MOST Ancillary Study 10-07 (AS10-07)
"Association of Central Sensitization with Pain Post-TKR" (Tuhina Neogi)

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1. Datast description and Analyst Notes

Dataset: AS1007_bioassay.sas7bdat
Observations: 1121 records (1080 participants, 3 assays, 3 visits)
Documentation:

- VariableGuide_AS1007_bioassay.pdf
- Distributions_AS1007_bioassay.pdf

AS1007plasma dataset contains 1121 records (multiple records per participant; unique record per participant and visit) with 3 assays results (Leptin, Adiponectin and TNFa) performed at the University of Vermont Laboratory for Clinical Biochemistry Research on baseline, 30-month or 60-month plasma:

- 134 records from baseline EDTA plasma blood sample
- 52 records from 30-month EDTA plasma blood sample
- 935 records from 60-month EDTA plasma blood sample

Note: the same participant could be eligible for more than one selection group if both knees had knee replacement (KR) surgery during the study and have different follow-up time periods.

Table 1.1. Participant eligibility and records included in the dataset

Eligibility	BL blood results included (VIS=V0)	30m blood results included (VIS=V2)	60m blood results included (VIS=V3)
Participants with KR between BL and 30m	**134 participants (pre-KR)**	2 participants (post-KR)*	6 participants (post-KR)*
Participants with KR between 30m and 60m	2 participants (pre-KR)*	**52 participants (50 pre-KR and 2 post-KR)**	**98 participants (post-KR)**
Participants with KR between 60m and 84m	6 participants (pre-KR)*	--	**89 participants (83 pre-KR and 6 post-KR)**
Participants without KR (as of 84m visit) – random sample	--	--	**750 participants (controls)**

*Blue text used to indicate if participant belongs to more than one selection group and longitudinal assay results are available from blood collected at different time points.

Participants with KR BL-30m and 30m-60m
There are 2 participants from the 134 participants who had KR BL-30m and 52 participants who had KR 30m-60m follow-up. The longitudinal results for KR BL-30m are included in this dataset.
Participants with KR BL-30m and 60m-84m
There are 6 common participants from the 134 participants who had KR BL-30m and 89 participants who had KR 60m-84m follow-up. The longitudinal results for KR BL-60m are included in this dataset.
Participants with KR 30m-60m
There are 33 participants who had KR 30m-60m and have longitudinal results from plasma collected at both visits.

Note: the laboratory performing the assay was blinded if there was two or more vials for the same participant from different visits and those vials may be processed in separate batches.

Analyst Notes:

- When assay results were not obtained, special missing values were used:

.L = below low detection level
.H = above high detection level

These results can be used in the categorical analysis only. Alternatively, an analyst can assign the special value above detection or below detection if requested by investigator.

- Each record is marked by variable VIS (values: V0, V2, V3) to indicate visit when blood sample was collected.

- Each record is marked by variable AnalysisType (values: pre-KR, post-KR, no KR-control) to indicate what type of analysis can be performed. For participants eligible for different groups due to knee replacement surgeries at different time periods, results from blood collected before the first surgery are marked as pre-KR and all results from blood collected after the first surgery are marked as post-KR regardless of the time of the second surgery.

2. Selection plan

Enrolled in MOST: N=3026 participants / 6052 knees

Select BL, 30m, and 60m plasma samples as outlined in Table 2.1 using KR time point to classify participants:

1. Knee replaced between BL and 30m– 168 participants: 134 participants projected for post-KR analysis
2. Knee replaced between 30 and 60m – 244 participants: 38 participants projected for pre-KR and post-KR analysis; 21 participants projected for pre-KR analysis only; 60 participants projected for post-KR analysis only
3. Knee replaced between 60 and 84m – 141 participants: 99 participants projected for pre-KR analysis
4. No KR – 2483 participants available: random sample of 750 participants projected for no-KR analysis

Table 2.1: Biospecimen Sample and KR Time Point Selection

Biospecimen Sample Time Point	KR BL – 30m	KR 30m – 60m	KR 60m – 84m	No KR BL -84m
BL plasma (250uL/ppt)N=134	134 (Table A)	-	-	-
30m plasma (250 uL/ppt)N=52	-	52 (Table B)	-	-
60m plasma (250 uL/ppt)N=937	-	98 (Table C)	89 (Table D)	750 (Table E)
Total N=**1123**	**134**	**150**	**89**	**750**

Detailed Inclusion/Exclusion Criteria for Each Group:

A. **BL PLASMA and KR BL-30M**: Participants with BL plasma available, had a knee replaced between BL and 30m, have WOMAC score non-missing at both time points.

Inclusion	Notes	Excluded
168 participants with KR between BL and 30m	10 participants with baseline KR in contralateral knee	
		N=**2** no BL plasma[2]
		N=**32** participants missed 30m visit
134 selected	134 participants have BL+30m CV done and WOMAC available	

B. **30M PLASMA and KR 30M-60M**: Participants with 30m plasma available, had a knee replaced between 30m and 60m; have WOMAC score non-missing at both time points.

Inclusion	Notes	Excluded
244 participants with KR between 30m and 60m	48 participants with KR prior to 30m in contralateral knee	
(7 can be used in analysis)	participants have 30+60m CV done and WOMAC available	N=**7** leptin and other assays from plasma (Lewis)
		N=**16** missed 30m visit
		N=**148** no plasma collected 30m[2]
		N=**3** plasma hemolyzed[2]
		N=**18** missed 60m visit
52 selected (***59*** *projected for analysis)*	59 participants have 30+60m CV done and WOMAC available	

C. **60M PLASMA and KR 30M-60M**: Participants with 60m plasma available, had a knee replaced between 30m and 60m; have WOMAC score non-missing at both time points.

Inclusion	Notes	Excluded
244 participants available with KR between 30m and 60m	48 participants with KR prior to 30m in contralateral knee	
		N=2 deceased or withdrawn prior to 60m visit
		N=59 missed 60m visit
		N=84 no plasma collected 60m[2]
		N=1 no WOMAC score at 30m
98 selected	98 participants have 30+60m CV done and WOMAC available; No participants with pain sensitivity exam fully done[1]	

D. **60M PLASMA and KR 60M-84M**: Participants with 60m plasma available, had a knee replaced between 60 and 84m; have WOMAC score non-missing at both time points.

Inclusion	Notes	Excluded
141 participants available with KR between 60m and 84m	12 participants with KR prior to 60m in contralateral knee	
10 can be used in analysis (results from Group C)	18 participants with KR between 30m and 60m in contralateral knee	8 missed 84m visit; (missing 84m WOMAC score)
		N=19 missed 60m visit
		N=3 no plasma collected 60m[2]
		N=**1** plasma hemolyzed[2]
		N=1 WOMAC missing at 60m
		N=15 missed 84m visit
89 selected *(99 projected for analysis)*	99 participants have 60+84m CV done and WOMAC available; 72 participants with pain sensitivity data at 60m[1]	

E. **60M PLASMA and NO KR (Random sample from available)**: Participants with 60m plasma available and neither entered the study with a KR nor had a knee replaced prior to the 84m visit; have WOMAC score and pain sensitivity exam data available at both time points: 60m and 84m.

Inclusion	Notes	Excluded
2483 participants available with no KR prior to 84m	58 participants with known post-84m KR (as of today)	
		N=**255** deceased or withdrawn prior to 84m
		N=**453** missed 84m visit
		N=**96** missed 60m visit
		N=56 no plasma collected 60m[2]
		N=13 plasma hemolyzed[2]
		N=**69** pain sensitivity exam not done[1]
		N=**220** pain sensitivity exam done partially (either 60m or 84m but not both time points)[1]
1321 remain available, **750** randomly selected	All participants have 60+84m CV done, WOMAC and pain sensitivity exam data available during 60m and 84m visit[1]	

Footnotes

Note 1: Pain sensitivity exam marked as done if temporal summation is complete in all 3 locations and PPT data available for arm and 2 patellas; exam done partially if some values are present and some are missing.

Note 2: Notation "no plasma" means no plasma was collected, or plasma marked as hemolyzed, or plasma excluded due to freeze/thaw cycles, or there is no plasma currently available in storage.

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3. Appendix A. Assay documentation provided by laboratory

Table 3.1. By assay performed in 2014

Assay	Manufacturer	Method	Catalog#	Kit Lot	Volume	Sample Type	Range of Standard	Dilution	Low Detection	High Detection	Estimated Normal Range
Adiponectin (Plasma) 2014	R&D Systems	Elisa	DRP300	317355	15 uLs	EDTA	39 - 250 ng/mL		~390	~25,000	865 - 21,424
Leptin* (Plasma) 2014	R&D Systems	Elisa	DLP00	317538	15 uLs	EDTA	15.6 - 1000 pg/mL		~1500	~120,00	Males: 2205 - 11,149 Females: 3877 - 77,273
TNFa (Plasma) 2014	Millipore Systems	Luminex panel	HADK2MAG-61K	2454779	60 uLs	EDTA	0.13 - 2,000 pg/mL		~ 0.30	~2,000	~0.5 - 5.0

*Note, normal range for leptin results reported by sex/gender.

Table 3.2. Inter- and Intra- CV provided – by assay

Assay	Inter-Assay CVs	Intra-assay CV
	Mean value	Mean value
TNFa (Plasma) 2014	5.91%	4.94%
Adiponectin (Plasma) 2014	10.89%	7.93%
Leptin (Plasma) 2014	10.4%	6.25%

4. Appendix B. Quality Control report for plasma results by assay

The Coordinating Center created QC reports for the AS10-07 study results. Note: results marked with special values (below or above detection level) are missing and therefore not included in these report tables.

Table 4.1. Adiponectin by sex and visit

sex/gender	visit	N obs	Mean	Median	Min	Max	N within Normal range	% within Normal range	N below normal	% below normal	N above normal	% above normal
Women	V0	92	13601.9	13331.4	2679.3	28832.7	80	85.1%	0	0.0%	12	12.8%
	V2	35	9957.2	9661.4	2442.3	21175.1	35	100.0%	0	0.0%	0	0.0%
	V3	550	11301.7	10297.2	1584.6	30608.2	507	91.2%	0	0.0%	43	7.7%
Men	V0	40	9677.1	8942.9	2517	23754.4	38	95.0%	0	0.0%	2	5.0%
	V2	17	7799.2	6792.8	1946.2	24084.2	16	94.1%	0	0.0%	1	5.9%
	V3	379	7416.7	6373.5	598.1	27222.6	370	97.1%	1	0.3%	8	2.1%
Adiponectin ng/ml		1113	10014.7	8908.5	598.1	30608.2	1046	93.1%	1	0.1%	66	5.9%

Table 4.2. Leptin by sex and visit

Sex/gender	vis	N obs	Mean	Median	Min	Max	N within Normal range	% within Normal range	N below normal	% below normal	N above normal	% above normal
Women	V0	94	41495.6	36760.9	2885.4	101035	81	86.2%	1	1.1%	12	12.8%
	V2	35	46538.6	43623.6	11109	107888.7	31	88.6%	0	0.0%	4	11.4%
	V3	555	36077.1	30995	2176	121838.7	503	90.5%	11	2.0%	41	7.4%
Leptin pg/ml		684	37357.1	32186	2176	121838.7	615	89.8%	12	1.8%	57	8.3%
Men	V0	38	12911.4	8383.3	1479.3	51063.7	19	47.5%	2	5.0%	17	42.5%
	V2	16	9670.8	7789.3	2167.5	30766.2	8	47.1%	1	5.9%	7	41.2%
	V3	373	14557.1	9277.1	1369.2	102166.1	202	53.0%	10	2.6%	161	42.3%
Leptin pg/ml		427	14227.6	9265.5	1369.2	102166.1	229	52.3%	13	3.0%	185	42.2%

Table 4.3. TNF-a by sex and visit

sex/gender	vis	N obs	Mean	Median	Min	Max	N within Normal range	% within Normal range	N below normal	% below normal	N above normal	% above normal
Women	V0	94	3.6	3.4	0.8	10	76	80.9%	0	0.0%	18	19.1%
	V2	35	3.5	3.1	1.1	11.1	30	85.7%	0	0.0%	5	14.3%
	V3	556	3.6	3.3	0.5	39.5	478	86.0%	1	0.2%	77	13.8%
Men	V0	40	3.4	3.1	0.6	13.3	38	95.0%	0	0.0%	2	5.0%
	V2	17	2.8	2.3	1.1	7.4	15	88.2%	0	0.0%	2	11.8%
	V3	381	3.6	3.1	0.7	44.2	338	88.7%	0	0.0%	43	11.3%
TNF-a pg/mL		1123	3.6	3.2	0.5	44.2	975	86.8%	1	0.1%	147	13.1%

5. Appendix C. Additional Information

The AS10-07 plan indicates the following: plasma results obtained for AS05-01 (Lewis) will be used to supplement the available sample of this study for participants eligible for one or more selection groups. The Coordinating Center identified 53 records (3 from baseline plasma and 50 from 30m plasma) with results obtained in both studies.

Reliability analysis for 51 samples (with non-missing values for Adiponectin and TNF-a) was performed and reliability results are described in Table 5.1.

Table 5.1. Reliability analysis - ICC – calculated

Assay	Comparison AS05-01 from 2010 AS10-07 from 2014	N of samples used for re-run	ICC Single score	ICC Random set	ICC Fixed set
Adiponectin	Plasma 2010 vs Plasma 2014	51	0.13787	0.33547	0.68058
TNF-a	Plasma 2010 vs Plasma 2014	51	0.45141	0.49099	0.5978
Leptin	Serum 2010 vs Plasma 2014	51	0.8662	0.86642	0.87038

Before combining the results from AS05-01 and AS10-07, please note that there is variation in the assay kit manufacturer, method and sample type (see assay documentation).

Table 5.2. Comparison of manufacturer/method and blood type used for the study

Assay	Manufacturer	Method	Catalog#	Kit Lot	Volume	Sample Type	Range of Standard	Dilution	Low Detection	High Detection	Estimated Normal Range
Adiponectin (Plasma) 2010	Millipore	Bio-Rad Luminex Flow Cytometry	HADK1-61K-A	1817775	2	EDTA	0.016 - 250 ng/mL	300	4.8	75,000	1198 - 19973 ng/ml
Adiponectin (Plasma) 2014	R&D Systems	Elisa	DRP300	317355	15 uLs	EDTA	39 - 250 ng/mL		~390	~25,000	865 - 21,424
Leptin* (Serum) 2010	R&D Systems	Elisa	DLP00	279515	10	Serum	15.6 - 1000 pg/mL	100	~1300	~120,000	Males(,2205-11,149 pg/mL) Females (3,877 - 77,273 pg/mL)
Leptin* (Plasma) 2014	R&D Systems	Elisa	DLP00	317538	15 uLs	EDTA	15.6 - 1000 pg/mL		~1500	~120,00	Males: 2205 - 11,149 Females: 3877 - 77,273
TNFa (Plasma) 2010	Millipore	Bio-Rad Luminex Flow Cytometry	HADK2-61K-B	1817776	75	EDTA	0.32 - 10,000 pg/mL	1	0.32	10,000	ND - 4.2 pg/mL
TNFa (Plasma) 2014	Millipore Systems	Luminex panel	HADK2MAG-61K	2454779	60 uLs	EDTA	0.13 - 2,000 pg/mL		~ 0.30	~2,000	~0.5 - 5.0

*Note: normal range for leptin results reported by sex/gender.