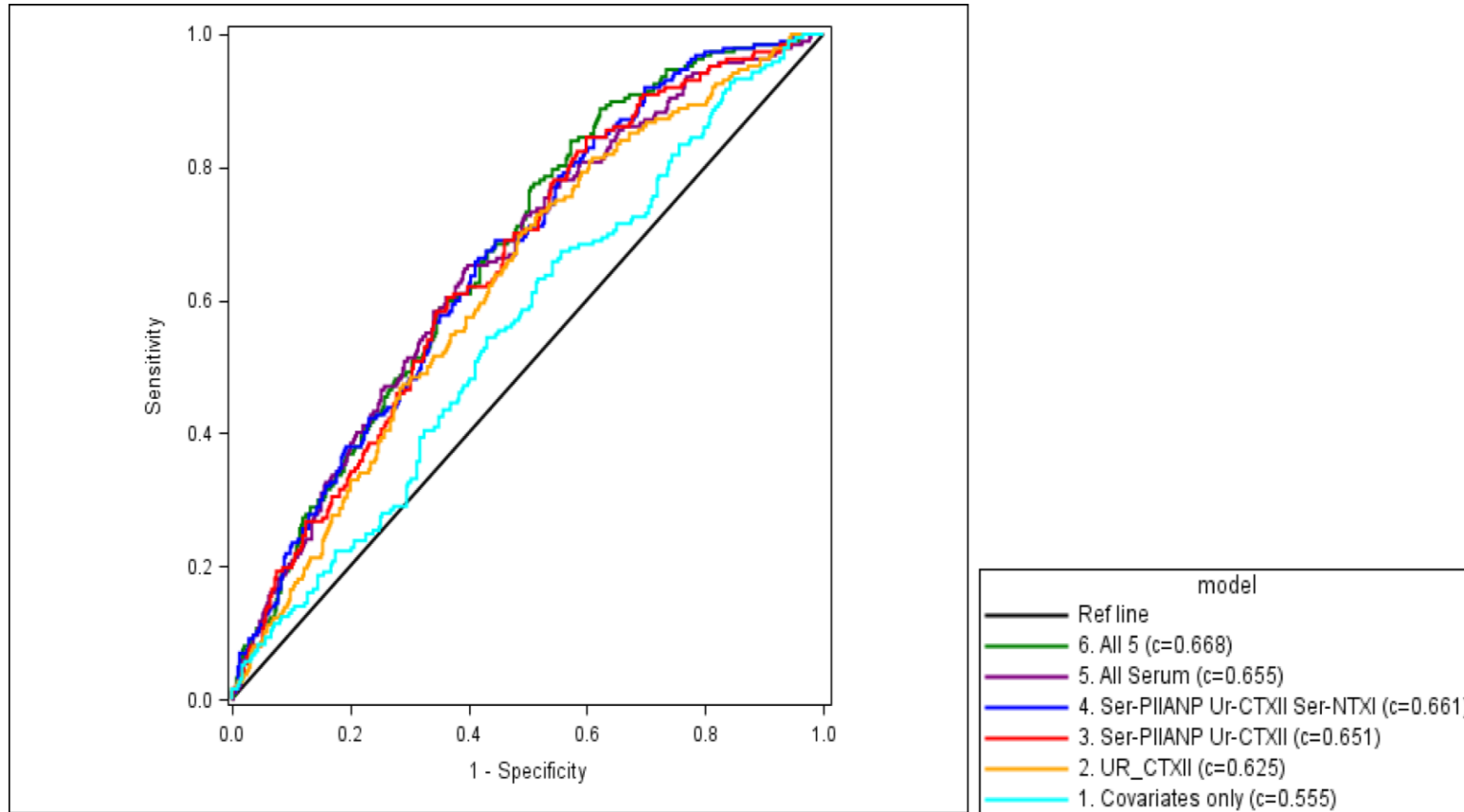
Soluble BM: Combinatorial Analysis-24 month TIC Predicting Case Status with Cross-Validation

model	C-statistic (no CV)	10-fold CV C-statistic - mean	10-fold CV C-statistic – range (min)	10-fold CV C-statistic – range (max)
Covariates Only	0.555	0.511	0.488	0.535
U-CTXII	0.625	0.594	0.577	0.607
Ser-PIIANP Ur-CTXII	0.651	0.620	0.606	0.631
Ser-PIIANP Ur-CTXII Ser-NTXI	0.661	0.628	0.614	0.638
All Serum 4*	0.655	0.613	0.594	0.627
All 5	0.668	0.627	0.614	0.640

CV=cross-validation;
serum 4: PIIANP, NTXI, CTXI, HA;   all 5: serum 4+urine CTXII

BM Analysis: Conclusions I

- The catabolic biomarkers (CTXII, CTX-I and NTX-I) were positively associated with OA progression while the anabolic biomarker (PIIANP) was negatively associated with OA progression.

- These type I collagen biomarkers along with two representative of type II collagen (CTXII and PIIANP) were the most predictive of case status.

- These results are consistent with the long recognized association of OA with bone abnormalities and the promise shown for a number of bone-acting agents for treating OA.

- The inflammatory biomarker (HA) was positively but not significantly associated with OA progression on its own but did contribute to the combinatorial prediction.

BM Analysis: Conclusions II

- The dynamic nature of biomarkers necessitated appropriate approaches to longitudinal analysis including the Time-Integrated-Concentration (TIC).

- The 24 month TIC of several biomarkers (singly and in combination) was superior to baseline covariates for predicting case status at 48 months.

- Although two of the markers (CTXI and NTXI) are *in vitro* diagnostics approved for osteoporosis, none of these biochemical markers are yet approved for clinical use for OA.

Project Data Access

David J. Hunter

Public Data Release – OAI Database

- Imorphics, Qmetrics & Chondrometrics imaging datasets were publically released on February 27th

- BICL and BioMediq datasets were publically released on the OAI Database on April 10th

- The Scaffold files (MSBioworks) – data and associated epitope mapping methodology were publically released on April 10th
  - Richard Jones has provided an instructional slide deck that walks users through downloading the free Scaffold software and opening the data files for individual use
  - Files viewed/accessed through Scaffold can be saved into Excel for further manipulation

- The Biochemical (serum/urine) and FSA datasets will be released May 29th
  - All OA Partners currently have access to these datasets; many have requested and been using them for some time
  - UCSF/OAI is working on final QC of data, uploads, etc. – may be able to release a week sooner.

Next Steps and Closing Remarks

David J. Hunter

Virginia Byers Kraus

Thumbnail Summary of Publications

- All biomarker measurements are completed
- Statistical analyses are ongoing

Publications

- Study design paper has been published: [Best Practice & Research Clinical Rheumatology 28 (2014) 61–71](#)

- Preliminary Assessment of Predictive Validity Periarticular Bone Area and Shape Markers in Knee OA, #336
  - **(Poster/Abstract, OARSI 2013)**

- Establishment of Reference Intervals for Osteoarthritis Related Biomarkers – The FNIH/OARSI OA Biomarkers Consortium
  - **(Podium Pres/Abstract, OARSI 2014)**

- Preliminary Assessment of Predictive Validity of Semi-quantitative MRI Biomarkers in Knee OA – The FNIH Biomarkers Consortium
  - **(Poster/Abstract, OARSI 2014)**

- Preliminary Assessment of Predictive Validity of Cartilage Thickness MRI Biomarkers in Knee OA – the FNIH OA Biomarkers Consortium
  - **(Poster/Abstract, ACR 2014)**

4

Biomarkers for osteoarthritis: Current position and steps towards further validation

David J. Hunter [a,*], Michael Nevitt [b], Elena Losina [c,d], Virginia Kraus [e]

[a] Department of Rheumatology, Royal North Shore Hospital and Northern Clinical School, Kolling Institute, University of Sydney, Reserve Road, St Leonards, Sydney, NSW 2065, Australia
[b] Department of Epidemiology and Biostatistics, OAI Coordinating Ctr., University of California, San Francisco (UCSF), San Francisco, CA, USA
[c] Orthopaedic and Arthritis Center for Outcomes Research, Department of Orthopedic Surgery, Brigham and Women's Hospital, Boston, MA, USA
[d] Harvard Medical School, Boston, MA, USA
[e] Department of Medicine, Duke University, Rheumatology & Immunology, Durham, NC, USA

Keywords:
Osteoarthritis
Biomarkers

A B S T R A C T

Historically disease knowledge development and treatment innovation in osteoarthritis (OA) has been considered to be slow. One of the many reasons purported as responsible for this slow pace has been the alleged lack of valid and responsive biomarkers to ascertain efficacy, which itself has been dependent upon the slow evolution of the understanding of the complex nature of joint tissue biology. This narrative review outlines the rationale for why we need OA biomarkers with regard to biomarker validation and qualification. The main biomarkers in current development for OA are biochemical and imaging markers. We describe an approach to biomarker validation and qualification for OA clinical trials that has recently commenced with the Foundation of NIH OA Biomarkers Consortium study cosponsored by the Osteoarthritis Research Society International (OARSI). With this approach we endeavor to identify, develop, and qualify biological markers (biomarkers) to support new drug development, preventive medicine, and medical diagnostics for osteoarthritis.

© 2014 Elsevier Ltd. All rights reserved.

Summary of Publications – Submitted & In Preparation

- PRELIMINARY ASSESSMENT OF PREDICTIVE VALIDITY OF PERIARTICULAR BONE AREA AND SHAPE MARKERS IN KNEE OSTEOARTHRITIS
  - ➤ **Submitted to Annals of Rheumatic Disease 3/16**

- CARTILAGE THICKNESS CHANGE AS AN IMAGING BIOMARKER OF KNEE OSTEOARTHRITIS PROGRESSION – DATA FROM THE FNIH OA BIOMARKERS CONSORTIUM
  - ➤ **Submitted to Arthritis & Rheumatology 3/25**

- PREDICTIVE VALIDITY OF BIOCHEMICAL BIOMARKERS IN KNEE OA - THE OARSI / FNIH OA BIOMARKERS CONSORTIUM
  - ➤ **Final analysis and draft in progress**

- PREDICTIVE VALIDITY OF RADIOGRAPHIC BONE TRABECULAR INTEGRITY IN KNEE OA - THE OARSI / FNIH OA BIOMARKERS CONSORTIUM
  - ➤ **Final analyses and draft in progress**

- SEMI-QUANTITATIVE MRI ASSESSMENT IN THE FNIH BIOMARKERS CONSORTIUM STUDY: OVERVIEW OF METHODOLOGY AND DEFINITION OF CHANGE
  - • **Analysis Plan to be distributed, ongoing analysis**

Summary of Publications – In Preparation

- PRELIMINARY ASSESSMENT OF PREDICTIVE VALIDITY OF SEMI-QUANTITATIVE MRI BIOMARKERS IN KNEE OA: FNIH OA BIOMARKERS CONSORTIUM
  - **Analysis Plan to be distributed, ongoing analysis**

- PREDICTIVE VALIDITY OF CARTILAGE AND MENISCAL VOLUME IN KNEE OA - THE FNIH OA BIOMARKERS CONSORTIUM
  - **Ongoing analysis**

- SUMMATIVE PROJECT MANUSCRIPT – RESPONSIVENESS OF MRI MEASURES – THE FNIH OA BIOMARKERS CONSORTIUM
  - **Ongoing analysis**

- SUMMATIVE PROJECT MANUSCRIPT – IMAGING AND FLUID BIOMARKERS OF OSTEOARTHRTIS
  - **Ongoing analysis**

- ESTABLISHMENT OF REFERENCE INTERVALS FOR OSTEOARTHRITIS RELATED BIOMARKERS
  - *Final analyses and draft in progress*

OA Biomarkers Project - Phase 2 Aims

- The overarching goal of this proposal is to pursue formal FDA and EMA qualification of OA biomarkers

- Our objective is to pursue qualification of biomarkers pertinent to knee OA for:
  - Prognostic biomarkers (baseline predicting progression of pain and structure and longitudinal burden predicting JSW change in long-term in placebo group);
  - Efficacy of intervention (predictive of treatment response) — short term predicting long term response in radiograph

- This will be pursued by deploying best novel biomarker measures in extant clinical trials to determine if they have greater prognostic ability and are more predictive of treatment response than the existing gold standard of radiographic JSW

Direct Benefits of OA Biomarkers

- Will provide a rich set of qualified drug development tools
  - Stratification of OA subjects who are progressors; will allow for enrichment of clinical trials with identified progressors
  - Will provide potential biomarker surrogates to take the place of the current radiographic joint space narrowing

- Will facilitate smaller, shorter trials more closely linked to clinical outcome endpoints, thereby dramatically reducing OA clinical trial costs

- Will inform the biological and clinical context of marker performance

Timelines for Phase 2 Project

■ **Proposal Development**

- Decision on imaging and biochemical BMx to include – May 2015
  - Need to establish criteria for inclusion/exclusion
- Outreach with Phase 1 data to potential funding partners
  - Q2/Q3 2015
- Work with regulatory agency liaisons (FDA/EMA)

■ **Concept Proposal submission to IISC**

- Mid 2015

■ **Funding Commitments**

- Funding commitments (letters of intent) finalized after full Project Plan approval by Biomarkers Consortium IISC and Executive Committee in Q4 2015
- Project ready for launch and contracts executed in Q1 2016

Acknowledgements