MOST Ancillary Study 05-01 (AS05-01)
"Inflammation and Knee Osteoarthritis" (Beth Lewis)

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1. Dataset description and Analyst Notes

Dataset: AS0501_bioassay.sas7bdat
Observations: 2348 records (1174 participants: 17 assays or 9 assays; 2 visits)
Documentation:

- VariableGuide_AS0501_bioassay.pdf
- Distributions_AS0501_bioassay.pdf

AS0501_bioassay dataset contains 2348 records; two records per participant; unique record per participant and visit. Assays were performed at the University of Vermont Laboratory for Clinical Biochemistry Research on baseline and 30-month plasma or serum paired samples.

Note: the laboratory performing the assays was blinded to subject ID but provided with paired link to ensure that both samples collected at baseline and 30-month time points from the same participant were included in the same batch (each sample, each assay).

Analyst Notes:

- When assay results were not obtained, special missing value were used:
  .L = below low detection level
  .H =above high detection level

  For categorical analyses, the values coded as undetectable low (.L) or undetectable high (.H) can be included in analysis using the cut point value indicated in the literature. For example, an adiponectin value of .H could be recoded to 75,000 ng/mL for inclusion in this analysis. Zero is not a valid result for any of these analytes.

- Each record is marked to indicate visit when sample of blood was collected using variable VISIT (values: V0 (baseline), V2 (30-month)).

- IMPORTANT NOTE: Leptin assays were done on plasma (2008) and on serum (2010), therefore there are two separate variables included in the dataset (leptin for the plasma assay results and leptin_se for the serum assay results). It is not recommended by laboratory to combine both in the same analysis unless properly controlled.

- If there was insufficient volume or some other reason assay could not be performed, and all assay values are missing, the record is not included in the analytical dataset.

2. Selection plan

Baseline and 30-month paired sample lab analysis will be done for all selected participants.

Selection criteria:

2008 Pilot Study Inclusion (n=100 random sample)

- Female
- Caucasian
- Gadolinium 1.5T MRI done at 30-month clinic visit (see selection criteria for D. Felson MOST Ancillary Study (AS04-07) entitled “Structural Correlates of Knee Pain”
- Baseline and 30-month serum, plasma, and urine samples available

2010 Study Inclusion (n=1076)[1]

- Non-hemolyzed baseline and 30-month serum and plasma samples available[2]
- Baseline buffy coat collected and consented for DNA testing

[1] Participants selected for the 2008 pilot study did not have their specimens retested in 2010.

[2] Urine was not needed for the 2010 lab assays.

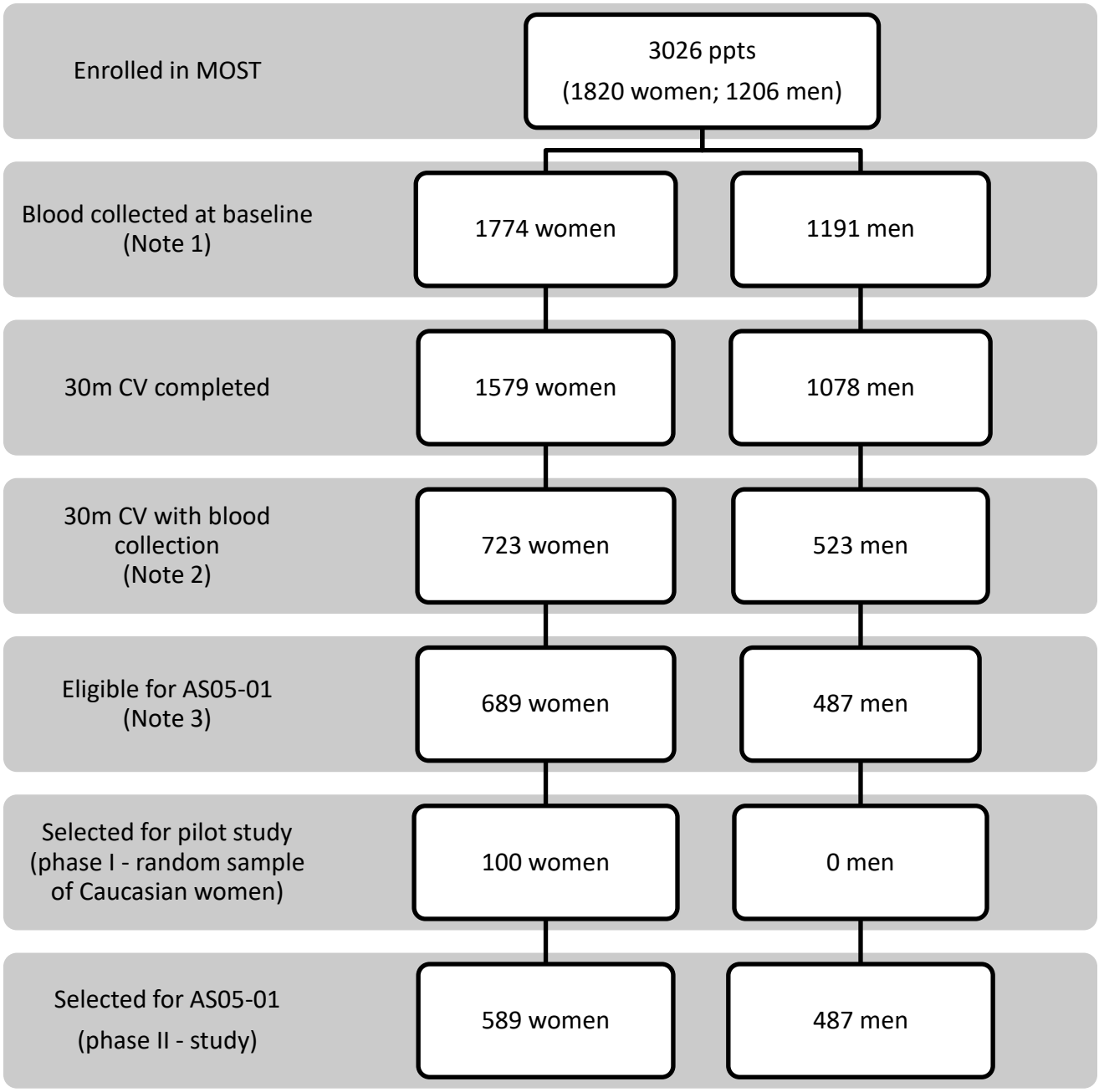
3. Assays

Based on results of the pilot study in 2008, a subset of assays were selected for the study done in 2010.

2008 Pilot	2010 Study	Assay	Blood type
X	X	TNFa (Tumor Necrosis Factor alpha)	EDTA plasma
X	X	Adiponectin Total	EDTA plasma
X	X	CRP (C-Reactive Protein)	EDTA plasma
X	X	Ox LDL (Oxidized Low-Density Liproprotein)	EDTA plasma
X*	X*	Leptin*	EDTA plasma / Serum
X		MCP-1 (Monocyte Chemotactic Protein 1)	EDTA plasma
X		PAI-1 Total (Total Plasminogen Activator Inhibitor-1)	EDTA plasma
X	X	MMP3 (Matrix Metalloproteinase-3)	Serum
X	X	TIMP-1 (Tissue Inhibitor of Metalloproteinase-1)	Serum
X	X	ICAM-1 (Intercellular Adhesion Molecule-1)	Serum
X	X	COMP (Cartilage Oligomeric Matrix Protein)	Serum
X		IGF-1 (Insulin-like Growth Factor-1)	Serum
X		IGFBP-3 (Insulin-like Growth Factor Binding Protein-3)	Serum
X		TGFb (Tissue Growth Factor beta)	Serum
X		Estradiol	Serum
X		SHBG (Sex Hormone Binding Globulin)	Serum
X		DHEA-s (Dehydroepiandrosterone-sulfate)	Serum

* Leptin assays done on plasma (2008) and on serum (2010). It is not recommended by laboratory to combine both in the same analysis unless properly controlled.

4. Flow-chart



Notes:

1) All participants enrolled in the study were eligible for blood collection during baseline visit; only a few participants were unable to complete the blood collection process.

2) Only random sample of participants (approx. 50%) were eligible for blood collection at the 30-month visit due to parent study funding limitations. Participants with known knee replacement (prior to the 30-month visit) were not eligible to be selected for blood collection.

3) Additional selection criteria for ancillary study eligibility: paired blood sample cannot be hemolyzed, participants are required to have DNA consent on file and buffy coat sample collected.

5. Appendix A. Assay documentation provided by laboratory

Table 1: By assay performed in 2008 (pilot study) and 2010 (main study).

Study batch	Assay	Manufacturer	Method	Catalog#	Volume	Sample Type	Range of Standard	Dilution	Low Detection	High Detection	Estimated Normal Range
Pilot	TNFa (Tumor Necrosis Factor alpha)	Millipore	Bio-Rad Luminex Flow Cytometry	HADK2-61K-B	75	EDTA	0.64 - 10,000 pg/mL	1	0.64	10000	ND - 4.2 pg/mL
Main		Millipore	Bio-Rad Luminex Flow Cytometry	HADK2-61K-B	75	EDTA	0.32 - 10,000 pg/mL	1	0.32	10,000	ND - 4.2 pg/mL
Pilot	Leptin*	Millipore	Bio-Rad Luminex Flow Cytometry	HADK2-61K-B	75	EDTA	16 - 250,000 pg/mL	1	16	250000	Male: 2205 - 11149 pg/ml: Females: 3877 - 77273 pg/mL
Main		R&D Systems	Elisa	DLP00	10	Serum	15.6 - 1000 pg/mL	100	!1300	~120,000	Serum Males(,2205-11,149 pg/mL) Females (3,877 - 77,273 pg/mL)
Pilot	Adiponectin Total	Millipore	Bio-Rad Luminex Flow Cytometry	HADK1-61K-A	2	EDTA	0.016 - 250 ng/mL	300	4.8	75000	1198 - 19973 ng/ml
Main		Millipore	Bio-Rad Luminex Flow Cytometry	HADK1-61K-A	2	EDTA	0.016 - 250 ng/mL	300	4.8	75,000	1198 - 19973 ng/ml
Pilot	CRP (C-Reactive Protein)	Seimens	BNII: Nephelometry		40	EDTA	0.0073 - 0.4675 mg/L	20	0.16	no limit	< 3 mg/L
Main		Seimens	BNII: Nephelometry		40	EDTA	0.0073 - 0.4675 mg/L	20	0.16	no limit	< 3 mg/L
Pilot	Ox LDL (Oxidized Low-Density Liproprotein)	Mercodia	Elisa	10-1158-01	25	EDTA	0.4-8.1U/L	41	16.4	332.1	
Main		Mercodia	Elisa	10-1158-01	25	EDTA	0.4-8.1U/L	41	16.4	332.1	none given
Pilot	MCP-1 (Monocyte Chemotactic Protein 1)	Millipore	Bio-Rad Luminex Flow Cytometry	HADK2-61K-B	75	EDTA	0.64 - 10,000 pg/mL	1	0.64	10000	72 - 295 pg/mL
Pilot	PAI-1 Total (Total Plasminogen Activator Inhibitor-1)	Millipore	Bio-Rad Luminex Flow Cytometry	HADK1-61K-A	2	EDTA	.003 - 50 ng/mL	300	0.9	15000	5 - 80 ng/mL
Pilot	MMP3 (Matrix Metalloproteinase-3)	R&D	Elisa	DMP300	50	Serum	0.15 - 10 ng/mL	10	1.5	100	Serum (2.10 - 64.4 ng/mL)
Main		R&D Systems	Elisa	DMP300	50	Serum	0.15 - 10 ng/mL	10	1.5	~120	Serum (2.10 - 64.4 ng/mL)
Pilot	TIMP-1 (Tissue Inhibitor of Metalloproteinase-1)	R&D	Elisa	DTM100	10	Serum	0.156 - 10.0 ng/mL	100	15.6	1000	Serum (87-524 ng/mL)
Main		R&D Systems	Elisa	DTM100	10	Serum	0.156 - 10.0 ng/mL	100	15.6	1,000	Serum (87-524 ng/mL)
Pilot	ICAM-1 (Intercellular Adhesion Molecule-1)	R&D	Elisa	DCD540	20	Serum	1.56 - 50 ng/mL	20	31.2	1000	Serum (98.8 - 320 ng/mL)
Main		R&D Systems	Elisa	DCD540	20	Serum	1.56 - 50 ng/mL	20	2	1,000	Serum (98.8 - 320 ng/mL)
Pilot	COMP (Cartilage Oligomeric	AnaMar Medical	Elisa	14-1006-71	20	Serum	0.4 - 3.2 U/L	10	4	32	Approximately 2 to 20 with a mean of 10.2

Study batch	Assay	Manufacturer	Method	Catalog#	Volume	Sample Type	Range of Standard	Dilution	Low Detection	High Detection	Estimated Normal Range
Main	Matrix Protein)	AnaMar Medical	Elisa	14-1006-71	20	Serum	0.4 - 3.2 U/L	10	4	32	Approximately 2 to 20 with a mean of 10.2
Pilot	IGF-1 (Insulin-like Growth Factor-1)	DSL	Elisa	DSL-10-2800	20	Serum	11 - 600 ng/mL	1	11	600	Age 50-59 (66 - 310 ng/mL)
Pilot	IGFBP-3 (Insulin-like Growth Factor Binding Protein-3)	DSL	Elisa	DSL-10-6600	10	Serum	1.4 - 87 ng/mL	100	140	8700	Males (1500 - 4600 ng/mL) Females (2670 - 5580 ng/mL)
Pilot	Estradiol	ALPCO	Elisa Ultrasensitive	20-DR-4399	200	Serum	0 - 200 pg/mL	1	0.01	200	Males: 10 - 36 pg/mL, Pre-Menopause: 13-191 pg/mL; Post-menopause: 11-65 pg/mL
Pilot	SHBG (Sex Hormone Binding Globulin)	ALPCO	Elisa	11-SHBHU-E01	10	Serum	3.3 - 295 nmol/L	10	33	2950	Males (7 - 70 nmol/L) Females (15 - 120 nmol/L)
Pilot	DHEA-s (Dehydroepiandrosterone-sulfate)	ALPCO	Elisa	11-DHEHU-E01	50	Serum	0.5 - 1000 ug/dL	1	0.5	1000	Males (0.39 - 4.63 ug/mL) Females (0.46 - 2.75 ug/mL) Postmenopausal Females (0.48 - 2.08 ug.mL)

*Note, leptin results obtained from different type of sample (EDTA plasma for pilot and Serum for main study). It is not recommended to combine those results within the same analysis.

Table 2: Inter- Assay CV provided by laboratory

	Inter-Assay CVs			
Assay	Control 1	Control 2	Control 3	Control 4
TNFa	7.56%	7.03%	10.10%	19.12%
MCP-1	3.90%	3.95%	7.75%	5.56%
Leptin	11.14%	4.87%	4.57%	4.40%
Adiponectin	10.91%	4.62%	2.37%	6.58%
PAI-1 Total	6.16%	1.30%	7.74%	9.92%
CRP	2.78%	4.45%	2.96%	N/A
MMP3	12.89%	19.83%	5.17%	N/A
TIMP-1	8.16%	5.27%	3.67%	N/A
ICAM-1	13.93%	4.21%	5.17%	5.26%
IGF-1	9.60%	4.97%	2.31%	3.67%
IGFBP-3	4.23%	11.68%	6.28%	2.88%
Ox LDL	13.60%	17.40%	6.60%	N/A
TGFb	7.62%	14.74%	N/A	N/A
Estradiol	8.67%	19.48%	10.97%	N/A
SHBG	9.13%	7.50%	5.72%	5.30%
DHEA-s	15.43%	13.24%	5.77%	N/A

N/A = Not run for that particular assay

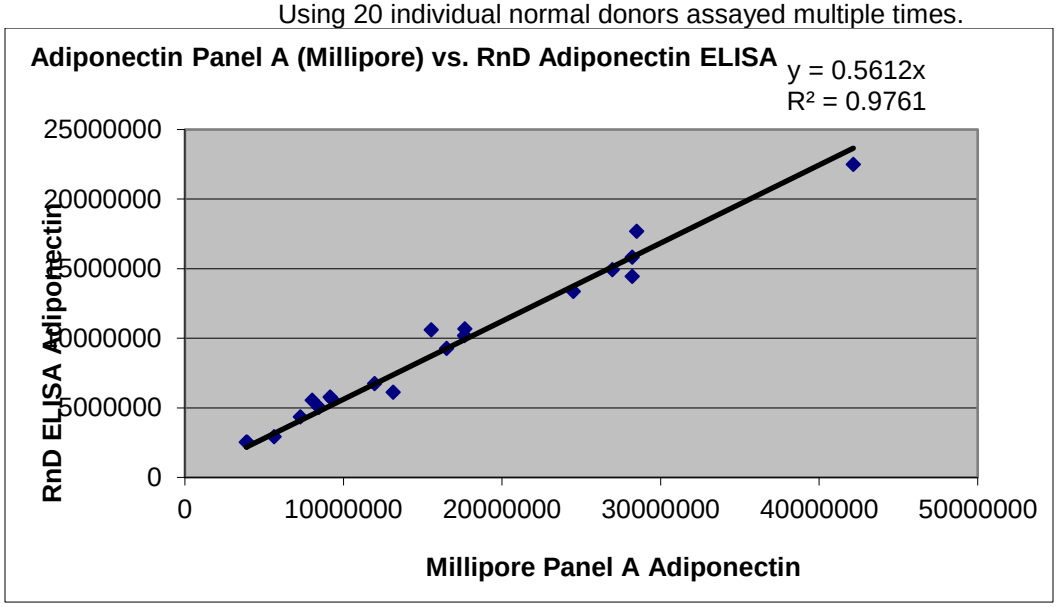
6. Appendix B. Note about Adiponectin assay

Adiponectin Data on method comparison between Millipore multiplex Panel B vs R&D System Elisa

Note: Although highly correlated, the Millipore multiplex method yields values almost twice as high as the R&D Systems Elisa

Method:	Millipore Adipokine Panel B	R&D Systems Elisa Catalog# DRP300
	Panel A Serum Set Average (ng/ml)	ELISA Serum Set Average (ng/ml)
	23 Sets (1/24/2006 - 5/22/09)	5 Sets (9/3/2004 - 5/11/09)
1	11969.34	6734.42
2	9162.42	5782.94
3	28488.27	17688.58
4	3875.81	2519.80
5	28216.45	15823.07
6	26958.18	14919.92
7	5622.27	2913.81
8	8021.64	5538.93
9	17661.87	10678.85
10	9271.75	5617.78
11	16494.89	9265.76
12	8455.92	5031.44
13	13142.81	6119.05
14	28213.58	14437.17
15	24501.31	13360.84
16	7288.33	4357.58
17	17635.82	10195.41
18	42154.11	22493.29
19	3936.15	2532.15
20	15536.72	10598.91
Average	16,330.38	9,330.49

Using 20 individual normal donors assayed multiple times.



7. Appendix C. Additional Information (Leptin)

UVM Laboratory for Clinical Biochemistry Research

Most Pilot Study (n=200) Assays were run from Dec '08 to Apr '09

MOST II (n =2152) Assays were run from Nov'10 to Apr'11

Due to known potential variation in reagent lots, it was decided to rerun a plate (approximately 36 samples) from the original Pilot study using the reagents purchased for the MOST II study. Direct comparison of these original results and rerun results can be used to determine if any 'drift' has been introduced to any particular assay in the two sets of data. Our laboratory generally uses a linear regression model for these analyses. Our analysis determined that adjustments are most likely necessary for Leptin if the data from the Pilot and MostII need to be directly compared.

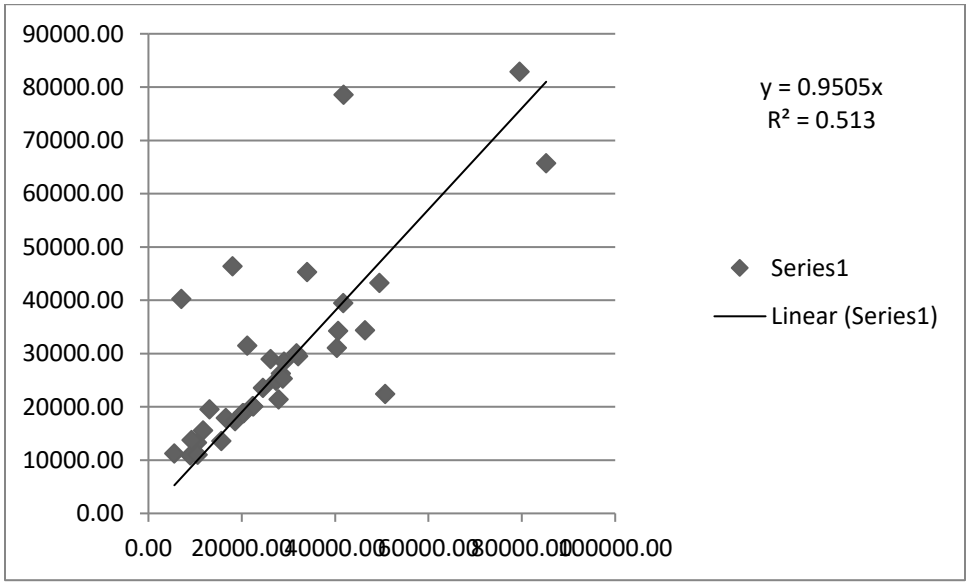


Table 1: Leptin comparison.

Visit	VT ID	Original Leptin pg/mL	Rerun Leptin pg/mL
BL	40	22311.64	20144.93
BL	41	28346.85	26253.48
BL	42	5552.75	11257.52
BL	43	21171.95	31492.65
BL	44	24556.35	23575.23
BL	45	10379.50	13290.14
BL	46	85218.16	65708.67
BL	47	32079.58	29506.14
BL	48	49470.93	43237.21
BL	49	20425.04	18812.22
BL	50	40649.48	34223.54
BL	51	27320.06	24848.29
BL	52	13083.91	19519.97
BL	53	16541.41	17893.27
BL	54	15602.37	13581.39
TH	55	18571.11	17285.25
TH	56	50712.81	22438.33
TH	57	46367.97	34362.80
TH	58	8973.03	10860.97
TH	59	79491.65	82909.85
TH	60	40329.69	31038.34
TH	61	31749.83	30016.66
TH	62	11685.42	15553.44
TH	63	28775.67	25298.46
TH	64	22456.66	20083.87
TH	65	10552.67	10976.31
TH	66	7015.07	40226.95
TH	67	9246.89	13752.00
TH	68	26147.31	28939.95
TH	69	18040.84	46369.23
TH	70	27875.50	21373.73
TH	71	41780.61	78535.65
TH	72	20183.42	18812.04
BL	73	33979.45	45316.01
BL	74	41761.10	39485.54
BL	75	29043.42	28472.85

Regression equation (after outlier was removed):
rerun Leptin (serum) = 0.9505* (original Leptin (plasma)
$R^2 = 0.513$

8. Appendix D. Additional Information (7 assays)

UVM Laboratory for Clinical Biochemistry Research

Most Pilot Study (n=200)	Assays were run from Dec '08 to Apr '09
MOST II (n =2152)	Assays were run from Nov'10 to Apr'11

Due to known potential variation in reagent lots, it was decided to rerun a plate (approximately 36 samples) from the original Pilot study using the reagents purchased for the MOST II study. Direct comparison of these original results and rerun results can be used to determine if any 'drift' has been introduced to any particular assay in the two sets of data. Our laboratory generally uses a linear regression model for these analyses. Our analysis determined that adjustments are most likely necessary for COMP, OxLDL, Adiponectin, TNFa, and ICAM if the data from the Pilot and MostII need to be directly compared.

Table 1. TNF-a comparison.

VT ID	Visit	Original TNF-a pg/mL	Rerun TNFa pg/mL	Comments
1	BL	3.11	6.04	
2	BL	2.31	5.94	
3	BL	4.64	9.72	
4	BL	2.03	3.94	
5	BL	4.65	9.02	
6	BL	3.03	5.86	
7	BL	5.76	10.06	
9	BL	7.52	8.60	
11	BL	4.51	7.72	
12	BL	2.33	4.45	
13	BL	2.77	6.60	
14	BL	2.96	4.27	
15	BL	1.48	2.89	
17	BL	8.26	16.13	
18	BL	3.80	8.28	
19	TH	5.45	10.75	
20	TH	4.46	8.73	
21	TH	86.09	5.63	Outlier?
22	TH	5.09	10.68	
23	TH	5.69	8.77	
24	TH	5.95	11.17	
25	TH	4.73	6.75	
26	TH	1.89	2.29	
27	TH	1.73	3.47	
28	TH	2.80	4.51	
29	TH	2.52	3.80	
30	TH	4.96	4.80	
31	TH	2.90	3.39	
32	TH	1.20	2.87	
33	TH	1.95	3.74	
34	TH	3.70	6.38	
35	TH	3.09	5.79	
36	TH	2.84	4.35	
37	BL	1.95	3.57	
38	BL	2.34	3.21	
39	BL	1.47	2.50	

Regression equation (after outlier was removed):
rerun TNF-a = 1.7314* (original TNF-a)
R^2 = 0.7953

Table 2. Adiponectin comparison.

VT ID	Visit	Original Adiponectin ng/mL	Rerun Adiponectin ng/mL	Comment
1	BL	22298.42	19169.03	
2	BL	54115.55	39280.63	
3	BL	52162.63	37688.19	
4	BL	34454.19	30263.06	
5	BL	30597.78	26689.85	
6	BL	18723.75	17147.98	
7	BL	27530.44	27188.36	
9	BL	18327.66	16663.20	
11	BL	43766.87	37071.98	
12	BL	51130.84	32684.89	
13	BL	17057.74	15549.40	
14	BL	17983.47	14526.39	
15	BL	58200.84	39326.27	
17	BL	11832.29	10150.27	
18	BL	33727.76	27749.50	
19	TH	31385.18	26995.10	
20	TH	35139.69	25520.32	
21	TH	17097.08	14490.41	
22	TH	19395.23	17707.81	
23	TH	26464.47	22567.79	
24	TH	11875.87	12186.30	
25	TH	41797.83	40922.49	
26	TH	27367.93	28042.89	
27	TH	33372.23	27914.75	
28	TH	27149.71	29191.88	
29	TH	44291.61	37241.51	
30	TH	24835.54	25312.38	
31	TH	45420.23	43269.42	
32	TH	23976.47	18227.31	
33	TH	48511.27	40335.92	
34	TH	12498.56	11871.52	
35	TH	27225.96	24366.61	
36	TH	18831.02	15683.24	
37	BL	29207.85	24187.30	
38	BL	19776.58	15925.83	
39	BL	39916.84	35161.58	

Regression equation:
rerun Adiponectin = 0.8244* (original Adiponectin)
$R^2 = 0.8542$

Table 3. OxLDL comparison.

VT ID	Visit	Original OxLDL U/L	Rerun Ox LDL U/L	Comment
1	BL	76.68	72.12	
2	BL	60.61	47.45	
3	BL	94.36	72.99	
4	BL	68.34	45.16	
5	BL	89.25	73.11	
6	BL	49.51	33.50	
7	BL	81.04	80.86	
8	BL	57.27		Insufficient Volume
9	BL	76.72	98.41	
10	BL	75.88		Insufficient Volume
11	BL	60.06	57.19	
12	BL	110.25	46.20	Outlier?
13	BL	76.70	66.43	
14	BL	83.80	67.98	
15	BL	56.56	41.70	
16	BL	96.00		Insufficient Volume
17	BL	68.00	67.21	
18	BL	81.25	72.52	
19	TH	86.76	81.21	
20	TH	68.67	66.74	
21	TH	61.86	44.79	
22	TH	94.39	94.16	
23	TH	69.42	68.37	
24	TH	70.11	64.35	
25	TH	55.65	57.40	
26	TH	73.38	59.68	
27	TH	76.28	33.53	
28	TH	59.93	50.85	
29	TH	65.02	57.95	
30	TH	88.93	100.09	
31	TH	62.60	43.80	
32	TH	82.77	87.88	
33	TH	44.53	40.21	
34	TH	53.39	47.49	
35	TH	75.05	69.81	
36	TH	70.42	62.99	
37	BL	55.74	38.96	
38	BL	59.05	41.13	
39	BL	59.85	38.92	

Regression equation (after outlier was removed):
rerun OxLDL = 0.8822* (original OxLDL)
R^2 = 0.6003

Table 4. ICAM comparison.

VT ID	Visit	Original ICAM-1 ng/ml	Rerun ICAM ng/mL	Comment
1	BL	281.76	193.29	
2	BL	204.22	189.08	
3	BL	311.52	225.59	
4	BL	224.46	199.06	
5	BL	289.98	242.43	
6	BL	139.10	125.30	
7	BL	336.76	246.76	
8	BL	210.27		Insufficient Volume
9	BL	293.06	227.39	
10	BL	230.53		Insufficient Volume
11	BL	315.21	302.35	
12	BL	229.30	221.77	
13	BL	225.72	214.47	
14	BL	294.50	255.95	
15	BL	235.79	224.68	
16	BL	318.29		Insufficient Volume
17	BL	208.14	172.10	
18	BL	318.90	248.57	
19	TH	218.74	217.94	
20	TH	276.83	265.12	
21	TH	268.78	263.68	
22	TH	293.05	291.44	
23	TH	209.81	214.65	
24	TH	242.42	222.48	
25	TH	195.07	173.97	
26	TH	303.94	265.48	
27	TH	223.40	200.33	
28	TH	213.66	221.40	
29	TH	351.36	319.85	
30	TH	217.92	226.68	
31	TH	333.89	250.37	
32	TH	293.27	231.57	
33	TH	242.68	207.88	
34	TH	218.46	176.76	
35	TH	162.45	160.95	
36	TH	192.26	191.86	
37	BL	248.35	260.45	
38	BL	274.73	298.77	
39	BL	327.71	339.85	

Regression equation (after outlier was removed):
rerun ICM-1 = 0.8918* (original ICAM-1)
$R^2 = 0.6007$

Table 5. TIMP-1 comparison.

VT ID	Visit	Original TIMP1 ng/mL	Rerun TIMP-1 ng/mL	Comment
1	BL	122.62	121.63	
2	BL	109.03	112.13	
3	BL	105.61	106.80	
4	BL	158.71	171.88	
5	BL	158.36	170.47	
6	BL	97.79	99.33	
7	BL	117.44	120.32	
8	BL	118.99		Insufficient Volume
9	BL	184.75	190.66	
10	BL	125.91		Insufficient Volume
11	BL	125.73	127.29	
12	BL	104.59	101.14	
13	BL	150.49	138.95	
14	BL	153.71	141.08	
15	BL	156.56	148.45	
16	BL	153.52		Insufficient Volume
17	BL	120.37	118.26	
18	BL	179.79	170.47	
19	TH	113.15	127.10	
20	TH	146.07	148.64	
21	TH	147.85	136.82	
22	TH	189.72	182.24	
23	TH	125.39	118.83	
24	TH	167.72	166.05	
25	TH	132.01	138.94	
26	TH	148.54	149.44	
27	TH	124.52	121.07	
28	TH	143.23	139.33	
29	TH	124.35	126.72	
30	TH	124.35	128.43	
31	TH	123.83	138.44	
32	TH	163.38	157.69	
33	TH	153.34	159.09	
34	TH	154.06	146.50	
35	TH	139.36	141.26	
36	TH	142.35	130.89	
37	BL	115.48	100.42	
38	BL	189.26	177.75	
39	BL	93.92	89.30	

Regression equation (after outlier was removed):
rerun TIMP-1 = 0.9895* (original TIMP-1)
$R^2 = 0.9031$

Table 6. COMP comparison.

VT ID	Visit	Original COMP U/L	Rerun COMP U/L	Comment
1	BL	11.84	13.22	
2	BL	9.53	8.39	
3	BL	13.04	11.28	
4	BL	13.13	10.75	
5	BL	9.02	7.32	
6	BL	10.36	9.23	
7	BL	10.69	9.48	
8	BL	9.57		Insufficient Volume
9	BL	11.60	9.45	
10	BL	7.32		Insufficient Volume
11	BL	14.25	11.46	
12	BL	8.86	6.44	
13	BL	8.42	6.33	
14	BL	14.96	11.23	
15	BL	15.81	10.95	
16	BL	16.43		Insufficient Volume
17	BL	15.41	14.05	
18	BL	16.15	13.86	
19	TH	15.12	12.20	
20	TH	9.72	6.91	
21	TH	16.58	13.66	
22	TH	17.87	13.83	
23	TH	5.26	6.64	
24	TH	9.44	7.74	
25	TH	23.94	18.17	
26	TH	19.08	15.17	
27	TH	11.79	9.28	
28	TH	11.19	7.85	
29	TH	14.96	13.15	
30	TH	11.56	10.66	
31	TH	9.23	7.50	
32	TH	17.82	13.50	
33	TH	12.13	9.94	
34	TH	6.90	-333.00	Low
35	TH	13.54	8.32	
36	TH	11.12	11.50	
37	BL	8.56	7.27	
38	BL	13.26	10.83	
39	BL	15.17	11.56	

Regression equation (after outlier was removed):
rerun COMP = 0.8108* (original COMP)
R^2 = 0.8147

Table 7. MMP-3 comparison.

VT ID	Visit	Original MMP3 ng/mL	Rerun MMP3 ng/mL	Comment
1	BL	10.47	11.27	
2	BL	9.77	9.70	
3	BL	8.25	7.44	
4	BL	9.95	9.56	
5	BL	5.67	5.20	
6	BL	8.97	8.14	
7	BL	15.65	14.74	
8	BL	8.70		Insufficient Volume
9	BL	6.75	8.25	
10	BL	12.64		Insufficient Volume
11	BL	7.48	8.33	
12	BL	9.28	9.85	
13	BL	8.38	7.81	
14	BL	8.20	7.40	
15	BL	11.07	10.76	
16	BL	8.43		Insufficient Volume
17	BL	4.71	5.41	
18	BL	5.72	6.13	
19	TH	7.34	8.81	
20	TH	5.97	6.73	
21	TH	9.93	11.97	
22	TH	21.54	23.03	
23	TH	15.26	16.40	
24	TH	5.93	6.30	
25	TH	9.26	9.68	
26	TH	8.63	8.75	
27	TH	5.72	6.81	
28	TH	5.79	5.72	
29	TH	9.30	10.25	
30	TH	6.16	6.68	
31	TH	11.55	13.02	
32	TH	5.77	5.97	
33	TH	8.61	9.10	
34	TH	7.55	10.17	
35	TH	9.46	10.60	
36	TH	8.04	8.93	
37	BL	8.18	9.28	
38	BL	6.77	8.87	
39	BL	3.91	5.13	

Regression equation (after outlier was removed):
rerun MMP3 = 1.0595* (original MMP3)
R^2 = 0.9319

9. Appendix E. Q and A from communications between University of Vermont Laboratory for Clinical Biochemistry Research and the MOST Coordinating Center.

Q1.
We noted that the low detection level reported from the pilot is different from that at Phase II for these assays: TNFα and ICAM-1 (llght green highlighted column- Low detection). For example, TNFα is 0.64 pg/ml in pilot and 0.32 in phase II. Did the assays improve? They look to be same assay. Just checking to see that these are accurate. Are the data otherwise comparable between the pilot and phase II? If we combined the data, we might have to set the phase II levels < 0.64 as undetectable which may not be desirable.
A.
We extended the standard curve on TNFa to 0.32 in Phase II. The ICAM method changed standardization. Not unusual for manufacturers to modify their assays.
[Comment: the answer is they are both right, the assay became more sensitive. We will have to think about desirability of combining.]
Q2.
In the tab=Assay Methods provided the Leptin assay used in the pilot was the Millipore using EDTA plasma and then we changed it to the R&D systems ELISA assay using serum in the larger Phase II group, because my recollection is that there was a volume advantage to the ELISA. Can those results be combined? If so, is there a correction factor we should apply if we want to combine those two different assays into one analysis dataset? We note that the normal ranges are the same regardless of specimen type and assay method as documentation indicated- correct? In addition, leptin plasma vs serum results were provided for only 36 vials (selection criteria for the re-run is unknown – was it a random selection or selected from different parts of the distribution?). This may be the data we need to "convert" one set of data to the other – correct? We note that there is a sheet on adiponectin containing data on the difference between those two assays, but not for leptin.
A.
The comparison between the two leptin methods is in the rerun spreadsheet (see appendix C). Not surprisingly, there are numerous samples that don't correlate particularly well. Not unusual with different methods and antibodies.
Q3.
In running some descriptive analyses, we observed gender differences in specific assays: Adiponectin, CRP, MMP3. The statistician noted that the lab documentation does not report gender-specific normal ranges for these assays, and wondered about that. I am not as familiar with MMP3, but I believe that this is probably due to adiposity and there are not gender-specific normal ranges.
A.
We do not have gender specific 'normal' ranges. It's not unexpected to see gender differences.
Q4.
Documentation contains QC table (see table 2 below). Obviously, you were running quality control samples with both the pilot and the phase II runs. I think you were running "control" samples of known concentration with each batch and calculating the coefficient of variation (CV) for the standards for each of the batches. Is this correct? For some analytes you have controls 1-3 and for some controls 1-4 – so different analytes were run in 3 and in 4 batches? Or was everything run in one batch and you used different controls with different known concentration in the batch? For some analytes, the CVs differ quite a bit. For example, highlighted in light green, Leptin controls 1 CV=16.7% and controls 3 CV=5.8%. Is this typical for between run CVs?
A.
We provided these values just so you would have the inter-assay CVs for publication purposes. (ex. The inter-assay CV range for Leptin ranged from 4.40 to 11.14%) The inter-assay CV is calculated on ALL the control data across multiple runs. Yes control CVS can vary based on the type of control material, level, and other factors. The control materials do not always overlap so the assignment of Control 1 – 4 is not specific to a certain type of control. I think you're reading more into this than was intended. This was not intended for direct comparison of qc materials.
Q5.
There is data on the difference between two panels – Millipore multiplex and R&D ELISA (see Appendix B). We used the same adiponectin assay in both the pilot and the phase II studies, so I assume these are data for use in publications in case a reviewer wants to know how the Millipore method compares to the ELISA. Our big issue is leptin where we switched methods, so we need comparable data on those assays to see if it would be possible to use the pilot data or at least understand how the two sets of data differ.

10. Appendix F. References

2010 abstract booklet for ACR
https://acrabstracts.org/wp-content/uploads/2018/06/2010_ACR_ARHP_Abstract_Supplement.pdf
Arthritis & Rheumatism Vol 62 (2) p403 – abstract #951

Results: The safety population included 1,154 patients, the intent-to-treat population included 1,149 patients, and PGIC data at endpoint were available for 1,059 patients. The majority (61.4% [650/1,059]) of patients reported a change in overall status at endpoint of "very much improved" or "much improved," and an additional 25.7% (272/1,059) of patients reported that their overall status was "minimally improved." The most commonly reported (>10%) TEAEs included headache (13.1% [151/1,154]), nausea (11.8% [136/1,154]), and constipation (11.1% [128/1,154]).

Conclusion: Long-term treatment with tapentadol ER (100–250 mg bid) for up to 1 year (and up to 2 years for some patients) was associated with an improvement in overall status for the majority of patients in this open-label extension study of patients with moderate to severe chronic osteoarthritis pain or low back pain.

Disclosure: B. McCann: Johnson & Johnson, 1, 3; **R. Lange:** Grünenthal GmbH, 3; **B. Wagner:** Johnson & Johnson, 1, 3; **A. Steup:** Grünenthal GmbH, 3; **B. Lange:** Grünenthal GmbH, 3; **M. Etropolski:** Johnson & Johnson, 1, 3.

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Analysis of the Multicenter Osteoarthritis (MOST) Study. Jasvinder Singh[1], Tuhina Neogi[2], David T. Felson[3], James Torner[7], Kristin Baker[4], Michael C. Nevitt[5], Irina Tolstykh[5] and Cora E. Lewis[6]. [1]Birmigham VA Medical Center and University of Alabama, Minneapolis, MN, [2]Boston Univ Schl of Med, Boston, MA, [3]Boston University School of Medicine, Boston, MA, [4]Boston University School of Medicine, [5]UCSF, San Francisco, CA, [6]University of Alabama, [7]University of Iowa

Objective: OA has been labeled an inflammatory disorder but studies have examined only a limited repertoire of inflammatory markers with disease and have reported inconsistent findings. We assessed whether inflammation biomarkers and those representing adipokines are associated with synovitis and knee pain in participants in a cohort study of knee OA.

Methods: A subgroup of 100 Caucasian women were included from the NIH-funded Multicenter Osteoarthritis (MOST) Study. MOST participants were age 50 to 79 years at baseline and either with symptomatic knee OA or at high risk of disease. At baseline and 30 month follow-up, subjects were queried about the presence of knee pain on most days, completed a knee-specific Western Ontario McMaster Osteoarthritis Index (WOMAC), and had weight-bearing PA and lateral knee x-rays. New knee pain was present if a subject did not have knee pain on most days at baseline but did at follow-up irrespective of x-ray OA. At 30 months, participants underwent a gadolinium contrast-enhanced MRI using a 1.5 Tesla scanner to detect synovitis, categorized as none/questionable, mild, or a lot/extensive. WORMS readings of MRI's were used to score bone marrow lesions (BML's) and size of effusion. We assessed the multivariable-adjusted association of 1 standard deviation change in each biomarker level at baseline (blood or urine) with synovitis, prevalent pain and incident pain on most days on WOMAC scale at 30 months: TNF-alpha, MCP-1, leptin, adiponectin, PAI-1, CRP, MMP3, TIMP3, ICAM-1, IGF-1, IGF-BP3, oxidized LDL, TGF-beta 1, estradiol (total), DHEA-S, sex hormone binding globulin (SHBG) and cartilage oligomeric matrix protein (COMP).

Results: Mean age (standard deviation) was 59.5 years (7.2), body mass index (BMI) was 29 (4.8) kg/m^2, 57% had no radiographic OA at baseline, 20% with unilateral OA and 23% with bilateral OA and 68% were using an pain medications at baseline. Adjusted for site, age, BMI, baseline K/L grade, BML score (>0) and effusion score (>0), the following associations were significant for presence of a lot/extensive synovitis at 30-month visit: baseline adiponectin, OR 2.6 (95% CI:1.3, 4.9) and baseline TGF-beta 1, OR 0.3 (95% CI: 0.2, 0.7).

Significant predictors of outcomes in Multivariable adjusted Odds per 1 Standard deviation increase in each biomarker

	A lot /extensive Synovitis (ref: none/mild)		Prevalent knee pain on WOMAC (ref: none)		Incident knee pain on WOMAC (ref: none)	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Adiponectin	2.6 (1.3, 4.9)	0.005	1.4 (0.9, 2.4)	0.16	0.9 (0.4, 2.2)	0.84
TGF-B1	0.3 (2.0, 0.7)	0.002	1.2 (0.7, 2.1)	0.43	1.2 (0.5, 2.9)	0.71
TNF-alpha	1.2 (0.7, 2.1)	0.44	1.9 (1.1, 3.2)	0.015	2.4 (0.8, 6.6)	0.10
ICAM-1	1.1 (0.7, 1.8)	0.71	1.1 (0.7, 1.8)	0.57	0.3 (0.1, 0.97)	0.044

Similarly, in multivariable-adjusted analyses, the following associations were significant for any synovitis (versus none) at 30-month: baseline ICAM-1, OR 1.9 (1.1, 3.4) and baseline IGF-1, OR 1.9 (1.1, 4.1). Adjusted for all the variables above and use of pain medications, baseline TNF-alpha was significantly associated with prevalent knee pain at 30-months, OR 2.0 (1.2, 3.4) and ICAM-1 was associated with incident knee pain, OR 0.3 (0.1, 0.97).

Conclusions: In this hypothesis-generating study using a longitudinal cohort of Caucasian women, we found several biomarkers predictive of synovitis, prevalent and incident knee pain at 30-month follow-up.

Disclosure: J. Singh: None; **T. Neogi:** None; **D. T. Felson:** None; **J. Torner:** None; **K. Baker:** None; **M. C. Nevitt:** None; **I. Tolstykh:** None; **C. E. Lewis:** None.

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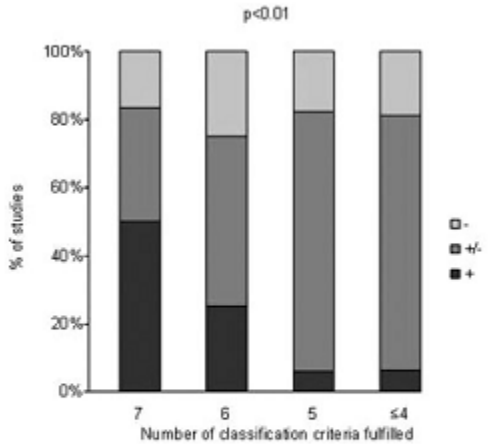
The Importance of Methodological Quality for the Association between Radiographic and Clinical Features of Hip and Knee Osteoarthritis: A Systematic Review. M. B. Kinds[2], E. P. Vignon[5], J. W. J. Bijlsma[4], M. A. Viergever[1], A. C. A. Marijnissen[4], F. P. J. G. Lafeber[4] and P. M. J. Welsing[3]. [1]Image Sciences Institute, University Medical Center Utrecht, The Netherlands, [2]Rheumatology & Clinical Immunology, Image Sciences Institute, University Medical Center Utrecht, The Netherlands, [3]Rheumatology & Clinical Immunology, Julius Center for Health Sciences & Primary Care, University Medical Center Utrecht, The Netherlands, [4]Rheumatology & Clinical Immunology, University Medical Center Utrecht, The Netherlands, [5]Rheumatology, University Hospital Lyon-Sud Pierre-Benite, France

Background: Still there is debate on the presumed association between radiographic and clinical features of osteoarthritis (OA). Inconsistency in reported associations might be caused by different definitions of clinical OA, and by different protocols and scoring methods for radiographic damage. Objective of this review was to evaluate whether there is an association between radiographic OA and clinical OA of hip and knee, accounting for the importance of disease definition, radiographic protocol, and standardized outcome measures.

Methods: A systematic literature search was performed with the keywords 'OA', 'hip', 'knee', 'radiographic', and 'clinical'. For comparison of study results, seven classification criteria for general study quality and methodological quality were developed. All studies were evaluated for an association between radiographic and clinical OA. Associations were classified as 'present' when statistically significant, as 'absent' when not statistically significant, and as 'non-evident' when not all performed comparisons were statistically significant. The influence of classification criteria on the association was investigated, by classifying both the number and the specific criteria fulfilled.

Results: The literature search resulted in 47 studies describing associations between radiographic and clinical OA. When all studies were evaluated, associations were present in 15%, absent in 19%, and non-evident in 66%. Associations were strongest in the six studies fulfilling all classification criteria; present in 50%, absent in 17%, and non-evident in 33% of studies. The frequency of studies with present associations significantly ($p<0.01$) diminished when the number of fulfilled criteria decreased.



Frequency of associations: + (association), +/− (non-evident association), and − (no association) in 6, 8, 17, and 16 studies fulfilling 7 (all), 6, 5, and ≤4 classification criteria respectively. Linear regression analysis with number of criteria as independent variable and association + vs association +/− and − as dependent variable: $p<0.01$.

Tuesday, November 9