

**AS14-06 “Fat, Fiber, and Osteoarthritis” (Felson, Misra)
“Magnesium and Chondrocalcinosis in Osteoarthritis:
the Multicenter Osteoarthritis Study (MOST)”**

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1. Dataset description and Analyst Notes

Dataset: AS1406mg_bioassay.sas7bdat

Observations: 215 records (215 participants, 1 assays; 1 visit)

Documentation:

- VariableGuide_ AS1406mg_bioassay.pdf
- Distributions_ AS1406mg_bioassay.pdf

AS1406mg_bioassay dataset contains 215 records (one record per participant). Magnesium assay was performed at the at the Cardiovascular Nutrition Laboratory Tufts University on baseline serum. This is additional selection to the assay parameters available in the dataset AS1406_bioassay.

Note: the laboratory performing the assays was blinded to subject ID and clinical characteristics.

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Dataset: AS1406 bioassay Pull #A (biospecimens sent to lab May 2018):

Selection was done for two outcomes (WKROA incidence and SxROA incidence) at three time points: 15m, 30m and 60m. All cases and random sample of controls (ratio 1 case:2 controls matched by clinic) were selected. Total 6 independent case/control risk sets. For details see the document “DatasetDescription_AS1406_bioassay.pdf”. Dataset AS1406_bioassay contains parameter Mg (magnesium) for 994 participants selected.

Dataset: AS1406mg_bioassay; Pull #B (biospecimens sent to lab July 2019):

An additional set of cases and controls were selected for magnesium assays based on chondrocalcinosis status at baseline, 30 months and 60 months, to supplement and be combined with Pull #A for analysis of magnesium and chondrocalcinosis status.

Total four case/control sets were selected: 1) incidence chondrocalcinosis at 30m; 2) cumulative incidence at 30m/60m; 3) incidence Sx chondrocalcinosis at 30m and 4) cumulative Sx chondrocalcinosis incidence at 30m/60m (ratio 1 case:1 control in each set).

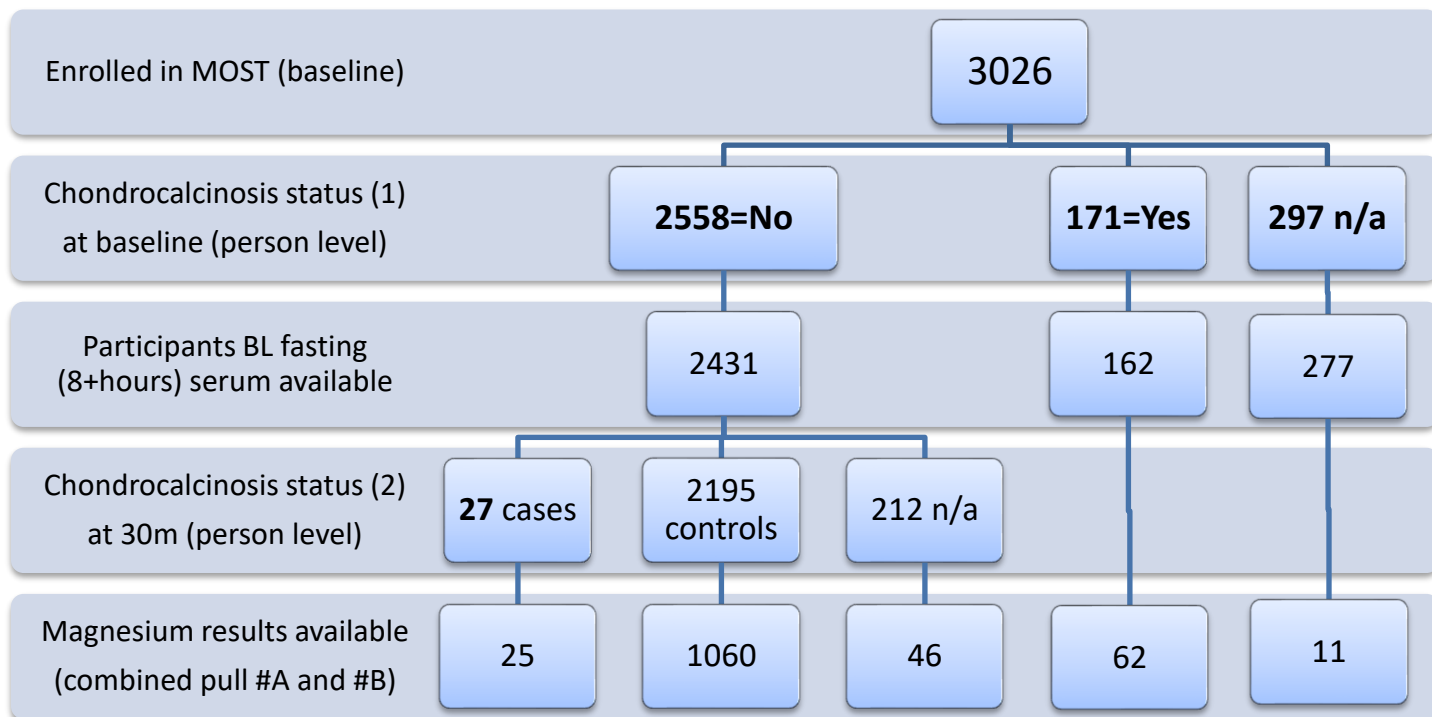
Due to the difference in the selection procedure for pull #A and pull #B, investigators should consider carefully the statistical issues involved in combining data in a single analysis.

Analyst Notes:

- If there was insufficient volume or some other reason assay could not be performed, and all assay values were missing, the record is not included in the analytical dataset.
- Indicator selection variables (#6-#9) were provided by BU analyst for additional selection (pull #B).

2. Chondrocalcinosis Status at Baseline and 30-month combining pull #A and pull #B

Figure 1 – Flow chart for study selection for 30m outcome.



(1) Chondrocalccinosis status at baseline:

- **No**=Participants with both knees without chondrocalcinosis on PA and lateral x-ray view
- **Yes**=Participants with at least one knee and at least one of the three chondrocalcinosis parameters (lateral view: V0LXR/LCHON, PA view: V0XR/LCHOL and V0XR/LCHOM) equal to 1
- **n/a**=Participants with missing chondrocalcinosis status or participants with baseline bilateral x-ray exclusion (RA, amputation, missing patella, necrosis) (not determined).

For participant to be eligible for incidence, both knees should be without chondrocalcinosis on PA and lateral x-ray view.

(2) Chondrocalcinosis status at 30m:

For participant to be eligible for chondrocalcinosis incidence, both knees need to be without chondrocalcinosis on PA and lateral x-ray view.

- **Case**=Participants with at least one knee and at least one of the three chondrocalcinosis parameters equal to 1
- **Control**=Participants with both knees without chondrocalcinosis on PA and lateral x-ray view
- **n/a**=Participants with missing chondrocalcinosis status or participants with 30m bilateral KR or other x-ray exclusion (RA, amputation, missing patella, necrosis) (not determined).

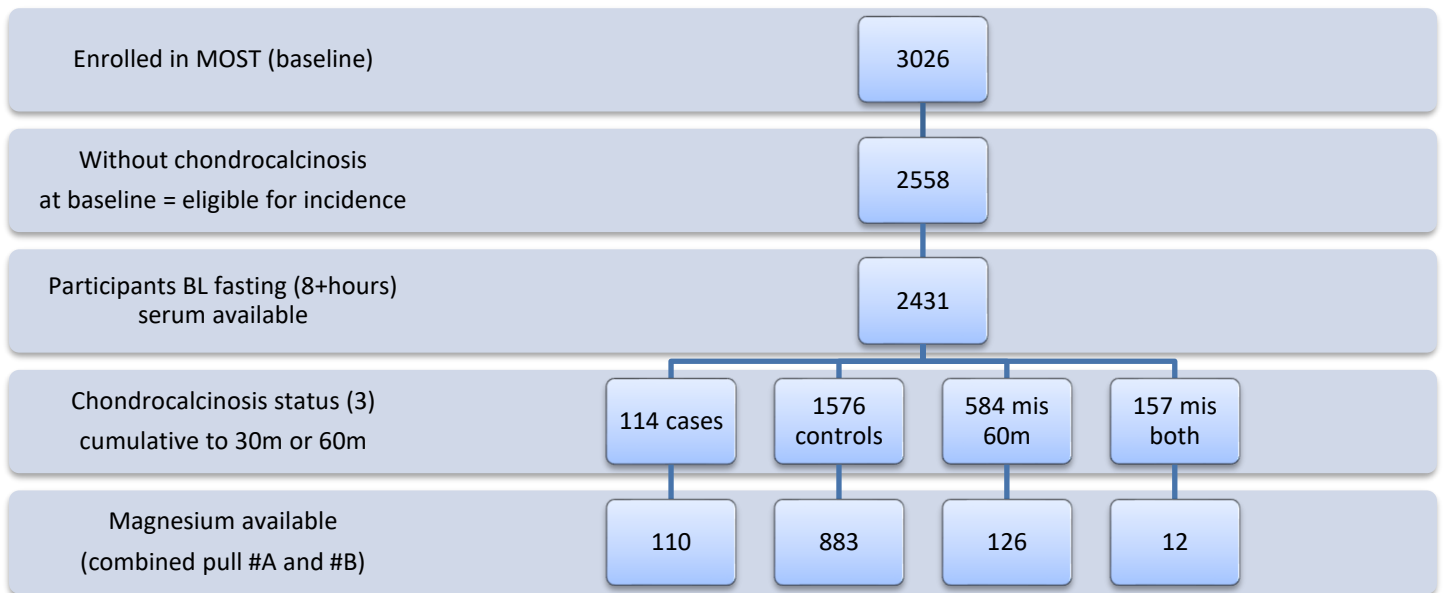
Note: If chondrocalcinosis was not present at baseline, participants were required to have x-ray obtained and read (PA view, left and right lateral view) and chondrocalcinosis condition determined in at least one knee.

Table 1. Magnesium assay completion rate by category

Chondrocalcinosis status at baseline and 30m	N participants	N participants with baseline serum	Magnesium results available	Magnesium completion rate
Without chondrocalcinosis at baseline, incidence case at 30m	29	27	25	86.2%
Without chondrocalcinosis at baseline, control at 30m	2302	2195	1060	46.1%
Without chondrocalcinosis at baseline, 30m status is not determined	227	212	46	20.3%
Subtotal: without chondrocalcinosis at baseline	2558	2431	1131	44.2%
With chondrocalcinosis at baseline	171	162	62	36.2%
Chondrocalcinosis at baseline is not determined	297	277	11	3.7%
Total enrolled in the MOST	3026	2870	1204	39.8%

3. Cumulative chondrocalcinosis incidence at 30m and/or 60m combining pull #A and pull #B

Figure 2 – Flow chart for study selection for cumulative 30m and 60m outcome.



(3) Cumulative chondrocalcinosis status at 30m and/or 60m:

Only eligible for chondrocalcinosis incidence (both knees need to be without chondrocalcinosis on PA and lateral x-ray view) are presented here:

- **Case**=Participants with at least one native knee and at least one of the three chondrocalcinosis parameters equal to 1 at one of the follow up visit (30m or 60m)
- **Control**=Participants with both knees without chondrocalcinosis on PA and lateral x-ray view at 60m
- **mis 60m**=Participants without chondrocalcinosis at 30m and missing chondrocalcinosis status at 60m or participants with 30m/60m bilateral KR or other x-ray exclusion (RA, amputation, missing patella, necrosis) (not determined)
- **mis both**=Participants with missing chondrocalcinosis status or participants with 30m/60m bilateral KR or other x-ray exclusion (RA, amputation, missing patella, necrosis) (not determined)

Note: If chondrocalcinosis was not present at baseline, to be included in analysis, participants were required to have x-ray obtained and read (PA view, left and right lateral view) and chondrocalcinosis condition determined in at least one native knee at one of the follow up visit (30m or 60m).

Table 2. Magnesium assay completion rate by category

Chondrocalcinosis status at baseline and 30m	N participants	N participants with baseline serum	Magnesium results available	Magnesium completion rate
Without chondrocalcinosis at baseline, incidence case at 30m	29	27	25	86.2%
Without chondrocalcinosis at baseline and 30m, incidence case at 60m	84	81	80	95.2%
Without chondrocalcinosis at baseline, missing 30m, incidence case at 60m	7	6	5	71.4%
Without chondrocalcinosis at baseline, cumulative incidence case at 30m or 60m	120	114	110	91.7%
Without chondrocalcinosis at baseline, control at 60m	1659	1576	883	53.2%
Without chondrocalcinosis at baseline, control at 30m, 60m is missing	614	584	126	20.1%
Without chondrocalcinosis at baseline, 30m/60m status is not determined	165	157	12	7.3%
Subtotal: without chondrocalcinosis at baseline	2558	2431	1131	44.2%

Note: Investigators should consider carefully the statistical issues involved in combining data in a single analysis.

4. Appendix 1. Magnesium Assay Documentation Provided by Laboratory (pull #A)

Magnesium	
Manufacturer	BeckmanCoulter
Instrument	AU480 Clinical Chemistry Analyzer
Method (as specified in procedural insert)	colorimetric, endpoint
Additional Reference	Mann et al
Catalog Number	OSR6189
Kit Lot (Reagent Lot)	#2569
Sample Size/Minimum Volume Required for Analysis	2 uL/175uL
Units of Measure	mEq/L
Low Detection Threshold	0.4 mEq/L
High Detection Threshold	6.6 mEq/L
Calibration Factor	2.6 mEq/L
Range of Standard (Range of QC Material Used)	1.4-4.29
Estimated Normal Range	1.3-2.6 mEq/L
Intra-assay Coefficient of Variance	2.2%
Inter-assay Coefficient of Variance	3.0%
References:	
Mann, C.K. and Yoe, J.H., Anal Chem, 28: 202-205, 1956.	

There were four samples that had clots and therefore we were unable to provide magnesium levels.

5. Appendix 2. Magnesium Assay Documentation Provided by Laboratory (pull #B)

Magnesium	
Manufacturer	BeckmanCoulter
Instrument	AU480 Clinical Chemistry Analyzer
Method (as specified in procedural insert)	colorimetric, endpoint
Additional Reference	Mann et al
Catalog Number	OSR6189
Kit Lot (Reagent Lot)	#2569
Sample Size/Minimum Volume Required for Analysis	2 uL/175uL
Units of Measure	mEq/L
Low Detection Threshold	0.4 mEq/L
High Detection Threshold	6.6 mEq/L
Calibration Factor	2.6 mEq/L
Range of Standard (Range of QC Material Used)	1.4-4.29
Estimated Normal Range	1.3-2.6 mEq/L
Intra-assay Coefficient of Variance	2.2%
Inter-assay Coefficient of Variance	3.0%

References:

Mann, C.K. and Yoe, J.H., Anal Chem, 28: 202-205, 1956.

NOTE: Our reference range is 1.30-2.60 mEq/L. Samples outside the range were repeated.

6. Appendix 3. Quality Control Report for Serum Magnesium Results by category.

The Coordinating Center performed QC procedures on the study results and generated this report. Note: the difference between “N obs” (column 2) and “N” (column 3) is due to missing assay results.

Table 1. Magnesium Assay by Laboratory Running Batch.

Analysis Variable : Magnesium									
Batch	N Obs	N	Mean	Std Dev	Minimum	25th Pctl	50th Pctl	75th Pctl	Maximum
1	81	81	1.83	0.17	1.23	1.73	1.83	1.95	2.19
2	81	80	1.83	0.13	1.51	1.76	1.84	1.9	2.2
3	81	80	1.93	0.2	1.33	1.84	1.93	2.05	2.4
4	81	81	1.91	0.17	1.53	1.75	1.93	2.04	2.23
5	81	81	1.92	0.24	1.3	1.76	1.9	2.11	2.39
6	81	81	1.99	0.16	1.65	1.88	1.98	2.09	2.35
7	81	79	1.9	0.2	1.26	1.81	1.89	2.02	2.45
8	81	81	1.99	0.17	1.6	1.88	2	2.1	2.36
9	81	81	1.87	0.17	1.32	1.78	1.87	1.98	2.42
10	81	81	1.87	0.15	1.54	1.78	1.89	1.97	2.31
11	81	81	1.78	0.16	1.34	1.67	1.76	1.88	2.39
12	103	102	1.83	0.18	1.32	1.74	1.82	1.93	2.37
100*	215	215	1.97	0.24	1.14	1.83	1.93	2.11	2.75

*pull #B results – all performed as one laboratory batch

Table 2. Magnesium Assay by Sex.

Analysis Variable : Magnesium									
Sex/Gender	N Obs	N	Mean	Std Dev	Minimum	25th Pctl	50th Pctl	75th Pctl	Maximum
Female	720	717	1.9	0.2	1.14	1.78	1.88	2.01	2.63
Male	489	487	1.91	0.2	1.34	1.77	1.9	2.02	2.75

Table 3. Magnesium Assay by chondrocalcinosis status at baseline.

Analysis Variable : Magnesium									
Baseline chondrocalcinosis	N Obs	N	Mean	Std Dev	Minimum	25th Pctl	50th Pctl	75th Pctl	Maximum
. (missing, not determined)	11	11	1.91	0.12	1.71	1.83	1.87	2.01	2.13
0	1136	1131	1.90	0.2	1.2	1.78	1.89	2.02	2.75
1	62	62	1.85	0.22	1.14	1.73	1.85	2	2.5

Table 4. Magnesium Assay by chondrocalcinosis status at 30m

Analysis Variable : Magnesium									
Chondrocalcinosis status at 30m	N Obs	N	Mean	Std Dev	Minimum	25th Pctl	50th Pctl	75th Pctl	Maximum
. (missing, not determined)	119	119	1.85	0.2	1.14	1.74	1.87	1.98	2.5
0	1065	1060	1.91	0.2	1.2	1.78	1.9	2.02	2.75
1	25	25	1.99	0.21	1.67	1.84	1.96	2.13	2.41

Table 5. Magnesium Assay by chondrocalcinosis status at 60m.

Analysis Variable : Magnesium									
Chondrocalcinosis status at 60m	N Obs	N	Mean	Std Dev	Minimum	25th Pctl	50th Pctl	75th Pctl	Maximum
. (missing, not determined)	220	218	1.87	0.2	1.14	1.75	1.87	2.01	2.56
0	886	883	1.90	0.19	1.23	1.78	1.9	2.01	2.63
1	103	103	1.93	0.24	1.2	1.78	1.91	2.05	2.75